



Isothermal SARS-CoV-2 Diagnostics: Tools for Enabling Distributed Pandemic Testing as a Means of Supporting Safe Reopenings

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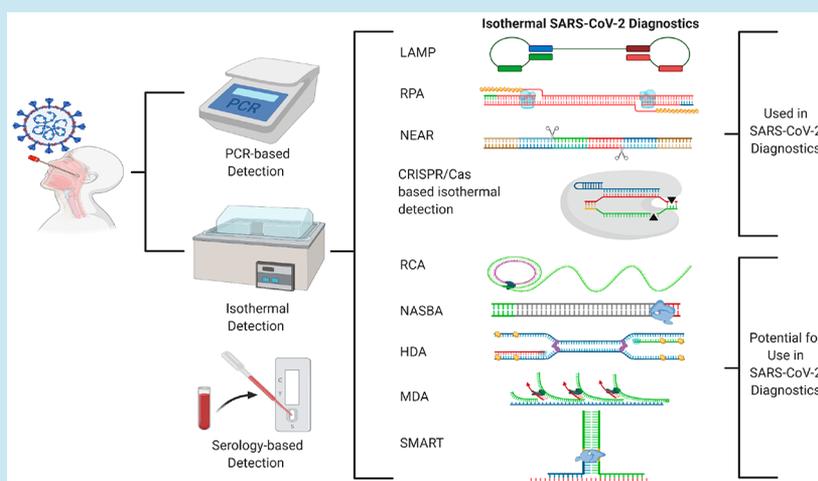


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ABSTRACT: The COVID-19 pandemic, caused by the SARS-CoV-2 virus, poses grave threats to both the global economy and health. The predominant diagnostic screens in use for SARS-CoV-2 detection are molecular techniques such as nucleic acid amplification tests. In this Review, we compare current and emerging isothermal diagnostic methods for COVID-19. We outline the molecular and serological techniques currently being used to detect SARS-CoV-2 infection, past or present, in patients. We also discuss ongoing research on isothermal techniques, CRISPR-mediated detection assays, and point-of-care diagnostics that have potential for use in SARS-CoV-2 detection. Large-scale viral testing during a global pandemic presents unique challenges, chief among them the simultaneous need for testing supplies, durable equipment, and personnel in many regions worldwide, with each of these regions possessing testing needs that vary as the pandemic progresses. The low-cost isothermal technologies described in this Review provide a promising means by which to address these needs and meet the global need for testing of symptomatic individuals as well as provide a possible means for routine testing of asymptomatic individuals, providing a potential means of safely enabling reopenings and early monitoring of outbreaks.

COVID-19 is the pandemic disease caused by a novel coronavirus first isolated in late 2019, SARS-CoV-2.¹ Belonging to the same family of viruses that caused the SARS (severe acute respiratory syndrome) epidemic of 2003 and the MERS (Middle East respiratory syndrome) outbreak of 2012, SARS-CoV-2 has proven to be highly transmissible.^{2–4} Just as SARS-CoV and MERS-CoV have animal reservoirs, SARS-CoV-2 is also thought to be of zoonotic origin, jumping from an animal to human host. Both a bat and pangolin coronavirus have been identified as the closest genetic relatives to SARS-CoV-2, but the exact route from animals to humans has yet to be elucidated.^{5,6} On March 11, 2020, the World Health Organization designated the outbreak to be a pandemic,⁷ and as of July 2020, there have been over 11.5 M cases and 500 K

deaths spanning the globe in 188 countries, demonstrating that the virus is a serious threat to global health.⁸

SARS-CoV-2 is an RNA virus with a ca. 30 kb genome (NCBI Reference Sequence: NC_045512.2). The genome encodes four structural proteins: the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleocapsid (N) protein. Around two-thirds of the genome consists of the *orf1ab*

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Table 1. Comparison of Different Diagnostic Test Classes

assay	positive characteristics	challenges
RT-PCR	current gold standard, ubiquitous in hospital laboratories	expensive, time-consuming, and energy-intensive with a requirement for high-cost instrumentation
one-step isothermal amplification/detection	lowest resource requirements, amenable to POC and field deployment, fast processing times	false positives and negatives present unique engineering challenges, primer design may be time-consuming and expensive in some cases
isothermal amplification/CRISPR/Cas-based detection	dual specificity enhancement afforded by two-step amplification/readout method	challenges associated with isothermal amplification techniques and those of Cas-based detection, readout is qualitative due to dependence on collateral activity of Cas enzymes
serological tests	only means of measuring immunity if neutralizing antibodies tested, rapid positive/negative result	patient must mount immune response before testing positive, distinction between neutralizing and non-neutralizing antibodies

Table 2. Comparison among Different Diagnostic Techniques for SARS-CoV-2

test	readout	limit of detection	enzymes	incubation temperature (°C)	accessibility	lyophilized reagents	detection time (min)	source
PCR-based techniques								
RT-PCR	fluorescence	0.136 copies/ μ L	Taq polymerase	25–95	available worldwide	yes	40–80	70
isothermal techniques								
RT-LAMP-based iLACO	pH change, colorimetric paper dipstick	0.5 copies/ μ L	reverse transcriptase, DNA polymerase	65	research use	possible	15–40	32
Abbott Laboratories' ID Now (NEAR)	fluorescence	0.125 copies/ μ L	nicking enzyme, DNA polymerase	54–58	available worldwide	yes	5–13	48, 71
COVID-19 Penn-RAMP	colorimetric	0.09 copies/ μ L	reverse transcriptase, recombinase, ligase, single-stranded binding proteins, DNA polymerase	38–68	research use	possible	60	34
isothermal techniques with CRISPR-based detection								
SARS-CoV-2 DETECTR lateral flow assay	lateral flow strip	7.8 copies/ μ L	reverse transcriptase, DNA polymerase, Cas12	37–62	research use	possible	<40	56, 72
CRISPR-based SHERLOCK	fluorescence and lateral flow	2 copies/ μ L	DNA polymerase, recombinase, Cas12b, RNase inhibitor, T7 RNA polymerase	22–60	FDA EUA approval	possible	40–70	64, 65

region, which codes for orf1ab polyproteins that are cleaved into various nonstructural proteins, composed of many proteins important for replication, such as an RNA dependent RNA polymerase (RdRp), replicase–transcriptase complex, and RNA helicase.^{9–11} SARS-CoV-2 infects by binding the angiotensin converting enzyme-2 (ACE2), present on the surface of a variety of cell types, with the S protein.¹² The virus is transmitted from person to person through aerosol droplets from an infected persons' cough, sneeze, or saliva.¹³ The pathological symptoms of the disease can vary but most commonly include respiratory illness, dry cough, fever, and cytokine-storm related complications.¹⁴ Further, many SARS-CoV-2 infected individuals are asymptomatic and capable of inadvertently spreading SARS-CoV-2, increasing the spread of the disease.^{15–18}

With no available vaccine and high transmissibility, proper detection and containment are key to helping mitigate the spread and devastation caused by SARS-CoV-2.¹⁹ The current most common diagnostic method used to identify SARS-CoV-2 infection is a molecular technique for detecting viral RNA through nucleic acid amplification, RT-PCR. Around the world, a variety of RT-PCR kits are in use, each with varying specificity. RT-PCR is a ubiquitous method for a myriad of disease detection, but the ability to employ this method in low resource areas lacking centralized care facilities or at the point of care (POC) is limited due to equipment and energy needs. Oftentimes, it is required to obtain a rapid result at the POC, allowing for easier containment of a pathogenic virus such as SARS-CoV-2.²⁰ This Review discusses current molecular and serological methods in use for SARS-CoV-2 detection, with

particular interest in isothermal detection platforms. We discuss their advantages and disadvantages relative to PCR-based and serological techniques (Table 1) as well as isothermal techniques not yet used in SARS-CoV-2 diagnostics that could allow for the expansion of testing capabilities as a means of supporting safe reopenings of businesses and schools as well as allowing for routine household testing.

PCR-BASED SARS-COV-2 DETECTION PLATFORMS

Nucleic acid amplification tests (NAATs) are the most common diagnostic tests used to detect pathogens, and many of the current SARS-CoV-2 detection techniques are primarily based on NAATs.²¹ NAATs involve nucleic acid amplification, a process that initiates with a small quantity of starting nucleic acids and uses primers that target specific, short nucleic acid sequences in conjunction with enzymes to amplify or increase the quantity of starting nucleic acids.²² The most common type of NAAT is the polymerase chain reaction (PCR), which amplifies DNA and can be used in reverse transcription PCR (RT-PCR) for detection of RNA or in quantitative PCR (qPCR) for measurement of copy number, and reverse transcription PCR (RT-PCR) for detection of RNA. Recently, Ellington and coworkers described a single-enzyme approach to RT-PCR detection of SARS-CoV-2. This approach uses an engineered thermostable reverse transcriptase/DNA polymerase based on a reverse transcription xenopolymerase (RTX) generated by directed evolution of Archaeal family-B DNA polymerases (polB).^{23,24}

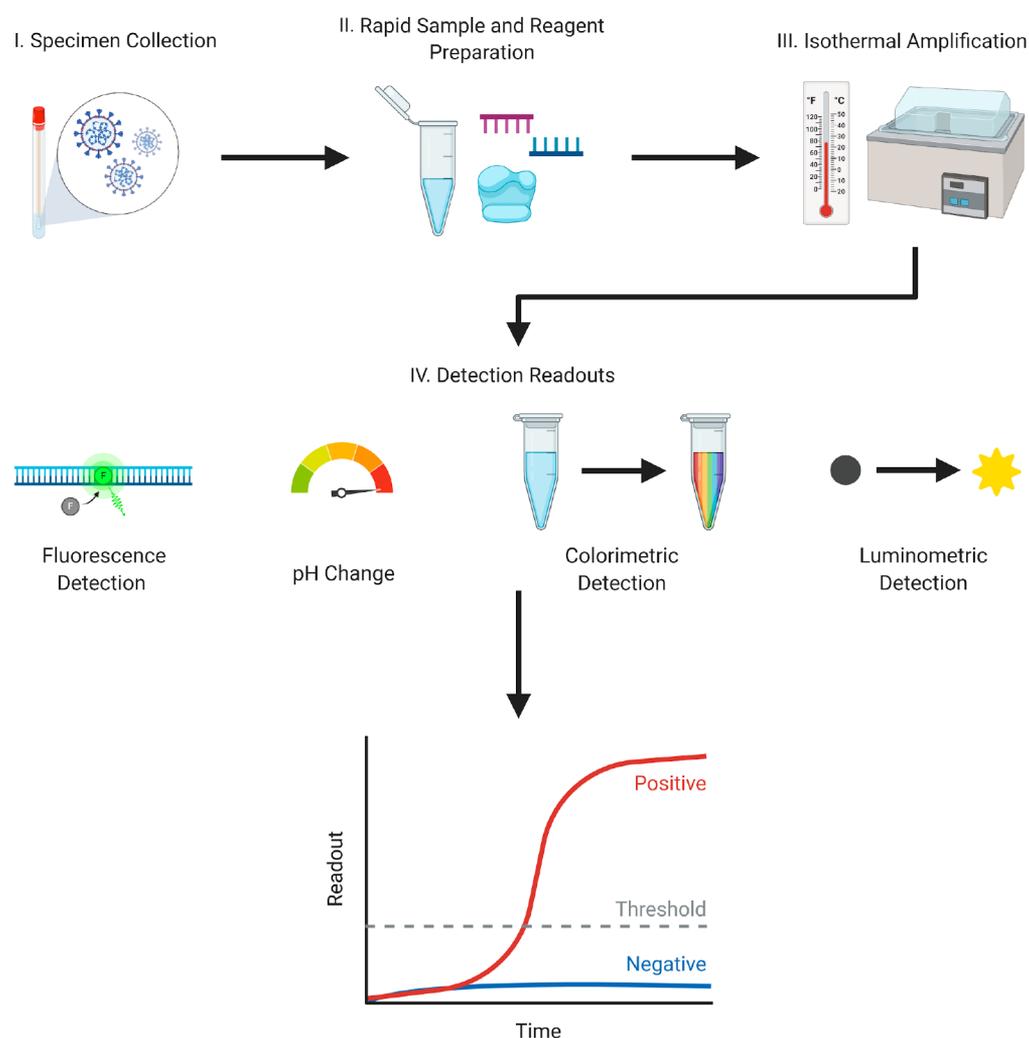


Figure 1. Workflow and readout for isothermal detection techniques. (I) Specimen collection from oropharyngeal swabs or serum collection from blood. (II) Rapid sample preparation and addition of specific primers and enzymes without the need for nuclear extraction methods. (III) Amplification of nucleic acids at a constant temperature. (IV) A variety of readouts are possible; some qualitative readouts only supply a positive or negative evaluation, while quantitative readouts measure the amount of infectious particles.

Advantages of PCR. PCR is the current “gold standard” in molecular diagnostics, and the multiple temperatures employed in the reaction allow for tuning a range of properties, including primer annealing temperature, denaturation temperature, and extension temperature. These can be adjusted at each successive cycle (e.g., touchdown PCR), and instruments exist that can screen a range of temperatures for a given step (e.g., gradient PCR). Fluorescence-monitored PCR instruments (e.g., real-time PCR instruments) exist, which can monitor reaction progress by either nonspecific dyes (e.g., SYBR Green) or dual-labeled probes (e.g., TaqMan).

Disadvantages of PCR. Owing to its requirement for repeated excursions to multiple annealing, extension, and denaturation temperatures, PCR requires bidirectional temperature control. This necessitates expensive instrumentation, typically using a Peltier-effect device as implemented in most thermal cyclers. Similarly, readout requires either electrophoresis followed by gel readout, requiring a skilled technician, or real-time fluorescence readout, requiring a real-time PCR instrument with associated excitation source(s), optics, and emission detectors. This necessarily increases costs.

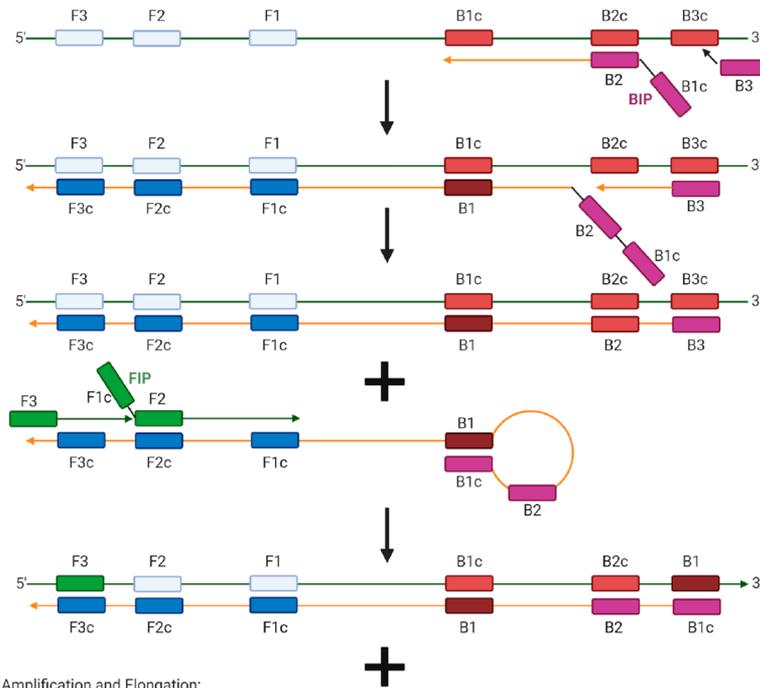
■ ISOTHERMAL SARS-COV-2 DETECTION PLATFORMS

Isothermal detection platforms make use of isothermal nucleic acid amplification methods that allow amplification at constant temperatures.²⁵ This type of NAAT can avoid the high temperatures associated with thermal cycling in PCR and are thus more applicable to low-resource environments, field applications, and laboratories lacking expensive, energy intensive PCR equipment. Isothermal detection techniques can undertake rapid sample and reagent preparation and can also be coupled with a variety of readouts, enhancing their ease-of-use and accessibility (Table 2, Figure 1).

■ LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

The loop-mediated isothermal amplification (LAMP) method uses a DNA polymerase enzyme in conjunction with 4–6 different primers, each recognizing different regions of the target DNA (Figure 2).²⁶ The DNA polymerase used in LAMP has high strand displacement activity and can be used in conjunction with multiplexed reactions and reverse transcription techniques

I. Production of LAMP Starting Material:



II. Cycling Amplification and Elongation:

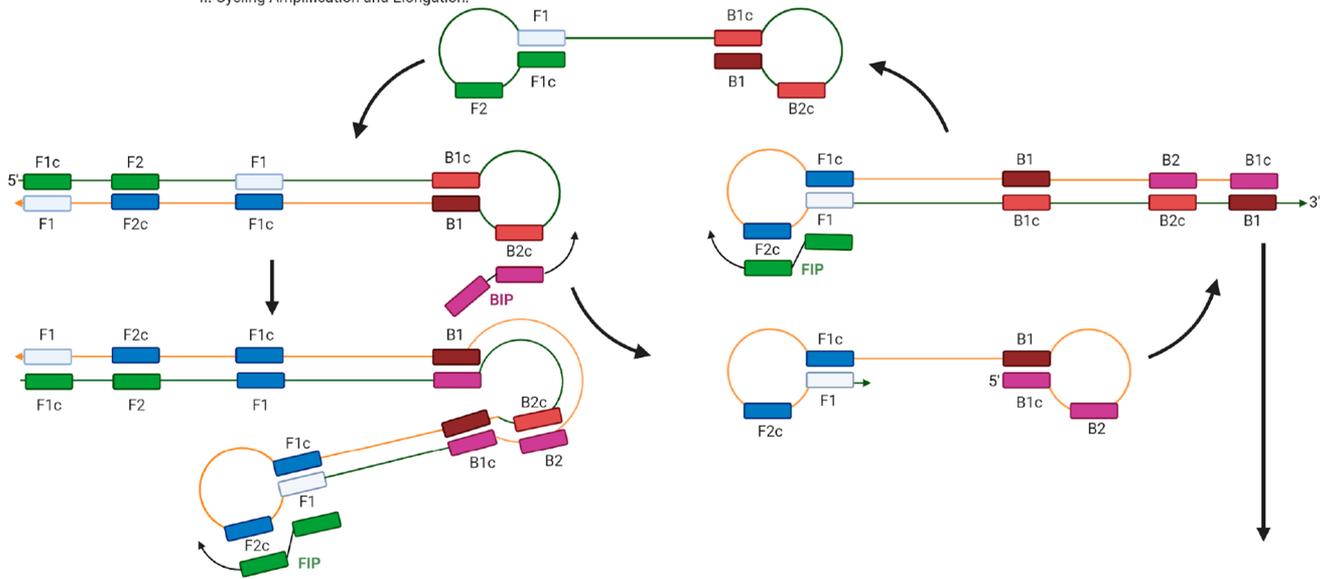


Figure 2. continued

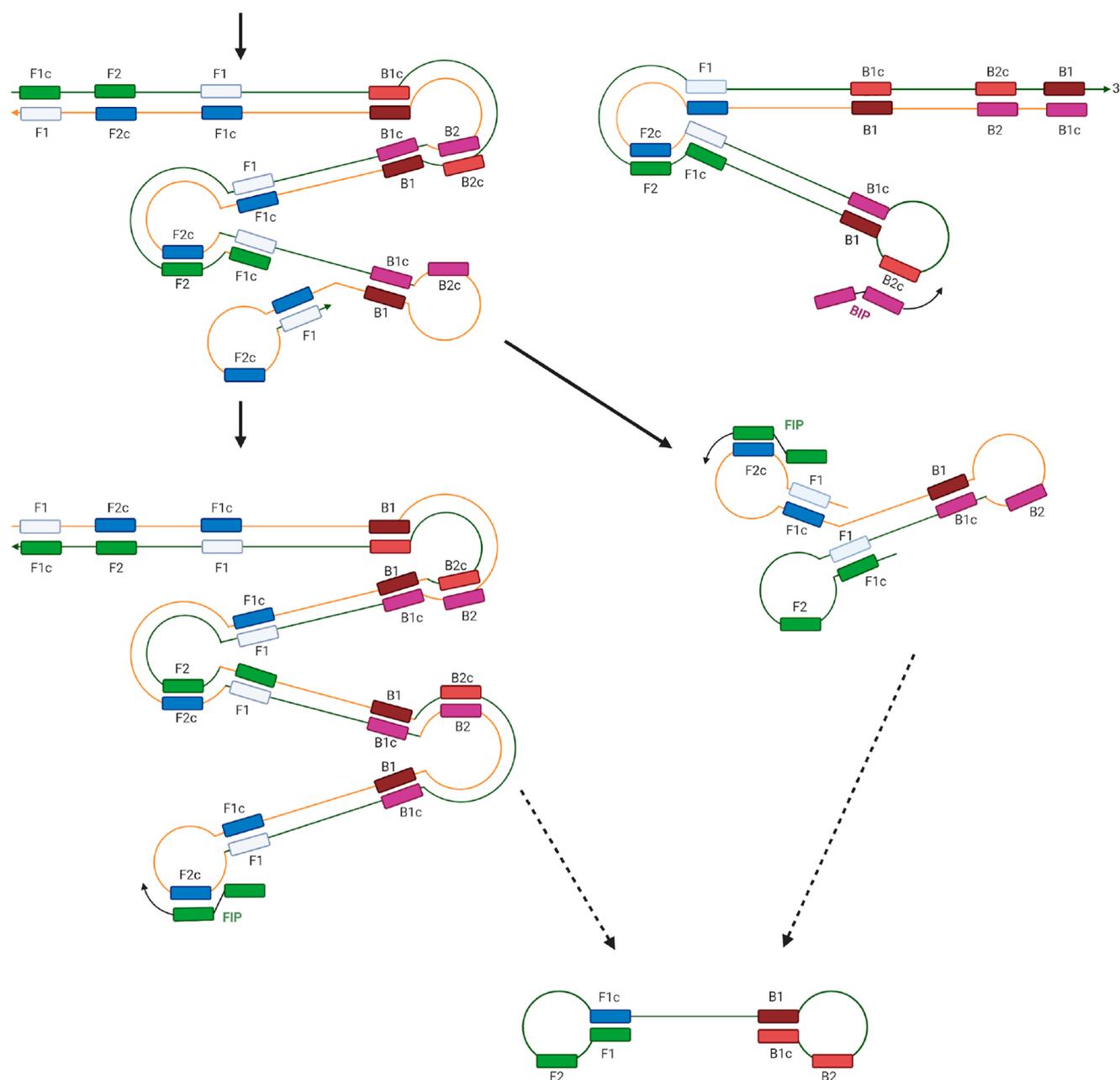


Figure 2. Loop-mediated isothermal amplification. (I) Production of LAMP starting amplicon: First, the forward internal primer (FIP) and the backward internal primer (BIP) anneal to the F2c region and the B2c region in the target DNA strand, respectively. Next, the primers are extended by a strand displacing DNA polymerase. Primers F3 and B3 hybridize to regions F3c and B3c, respectively, and are extended by a strand displacing DNA polymerase generating both double-stranded and single-stranded products. Self-primed DNA synthesis converts dumbbell-shaped DNA structures to stem-loop structures that are the starting material for LAMP. (II) Cycling amplification and elongation: Subsequent hybridization of backward inner primer generates gapped stem-loop DNA, which undergoes self-primed DNA synthesis, forming further loops for binding of forward inner primers. Dotted arrows show continuous reactions. Subsequent rounds of elongation and recycling give rise to multiple stem-loop DNA structures with both double- and single-stranded products.

to detect pathogenic agents.²⁷ Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is useful for detecting RNA-based viruses as it combines conventional LAMP with a reverse transcriptase enzyme, allowing for simultaneous reverse transcription and amplification. RT-LAMP is a one-step amplification reaction, allowing all the primers and necessary enzymes to be incubated isothermally in a

single step.²⁸ The final readout for LAMP can be real-time detection using fluorescence or absorbance instrumentation.

LAMP and SARS-CoV-2. RT-LAMP was recently shown to detect SARS-CoV-2 in <30 min, using patient samples such as urine, saliva, and oropharyngeal and nasopharyngeal swabs spiked with various concentrations of synthetic COVID-19 amplicon.²⁹ The low detection time is promising for the potential use of this technique in POC applications. This rapid

screening test used Lasergene MegAlign (DNASTAR) and BLAST 2 (Basic Local Alignment Search Tool) for analysis of 23 COVID-19 strains and other sequence-related coronaviruses to characterize areas of sequence convergence and design specific primers. Using six different primers, these investigators tested simulated clinical samples that were spiked with SARS-CoV-2, Middle East respiratory syndrome (MERS), betacoronavirus England-1 (BtCoV), or murine hepatitis virus (MHV). The study conducted qRT-PCRs for comparison and found that RT-LAMP was both specific and sensitive for SARS-CoV-2. By using fluorescence and gel-based detection techniques, they evaluated that a temperature of 63 °C for 30 min was optimal for amplification and detection. RT-LAMP was successful in detecting 27 different strains of SARS-CoV-2 and specificity was evaluated by ensuring that SARS-CoV-2 spiked samples were the only positive RT-LAMP reactions. Lack of accessibility to clinical samples limited the ability to test this technique on infected samples, and it was only tested on synthetic samples.

In another report, Lu et al. designed a RT-LAMP assay targeting the *RdRp* gene exhibiting a colorimetric readout based on the pH of the sample. This assay has a limit of detection of 30 copies of RNA and detection takes place in 40 min. Specificity was tested against clinical samples for other common respiratory illnesses, and no off-target amplification was seen.³⁰ A unique adaptation of LAMP was implemented in a barcoded RT-LAMP method called LAMP-seq that uses three isothermal steps to barcode amplicons.³¹ The protocol incorporates sample-specific barcodes by inserting barcode sequences into the LAMP primers, followed by pooling of reactions into batches, which are then subjected to a secondary amplification and barcoding with PCR. These amplicons are then purified and deep-sequenced, and analyzed using a software package, LAMP-Seq-Inspector, also reported in the manuscript. With a limit of detection of 105 RNA molecules/ μL and a cost of less than \$20 per test, LAMP-seq has the ability to pool and process these barcoded amplicons through mass sequencing, making the process scalable for millions of tests every day. This is especially relevant in combating virus spread through asymptomatic carriers.¹⁶ High-throughput techniques such as LAMP-seq could enable population-scale testing, which will be beneficial for monitoring cases in areas of SARS-CoV-2 resurgence and will enhance surveillance, limiting exponential spread.

RT-LAMP-based iLACO (isothermal LAMP-based method for COVID-19) is another recently reported LAMP-based technique developed for detection of SARS-CoV-2.³² iLACO is as sensitive as qPCR, with a detection limit of 10 copies of SARS-CoV-2, and a detection time of between 15 and 40 min. Since DNA polymerase-led nucleotide incorporation is accompanied by hydrogen ion release, iLACO, like the assay developed by Lu et al., combines RT-LAMP with a pH-based readout to enable colorimetric output. iLACO was tested on viral RNA extracted from patient samples and reverse transcribed using an engineered reverse transcriptase (SuperScript (III)). Primer design involved analysis of a region of the ORF1ab gene. Using five sets of different primers, samples were tested in both a thermal PCR cyler and a water bath maintained at 65 °C for 20–40 min. Primer specificity was ascertained with sequence comparison to other related coronaviruses and influenza viruses. The colorimetric detection entailed that negative reactions would remain at higher pH, resulting in the phenol red pH indicator remaining pink, while a reaction positive for SARS-CoV-2 would decrease in pH, resulting in a color change from pink to yellow.

iLACO is rapid; a signal for positive reactions can be detected in about 20 min. The test is also sensitive to up to 10 copies of the *ORF1ab* gene, and the test detection time correlated with qPCR cycle number; a qPCR Ct of 37 corresponded to a iLACO color change point of 37 min. The study also used several alternative detection methods such as fluorescence detection and gel imaging. Furthermore, the use of 43 clinical samples that were confirmed positive by RT-PCR methods is promising for the clinical use of the iLACO system. Forty-two of the 43 known-positive samples were detected successfully by iLACO within 40 min of incubation, when the RNA concentration in the sample was around 0.2–47 ng/ μL . With a short detection time of ca. 30 min, this test shows efficacy in detecting SARS-CoV-2 in both RNA and cDNA samples.

Another recent preprint from Ellington and coworkers uses RT-LAMP and seeks to overcome potential false positives due to aberrant amplification. This is a problem with nonspecific readouts, seen with pH probes or intercalating dyes. To overcome this, they used oligonucleotide strand exchange (OSD) probes. In the technique described, SARS-CoV-2 LAMP-OSD, fluorescent probes are used that are mostly double-stranded and contain a fluorescent probe-quencher pair at one end.³³ Strand exchange occurs when a toehold in the probe base pairs with the product of the LAMP reaction, initiating branch migration until the probe's fluorophore and quencher are separated. Bhadra et al. demonstrates this to work with probes previously designed in assays using nonspecific readouts.^{29,34,35} Building on these assays, Bhadra et al. created a one-pot reaction capable of detecting two targets, the *N* gene and the *ORF1ab* gene. With primers targeting both regions and probes for each amplicon, a positive signal was seen with as little as 10 genomic RNA copies/reaction, and no signal was seen when using 23 ng of human genomic DNA as the negative control.

Handyfuge-LAMP was recently developed as an optimized LAMP assay that is especially suited for POC diagnostics due to its low-cost and electricity-free centrifugation methods.³⁶ The group established a hardware system called "Handyfuge" that is cheap (<\$5 per unit) and easily assembled for achieving the high speed centrifugation needed for separation of inhibitory components from inactivated saliva samples. The reagents also include a chaotropic salt binding solution and a silica binding solution that enables viral RNA capture. This allows the whole procedure of sample processing and amplification to be carried out with an inexpensive Handyfuge and a water bath, along with LAMP reagents. It is thus able to avoid multiple-step sample handling and expensive centrifugation machines. The preprint claims a detection limit of 10–100 copies per μL in saliva, shown by colorimetric change to indicate presence of synthetic SARS-CoV-2 RNA.

Advantages of LAMP. The primary advantage of this rapid detection technique is that it is performed isothermally and hence is amenable to use with low-cost instrumentation. The use of LAMP with a pH-based readout showcases the adaptability of this reaction. Another important advantage is that the detection procedure can avoid the nucleic-acid extraction step, a procedure that is time-consuming and potentially contamination-inducing. LAMP has been shown to function with cell lysates.³⁵ However, extraction steps concentrates the nucleic acid as well, enhancing overall assay sensitivity. By using multiple primer sets in a single reaction, LAMP has been shown to be extremely specific for the target amplicon. Furthermore, LAMP reagent kits are amenable to lyophilization and have been

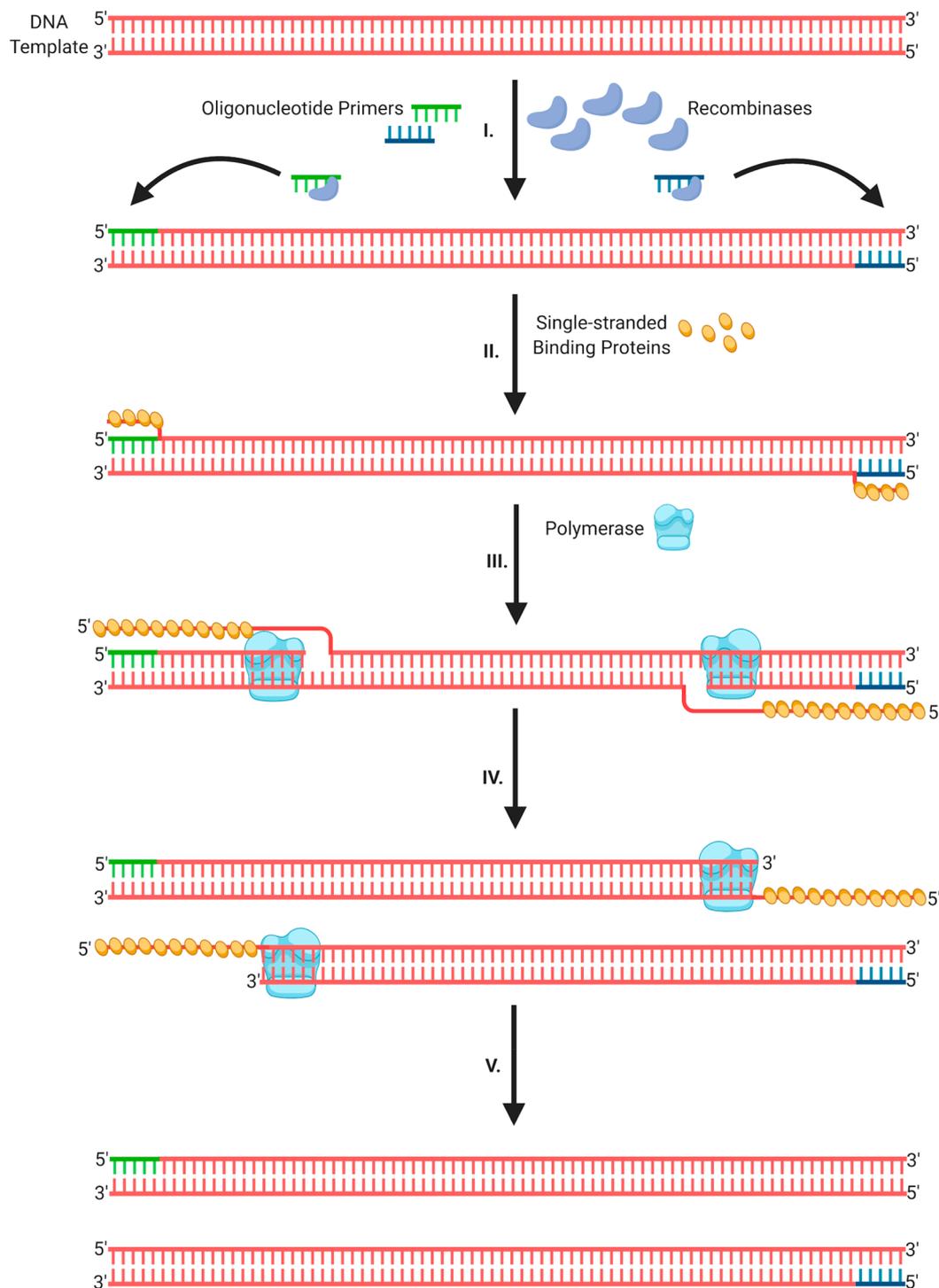


Figure 3. Recombinase polymerase amplification (RPA). (I) RPA initiates with binding of recombinase–nucleotide primer complexes to homologous sequences in the target DNA. (II) The D-loop is stabilized by binding of single-stranded binding proteins to the displaced strands. (III) Synthesis of new DNA strands is initiated by strand displacement polymerases. (IV) The parent DNA strands are separated. (V) DNA synthesis continues until two DNA duplexes are formed.

effective in detection of other RNA viruses such as HIV and the Ebola Zaire Virus.²⁷

Disadvantages of LAMP. Due to the number of primers needed, primer design is complicated, making it difficult to design new assays. It is also important to note that isothermal detection techniques can exhibit false positives and thus more

stringent controls may need to be in place than with RT-PCR methods.³⁷

■ RECOMBINASE POLYMERASE AMPLIFICATION

Recombinase polymerase amplification (RPA) is an isothermal amplification technique that involves two primers binding a double-stranded template with assistance of single-stranded

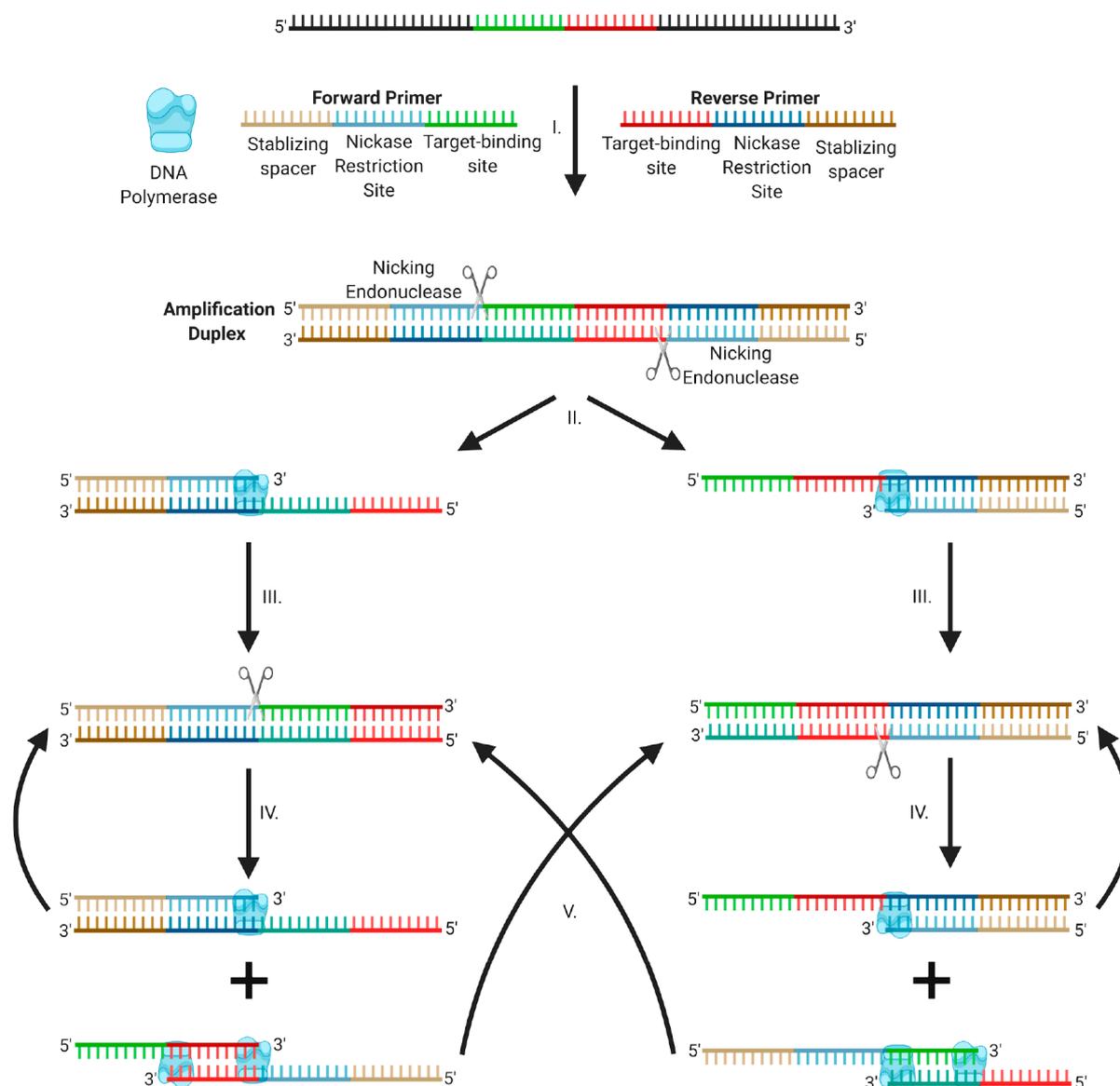


Figure 4. Nicking enzyme amplification reaction (NEAR). (I) The forward and reverse primers bind to complementary regions on the target amplification template. Polymerase extends the primers through strand displacement, and two restriction sites are created for nicking enzyme activity. This creates an amplification duplex for NEAR amplification, with recognition sites for the nicking endonuclease. (II) Nicking endonucleases conduct cleavage and allow for strand displacement amplification, forming a complex with a single-stranded target region, a stabilizing duplex region, and a site for nicking endonuclease activity. (III) A full-length duplex is formed again, which undergoes subsequent amplification through rounds of nicking, extension, and primer binding (IV–V).^{45,48,49}

binding proteins and a recombinase, followed by extension with DNA polymerase, to allow isothermal amplification on DNA targets of interest.³⁸ The process involves recombinase–primer complexes that are able to scan dsDNA, identify a primer-binding site, and enable strand invasion by the primer.³⁹ RPA circumvents the need for thermocycling by employing a recombinase enzyme and a strand displacing DNA polymerase. Via the use of loading factor proteins, primers anneal to their complementary region by the recombinase enzyme. Single-stranded binding proteins then complex the strand displaced by the primer, preventing reannealing and allowing for a strand displacing DNA polymerase to polymerize a new strand. As in PCR, the amplicon is comprised of the region spanning the two primers (Figure 3).

RPA and SARS-CoV-2. **COVID-19 Penn-RAMP** is a novel isothermal POC diagnostic method that combines the LAMP strategy with RPA.³⁴ This test incorporates a nested nucleic acid amplification technique showing higher sensitivity of detection than LAMP alone and conventional RT-PCR for minimally processed SARS-CoV-2 samples. Penn-RAMP avoids false negatives by implementing two stages of isothermal amplification - a first stage of RPA at 37 °C and a second stage of LAMP at 63 °C. In this closed-tube molecular test, RPA is conducted in the tube cap while LAMP is conducted inside the tube. The RPA reaction mix has primers, buffers and salts, and this mix is loaded in the tube cap alongside samples with various target concentrations. After the preliminary 15–20 min incubation at 37 °C for the RPA process, the tube is centrifuged or inverted multiple times to mix the RPA and LAMP reactions, followed by

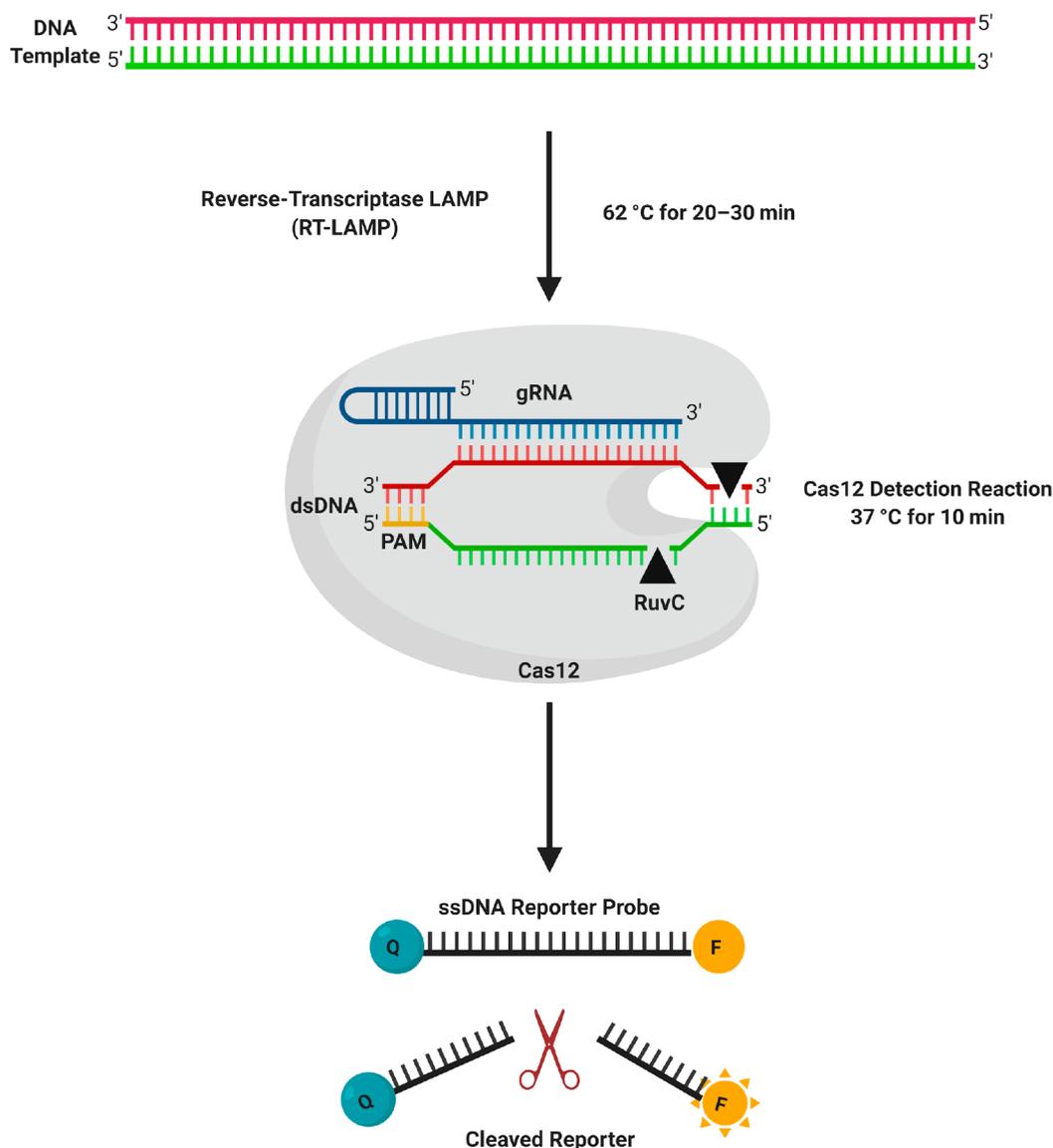


Figure 5. DNA endonuclease-targeted CRISPR trans reporter (DETECTR). DETECTR is a one-pot detection method that combines isothermal amplification with CRISPR-Cas-based detection. The first step is reverse-transcriptase LAMP (RT-LAMP)-based isothermal amplification of DNA at 62 °C for 20–30 min. This is followed by a Cas12a detection reaction at 37 °C for 10 min. Upon recognition of a thymine-rich PAM sequence, Cas12a is able to bind to the double-stranded DNA (dsDNA) template and becomes catalytically activated. Cas12 then uses its RuvC nuclease catalytic domain to generate a staggered cut with a 5'-overhang in the dsDNA, downstream of thymine-rich PAM sequences⁶² and complementary to the guide RNA sequence. Next, Cas12a participates in indiscriminate *trans* cleavage of ssDNA, and it releases the PAM-distal ssDNA cleavage products of the DNA strand from the active site of the RuvC.⁵⁶ Addition of an ssDNA fluorescence-quencher reporter (ssDNA-FQ) allows for production of a fluorescent signal upon cleavage.

an incubation at 63 °C for 40 min, and it is compatible with both fluorescent and colorimetric readout.

With primers targeting the *ORF1ab* gene in SARS-CoV-2, Penn-RAMP has a limit of detection of 7 copies per reaction and is highly specific to SARS-CoV-2. With the use of purified RNA, the authors obtained 100-fold lower detection limits than conventional PCR for SARS-CoV-2. Specificity was tested using other related coronaviruses such as *Alphacoronaviruses*, *Gammacoronaviruses* and *Deltacoronaviruses* as negative controls. For colorimetric detection, a leuco crystal violet (LCV) solution was used, which can be lyophilized.⁴⁰ The LAMP mix contained an aliquot of LCV solution along with the LAMP primers and polymerase, allowing for colorimetric detection at the end of the RPA and LAMP phases. The color change can be observed by

the naked eye and also monitored in real time using a camera or smartphone. The Penn-RAMP study used synthesized DNA to mimic the target sequence for SARS-CoV-2 due to lack of access to real patient samples. Thus, more research on clinical specimens may be needed to validate the specificity and sensitivity. Furthermore, a platform for quantification needs to be assimilated into the Penn-RAMP testing method, enabling quantification in real-time and increased ease of use.

Advantages of RPA. Primer design for RPA reactions is similar to PCR, and it is thus fairly straightforward to design new assays. Due to the reaction's isothermal nature, the melting temperature of the primer is not a factor, in contrast to PCR. TwistDx, a United Kingdom-based company, commercially produces reagents, with lyophilized kits available making

transport of reagents possible without a cold chain. No initial thermal denaturation step is required for RPA as well.⁴¹ RPA can be coupled to multiple different types of readout (e.g., fluorescent and colorimetric), allowing for adaptation to POC diagnostics. For example, a colorimetric readout technique that combines RPA with cell free transcription-translation systems could be modified for SARS-CoV-2 detection. The Lucks group recently showed successful detection of plant pathogens following this method by incorporating small transcriptional activators into the amplification reaction and subsequently activating transcription and translation of catechol 2,3-dioxygenase (CDO) for the conversion of catechol into hydroxyymuconic semialdehyde, producing a yellow positive signal.^{42,43}

Disadvantages of RPA. Multiple enzymes are needed to perform RPA, including strand displacing polymerase, single-stranded binding proteins, and recombinase, as well as cofactors and accessory proteins. This makes RPA more difficult from a manufacturing perspective than other methods employing fewer enzymes.⁴¹ In Penn-RAMP, RPA was coupled with a second isothermal amplification (LAMP) reaction to overcome false positives and increase sensitivity, further complicating primer design, increasing the reaction cost, and adding an additional isothermal step and temperature. RPA amplicons are typically 100–200 nt, with larger products amplifying more poorly due to the commercially available TwistAmp RPA kits being optimized for amplification speed.⁴⁴

■ NICKING AND EXTENSION AMPLIFICATION REACTION

The nicking and extension amplification reaction (NEAR) system amplifies short sequences of nucleic acids.^{45,46} The first use of NEAR was in 2003, and it was later amended for *in vitro* diagnostics in 2009.⁴⁶ Two primers, a DNA polymerase and a nicking enzyme, are used. The reaction temperature can vary depending on the polymerase and nicking enzyme used. Each primer consists of a binding region and a nicking enzyme recognition region. The first primer can bind the sequence of interest, enabling a DNA polymerase to extend it, creating a dsDNA product. The nicking enzyme then nicks only a single strand of the sequence allowing for DNA polymerase to elongate the primer. The second primer can bind the displaced product of the first strand and undergo extension by DNA polymerase.^{47,48} This continues to allow amplification of the region between the two primers, similar to PCR. NEAR is able to amplify 20–30mers 10⁸ to 10¹⁰ fold in under 10 min (Figure 4).⁴⁶

NEAR and SARS-CoV-2. The Abbott ID NOW COVID-19 test is an automated, instrument-based, POC SARS-CoV-2 test using a qualitative fluorescence readout technique enabled by NEAR. This test isothermally amplifies a portion of the *RdRp* gene. One study found Abbott ID NOW detected ca. 94% (90 out of 96) known positive samples,⁵⁰ whereas another study found Abbott ID NOW detected only one-third of the positive samples detected by an RT-PCR method,⁵¹ with dilution associated with the use of viral transport media providing one possible explanation for the diminished sensitivity.

Advantages of NEAR. The ID NOW test is rapid and takes place inside the manufacturer's instrument, with a reaction time of ca. 5 min, making this the fastest time-to-completion among approved tests.⁵² This speed is in part due to the small size of the amplicon compared to other NAATs. Fluorescently labeled molecular beacon probes provide a real-time readout. This

reaction can be adapted to different temperatures by the use of various primers, polymerases, and nicking enzymes.

Disadvantages of NEAR. As discussed above, NEAR has exhibited false negatives under some conditions. Some studies have suggested that dilution associated with the use of viral transport media prior to amplification could play a role.⁵³

■ ISOTHERMAL AMPLIFICATION COUPLED TO CRISPR/CAS-BASED DETECTION

Clustered regularly interspaced short palindromic repeats (CRISPR)-based disease detection methods utilize enzymes with nonspecific DNase or RNase activity, such as Cas12 or Cas13 from the bacterial immune system, along with guide RNAs to direct enzyme binding to specific target areas on pathogenic DNA or RNA sequences.⁵⁴ Addition of fluorescent reporter sequences to this ribonucleoprotein complex enables readout of enzyme activity which can be used for pathogen detection.

CRISPR/Cas and SARS-CoV-2. A novel CRISPR-Cas12-based SARS-CoV-2 detection system called SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) uses a lateral flow assay for detection of SARS-CoV-2 in under 40 min with respiratory swab RNA extracts.⁵⁵ The technique first uses RT-LAMP for reverse transcription and isothermal amplification of viral RNA, and then employs the Cas12a enzyme to identify sequences of SARS-CoV-2, allowing cleavage of a reporter molecule (Figure 5).

SARS-CoV-2 DETECTR uses primers that amplify regions of overlap in the *E* gene and *N* gene of SARS-CoV-2 and takes advantage of the nonspecific DNase activity of Cas12a for indiscriminate ssDNA cleavage and degradation.⁵⁶ This Cas12a activity allows for cleavage and activation of an ssDNA reporter. Design of the Cas12a guide RNAs (gRNAs) entailed designing three *E* gene gRNAs for identification of three SARS-related coronaviruses and one *N* gene gRNA that specifically targeted the *N* gene for SARS-CoV-2 detection, thus requiring identification of both *E* and *N* genes for detection. The specificity analysis showed that the test was specific for SARS-CoV-2 and not related coronaviruses such as bat-SL-CoVZC45 and SARS-CoV. The human *RNase P* gene was used as a control. The detection reaction involved an isothermal RT-LAMP phase at 62 °C for 20 min, followed by Cas12a-gRNA guided detection reaction at 37 °C for 10 min, the output from which could be visualized using a lateral flow assay in the form of an easily interpretable, qualitative, visual signal. The limit of detection for this test was found to be 10 copies/ μ L of reaction, with a 100% negative predictive value and a 95% positive predictive value. Importantly, this assay has been tested on RNA extracted from SARS-CoV-2 PCR-positive clinical patient samples.

Ding et al. recently described the use of RPA with Cas12a to detect SARS-CoV-2 in a single reaction, which they have termed All-in-One Dual CRISPR-Cas12a, AIOD-CRISPR.⁵⁷ This assay exhibits a limit of detection of 1.3 copies of the *N* gene synthetically derived from a plasmid in 40 min. The specificity was tested against SARS-CoV and MERS-CoV control plasmids. This assay was also tested against HIV-1 RNA. Another CRISPR-Cas12-based assay developed by Lucia et al. uses RPA in conjunction with reverse transcription and subsequent detection by the Cas12a endonuclease collateral activity.⁵⁸ With primers targeting the *ORF1ab* region, the authors' test reached a limit of detection of 10 viral copies/ μ L. This test was compatible with both fluorescence readouts and a paper-based strip assay. Furthermore, successful testing on spiked clinical saliva samples

Table 3. Comparison among Different Isothermal Amplification Techniques in Use for SARS-CoV-2 Detection or with the Potential to Be Used to Detect SARS-CoV-2

name	acronym	template	incubation temperature (°C)	enzymes needed	limit of detection	available readouts	detection time	commercialized tests	source
loop mediated isothermal amplification	LAMP	DNA or RNA	65	DNA polymerase with strand displacement activity, reverse transcriptase if required	0.24 copies/ μ L	colorimetric, pH, fluorescence	30 min	Meridian <i>illumigene</i> , malaria	26, 94
recombinase polymerase amplification	RPA	DNA or RNA	37–42	recombinase, recombinase loading factor, single-stranded binding proteins, DNA polymerase	0.38 copies/ μ L	fluorescence, gel electrophoresis, flocculation, chemiluminescence, silicon microring resonator, surface enhanced Raman scattering, lateral flow dipstick	10 min	TwistDx, multiple pathogens	95
nicking enzyme amplification reaction	NEAR	DNA or RNA	dependent on nicking enzyme and polymerase used, typically 37	nicking enzyme, DNA polymerase, reverse transcriptase if required	0.125 copies/ μ L	fluorescence	10 min	Abbott Alere i, influenza A and B, Abbott IDNOW, SARS-CoV-2	48, 71
rolling circle amplification	RCA	DNA or RNA	37	ligase, DNA/RNA polymerase	100 copies/ μ L	colorimetric, fluorescence, nanoparticles	0.5–3 h ^a	n/a	89, 96–98
nucleic acid sequence based amplification	NASBA	DNA or RNA	37–42	reverse transcriptase, RNase H, RNA polymerase	0.04 copies/ μ L	colorimetric, fluorescence, gel electrophoresis, lateral flow	1.5 h	bioMérieux, <i>M. pneumoniae</i> and <i>C. pneumoniae</i> , HPV, HSV, RSV, MRSA, HIV-1 viral load, Coris BioConcept OligoC-Test, enterovirus, <i>Leishmania</i> , <i>T. Cruzi</i>	99–101
helicase dependent amplification	HDA	DNA	37 or 60–65 with heat stable enzymes	helicase, single-stranded binding proteins, <i>MutL</i> , DNA polymerase (only required for 37 °C condition)	10 copies/ μ L	gel electrophoresis, fluorescence, electrochemical, lateral flow, hybridization assays	30 min	AmpliVue-Quidel, <i>B. pertussis</i> , <i>C. difficile</i> , <i>Herpes simplex virus</i> , <i>T. vaginalis</i>	85, 86
multiple displacement amplification	MDA	DNA or RNA	30	strand displacing DNA polymerase	5 copies/ μ L	fluorescence, gel electrophoresis, pH change	4–18 h ^a	n/a	89, 102
signal-mediated amplification of RNA technology	SMART	DNA or RNA	41	DNA polymerase, RNA polymerase	5000 copies/ μ L	ELISA, fluorescence	3 h	n/a	90, 91

^aDetectable results with increasing signal over this time.

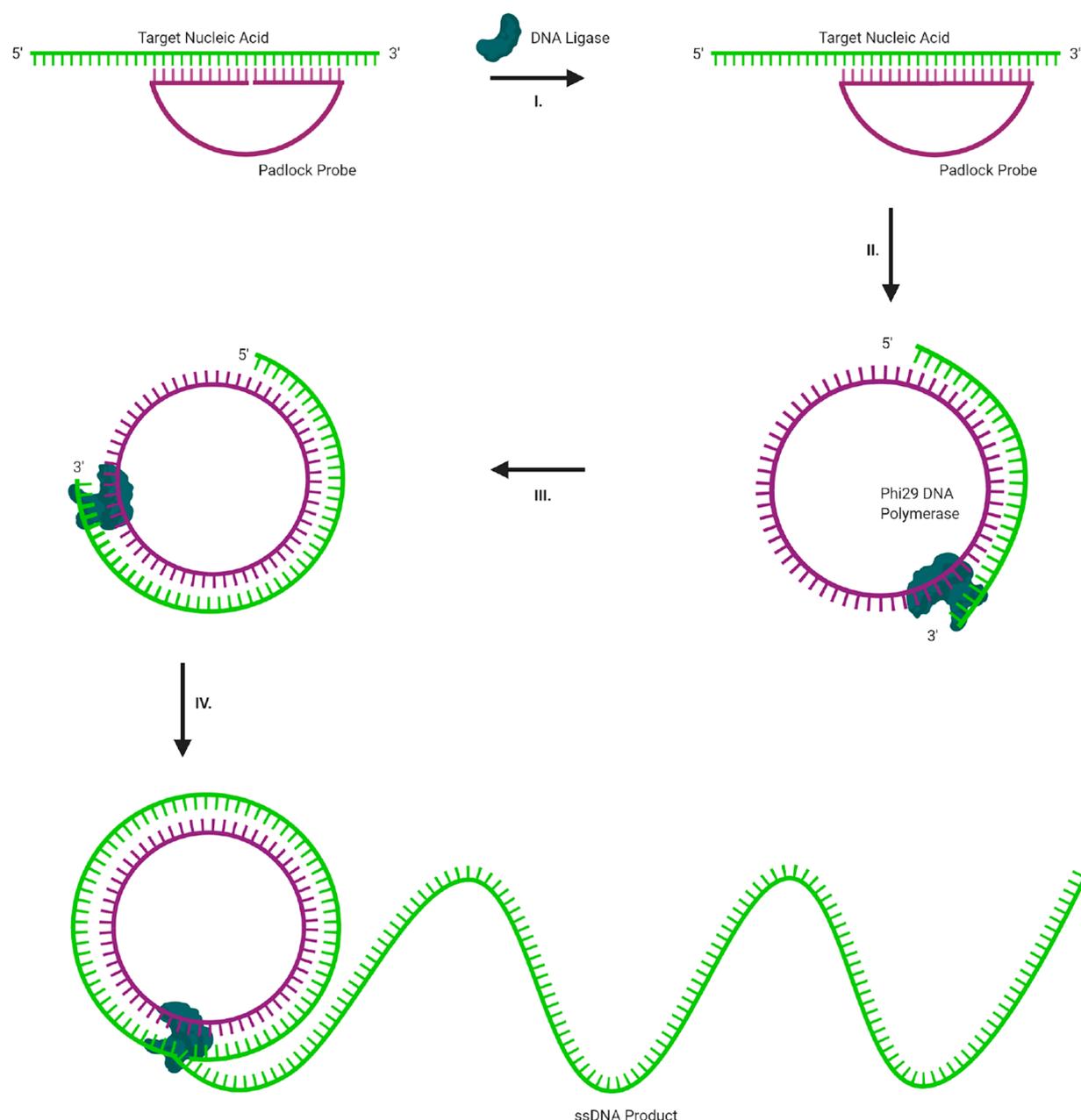


Figure 6. Rolling circle amplification (RCA). (I) Template-mediated enzymatic ligation synthesizes a circular DNA template from a linear DNA template. This is followed by annealing of a primer to a region of complementarity in the circular DNA template. (II) Phi29 DNA polymerase initiates DNA synthesis by elongation of the annealed primer. (III) DNA polymerase activity results in a long ssDNA with repeats of the DNA template sequence.⁷⁹

shows that the method is valid for easily obtainable saliva specimens and thus suitable for POC operations.

Another study on a CRISPR-based detection system, called the CASdetec (CRISPR-assisted detection) strategy,⁵⁹ shows improved sensitivity compared to Cas12a-based detection systems owing to the accuracy and specificity of dsDNA trans-cleavage by the Cas12b enzyme.⁶⁰ CASdetec uses reverse transcriptase recombinase aided amplification (RT-RAA) for a preamplification step, followed by the use of Cas12b with gRNA targeted to the *RdRp* gene, with a limit of detection of 10^4 SARS-CoV-2 pseudovirus copies/mL. The RAA assay is performed between 37 and 42 °C and employs single-stranded binding proteins, a recombinase and a DNA polymerase.⁶¹ To simplify the reaction, it was split within one vessel, with RT-RAA

conducted inside the reaction tube while the Cas-12b-based detection reagents were kept in the cap of the tube, followed by a spin-down mixing of the reagents and subsequent fluorescent readout with a total reaction time of about 30 min. The authors demonstrated that their readout was compatible with LED excitation, increasing usability in POC settings.

A novel CRISPR-based diagnostic for COVID-19 detection with recent U.S. Food and Drug Administration (FDA) approval is called STOPCovid (SHERLOCK Testing in One Pot COVID)⁶³ and uses the SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique.⁶⁴ STOPCovid targets the SARS-CoV-2 *N* gene and aims to be a POC assay, taking about an hour to process results and needing very little instrumentation. Similar to DETECTR, STOPCovid uses

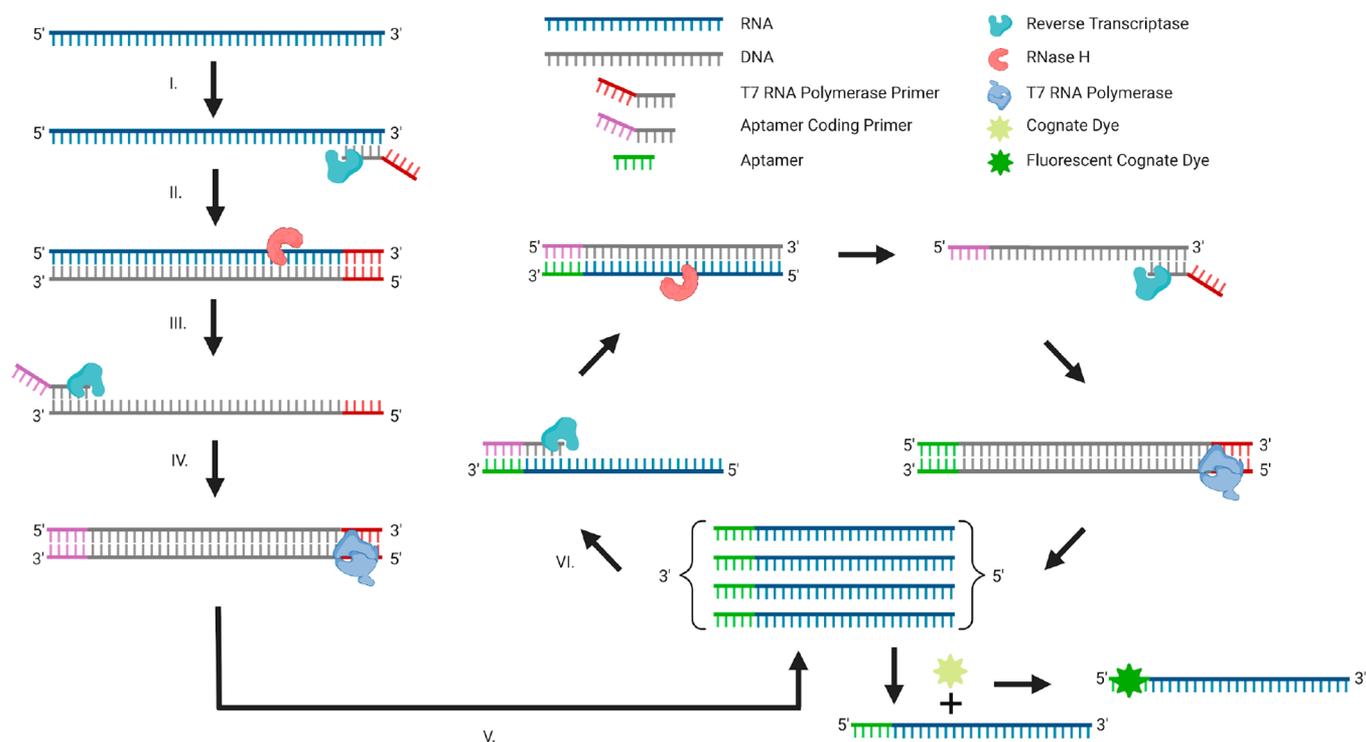


Figure 7. Aptamer-detected nucleic acid sequence-based amplification. (I) T7 RNA polymerase promoter-containing primer binds the amplicon of interest. Reverse transcriptase (RT) extends the primer, generating a DNA–RNA hybrid composed of the amplicon target sequence and an antisense DNA copy of the amplicon, with a T7 RNA polymerase (T7 RNAP) promoter. (III) RNase H degrades the original amplicon RNA of interest. (IV) The aptamer-coding primer binds and reverse transcriptase extends the aptamer-coding primer, generating a DNA duplex corresponding to the amplicon, with a T7 RNAP promoter (red) and an aptamer coding sequence (purple). (V) T7 RNAP generates an RNA fusion construct that contains the sequence antisense to the target sequence, fused at the 3' end with the aptamer coding sequence (green). (VI) Aptamer-coding primer binds this RNA, which again enters the RT–RNase H–T7 RNAP cycle. At each step, RNA is generated with multiple turnovers, enabling exponential amplification. In the conventional NASBA cycle, the aptamer coding sequence is omitted from the second, nonpromoter-containing primer.

LAMP for primer-specific isothermal amplification of the target RNA or DNA and follows this with guide RNA-mediated, CRISPR-based detection using the Cas-12b enzyme, with fluorescent (40 min) or lateral flow (70 min) readout.⁶⁴ The reaction can be conducted using a water bath or a heat block and uses taurine to confer thermostability to reaction components.⁶⁵ With an LOD of 100 copies of the SARS-CoV-2 genome per reaction, the test has a sensitivity of 97% and a specificity of 100%. Showing successful detection in healthy saliva samples spiked with standard concentrations of SARS-CoV-2 RNA, the test is highly amenable to POC assays with easily obtained saliva samples and does not require trained personnel.

Another recent study from Myhrvold and colleagues proposes assay designs for surveillance of SARS-CoV-2 and other related viruses and uses CRISPR-based nucleic acid detection.⁶⁶ This study is unique in that it addresses common challenges with virus surveillance such as false positives due to cross-reactivity of detection assay reagents with related coronavirus species, higher demand for tests than supply, and the complicating occurrence of coinfections in patients with SARS-CoV-2. The study developed a machine learning model and concurrent algorithms to evaluate the comprehensiveness, predicted sensitivity and predicted specificity of nucleic acid detection techniques. Comparing different SHERLOCK methods, the model identified a RPA-Cas13a-based SHERLOCK detection system to have the best performance and highest specificity.^{54,67,68} The study subsequently conducted this SHERLOCK assay to show detection of synthetic SARS-CoV-2 RNA at 10 copies/ μL .

Advantages of CRISPR-Based Detection. Employing the nuclease specificity of the CRISPR system for diagnostic readout allows the detection system to be designed for single base specificity.⁶⁴ Detecting RNA viruses can be a challenge due to their high rate of evolution and single nucleotide changes. Being able to detect such changes with CRISPR-based methods would allow for determining the path of infection a virus takes without the need for sequencing. CRISPR-based detection uses both primer-specific amplification and guide RNA directed detection, thus increasing sequence specificity in two different ways. Furthermore, the current readout for SARS-CoV-2 DETECTR and STOPCovid employs lateral flow detection, which has numerous advantages for POC applications.⁶⁹ CRISPR-based detection techniques can also be combined with a variety of isothermal detection methods, as showcased by the described methods using RT-LAMP and RPA with sensitivity compared to RT-PCR.

Disadvantages of CRISPR-Based Detection. RT-LAMP or another isothermal amplification method is required to amplify RNA of interest to generate sufficient signal. Accordingly, any difficulties associated with the chosen isothermal method would all apply CRISPR-based detection. As these assays rely on collateral activity of Cas enzymes, their readout is also qualitative.⁵⁵

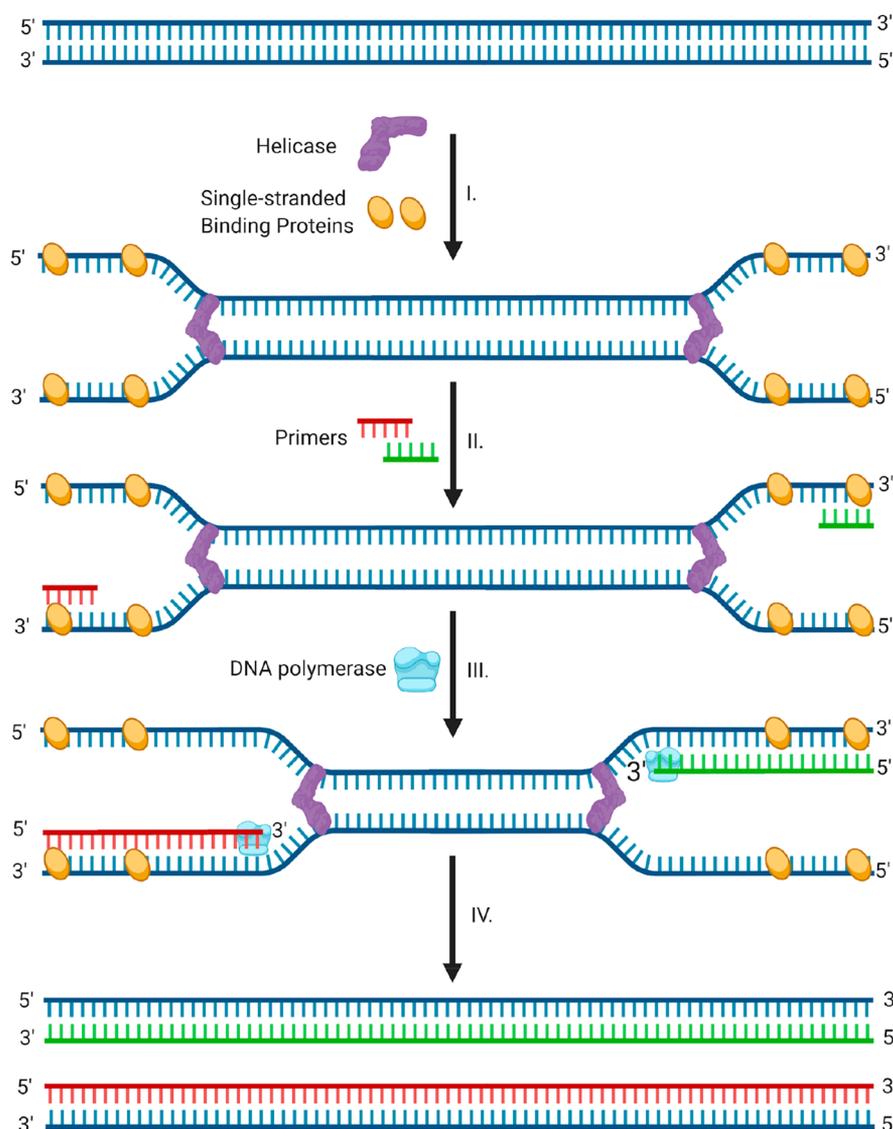


Figure 8. Helicase-dependent amplification (HDA). (I) Helicase unwinds dsDNA and ssDNA is stabilized by single-stranded binding proteins (SSBs). (II) Forward and reverse primers anneal to complementary sequences on ssDNA. (III) DNA polymerase extends the primers. (IV) One DNA duplex is amplified to form two DNA duplexes, and the cycle repeats.⁸⁵

■ POTENTIAL ISOTHERMAL TECHNIQUES FOR SARS-COV-2 DETECTION

Many useful isothermal amplification techniques exist which are not currently adapted to detect SARS-CoV-2, but have the potential to be beneficial for addressing the global pandemic. Isothermal amplification techniques such as rolling circle amplification (RCA), nucleic acid sequence based amplification (NASBA), multiple displacement amplification (MDA), helicase dependent amplification (HDA), and signal mediated amplification of RNA technology (SMART) would allow for efficient amplification of nucleic acids, followed by easily interpretable readouts (Table 3).

Rolling Circle Amplification. RCA and rolling circle transcription (RCT) techniques developed based upon the discovery that DNA and RNA polymerases could use small (less than 100 nucleotides) circular constructs as their template. Polymerases have been shown to work on templates as small as 26 nucleotides; templates that have a smaller diameter than the

polymerase's footprint suffice for amplification. Only a single primer is needed, and due to the primer seeding multiple copies of the same sequence, the ratios of template to primer differ greatly from those in PCR.⁷³ The primer binds to the circular template and allows for a strand displacing DNA polymerase to continually elongate the circular template (Figure 6). In the case of RCT, the primer binds, creating a double-stranded promoter sequence capable of recruiting the selected RNA polymerase. The resulting product is a long, repetitive nucleotide sequence. With the discovery of highly processive enzymes from bacteria, products can reach greater than 1000 bases. By adding a ligase and a specifically designed padlock probe, this technology has been used to detect microRNAs. RCT only amplifies a small part of the target, accompanied by an easily detectable sequence. The technique has been used in cells to detect let-7a microRNA, which is associated in regulation of development and tumor suppression,⁷⁴ with femtomolar sensitivity.⁷⁵ Similar to the methods used previously,⁷⁶ incorporating a hemin-binding G-Quadruplex into a rolling circle amplification (RCA) reaction

would enable colorimetric readout for SARS-CoV-2 samples and would be a useful POC diagnostic technique.

Advantages of RCA. The process of RCA is simpler than other most isothermal techniques due to simple primer design. The Phi29 DNA polymerase has catalytic activity at 30 °C or room temperature, making the process energy-efficient without the need for thermostable enzymes.⁷⁷ Furthermore, since amplification is preceded by a padlock-probe approach where specific ligase activity is needed to make the circular DNA template there is a high degree of specificity associated with RCA. This makes it suitable for scoring single-nucleotide polymorphisms (SNPs) and for high-throughput assays.⁷⁸

Disadvantages of RCA. RCA requires an additional ligase step, since it amplifies circular DNA templates and the circularization of the DNA template can be low-yielding if folding is not optimal.⁷³

Nucleic Acid Sequence Based Amplification. NASBA is an isothermal amplification reaction that can use either DNA or RNA as a template.⁸⁰ NASBA employs three enzymes: a reverse transcriptase, RNase H, and an RNA polymerase. Two primers are used, and in addition to a complementary region to the sequence of interest, one of the primers also encodes for an RNA polymerase promoter sequence. Through reverse transcriptase and RNase H activity, a dsDNA is created with an RNA polymerase promoter sequence. RNA polymerase transcribes this sequence, creating a pool of product RNA. Primers can bind this product RNA, again generating dsDNA with T7 promoter sequences through reverse transcriptase and RNase H activity providing exponential amplification (Figure 7).

Recently, two groups developed a method for tagging NASBA reactions with fluorescent aptamers, enabling genetically encoded fluorescent readout. Unrau and coworkers recently demonstrated the use of the Mango aptamer in Nested Mango NASBA,^{81,82} and Engelhart and coworkers showed multiplexed detection with cell phone camera-based readout using the Broccoli, Corn, and malachite green aptamers in Apta-NASBA.⁸³ These techniques enable an inexpensive fluorogenic real-time readout. NASBA has also been detected by an aptamer-based molecular beacon approach.⁸⁴

Advantages of NASBA. NASBA has the capability of starting with either DNA or RNA, with RNA as the main reaction product, enabling the use of functional nucleic acids such as aptamers and ribozymes in the reaction product, opening the door to using a variety of detectable signals.^{81,83} By applying fluorescent RNA aptamers for the readout, it is possible to obtain comparable specificity as a fluorescent probe, but with the cost of an intercalating dye such as SYBR Green. The optimal reaction temperature is 37–42 °C, lower than that of LAMP or RPA. The reaction is also compatible with lyophilization, mitigating the need for a cold chain in field applications.

Disadvantages of NASBA. False positives, as with many isothermal techniques, can complicate detection. Recently, Abdolazadeh et al. mitigated this issue through a nested procedure, and Aufdembrink et al. used a competitor DNA duplex to do so.^{81,83} As with other multienzyme isothermal reactions, the use of multiple enzymes necessarily adds complexity to manufacturing processes.

Helicase Dependent Amplification. HDA mimics the replication process in a cell, which isothermally separates DNA using helicase enzymes. In HDA, a helicase unwinds dsDNA, allowing for primers to bind, initiating elongation with a DNA polymerase. Single-stranded binding proteins are included, as in RPA, to stabilize the ssDNA unwound by the helicase (Figure

8).⁸⁵ Two primers are used to amplify specific sequences of DNA, but due to the isothermal nature of the reaction, a lower concentration of primers is typically used compared to PCR to minimize formation of primer dimers. Altered dNTPs have been shown to be successful in mitigating primer dimers in self-avoiding molecular recognition systems (SMARS) where, due to the base changes, primers can only pair with naturally occurring nucleotides and not themselves.⁸⁶

Advantages of HDA. HDA has been shown to work on a variety of samples, including genomic DNA, plasmid DNA, blood samples, bacteria cultures, and fecal samples.⁸⁶ It is also amenable to readouts used with PCR. AmpliVue assays have been commercialized for certain pathogens with a lateral flow device where the only additional equipment required beyond a heat source for the reaction is a heated lid.⁸⁶

Disadvantages of HDA. As with most isothermal reactions, false positives are an issue. The use of SMARS, as described above, helps diminish this. While there are many published methods using HDA for pathogen detection, there are a limited amount of validated tests. A reverse transcription reaction must be performed if trying to detect RNA, as is required to detect retroviruses such as SARS-CoV-2.

Multiple Displacement Amplification (MDA). MDA is a technique used in whole genome amplification which has proved useful in single-cell sequencing. It works by using a strand displacing polymerase and multiple primers. A denaturing step is used at the beginning to obtain single-stranded DNA. Primers bind their complementary region and a highly processive, strand displacement-capable DNA polymerase, typically Phi29 DNA polymerase,⁸⁷ synthesizes new DNA strands. Additional primers bind to the newly synthesized strand, or displaced strand, allowing for amplification of specific regions (Figure 9). Based on the primers used, certain sequences have preferential amplification.⁸⁸ By using multiple primers specific toward human papillomaviruses (HPV), this technique has been used on patient samples to identify novel HPV and thus has the potential to be used in detecting SARS-CoV-2 and novel mutations in the SARS-CoV-2 genome.⁸⁹

Advantages of MDA. MDA is capable of identifying novel viruses in patient samples through the use of many primers. As in LAMP, the use of multiple primers increases the specificity of the reaction compared to other isothermal methods.

Disadvantages of MDA. MDA requires a reverse transcription step to detect RNA. Multiple primers are needed for this reaction, adding complexity to primer design when setting up a new assay.

Signal Mediated Amplification of RNA Technology. SMART takes advantage of the requirement for T7 RNA polymerase to bind a double-stranded promoter to transcribe RNA. Targeting RNA, two probes create a three-way junction with the template of interest. The two probes each contain a sequence complementary to adjacent regions of the target of interest and a second mutually complementary sequence, enabling the formation of a three-way junction. The sequence complementarity between the two probes is not sufficient to allow probe annealing, and the template brings probes together via three-way junction formation, enabling primer extension.⁹⁰ The first probe, termed the extension probe, is shorter and acts as a primer for elongation using the second, longer probe, termed the template probe, as the template. The template probe contains the antisense template sequence, a T7 RNA polymerase promoter, and a sequence complementary to the sequence to be detected. When sequences hybridize, forming a three-way

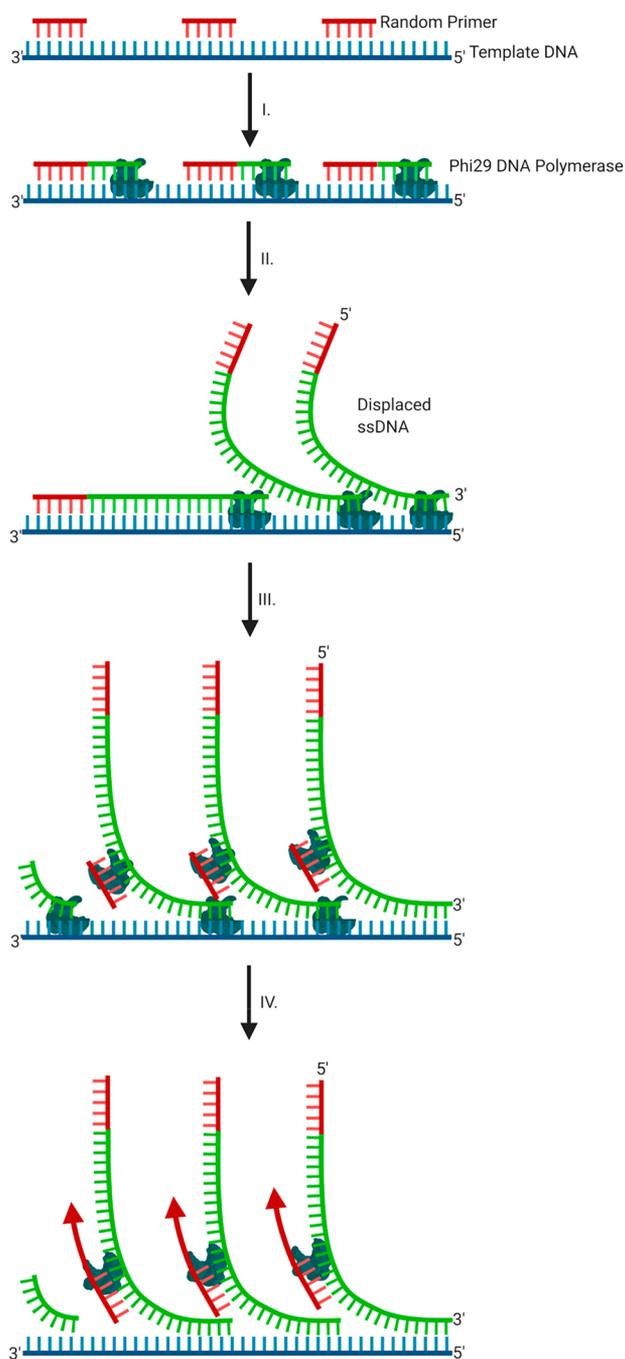


Figure 9. Multiple displacement amplification (MDA). MDA starts with primers annealing to different regions of the template DNA strand. (I) Phi29 DNA polymerase elongates each primer to produce a newly synthesized DNA strand. (II) Phi29 DNA polymerase continues elongation by displacing these new single-stranded DNA (ssDNA) strands. (III) Strand displacement and elongation reveals new sites for more primers to bind. (IV) Polymerase extension of newly annealed primers creates branching of the synthesized strand, allowing for more strand-displacement synthesis and exponential amplification.⁸⁷

junction, DNA polymerase is used to create a dsDNA template for T7 RNA polymerase to transcribe. T7 RNA polymerase can transcribe many copies of the specified sequence, giving an amplified signal (Figure 10). If further sensitivity is required, a third probe can be used for greater amplification. The third probe contains a single-stranded T7 RNA polymerase promoter

region and complementarity to the product formed via the three way junction transcription. This product acts as a primer for the DNA polymerase, allowing DNA polymerase to create another dsT7 RNA polymerase promoter. T7 RNA polymerase is then able to come in and create multiple copies of a second detectable sequence (Figure 10). While this step can increase sensitivity, it also increases complexity and hands on time, as it is done in two separate steps.⁹¹ This technique has the advantage of being easily amenable to many target sequences^{90,92} by altering only the sequence complementary to the target.

Advantages of SMART. This technique has the advantage of being easily amenable to many target sequences. Like NASBA, RNA is the main product and there is potential for a variety of functional sequences to be used for varying read outs.

Disadvantages of SMART. SMART lacks an exponential step, making it a linear amplification reaction. The product produced by SMART does not seed another reaction allowing for increased amplification.

■ SEROLOGY-BASED SARS-COV-2 RAPID DETECTION PLATFORMS

Serological tests use serum as a specimen for diagnosing whether an individual has been affected by a certain disease causing pathogen.¹⁰³ In contrast to NAATs, these tests' detection modalities are based on recognition of circulating antibodies in blood serum produced by the immune system in response to an invading foreign pathogen. Tests for SARS-CoV-2 mainly detect Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies, where IgM antibodies are first detectable in blood around day 3 of infection, while IgG antibodies first appear around day 5 of infection.¹⁰⁴ The rapid diagnostic test (RDT) is a serology test that comes in the form of a POC assay employing a lateral flow device for testing serum samples or blood samples collected from a finger prick.¹⁰⁵ These tests are set up to test the presence of IgM and IgG antibodies in the blood and are not suitable for quantitative results on the levels of these antibodies. There are currently 12 serological tests with FDA emergency use authorization that use a variety of lateral flow techniques, chemiluminescent immunoassays, or enzyme linked immunosorbent assays.¹⁰⁶

Advantages of Serological Tests. Most serological tests are easy to use for POC diagnostics. They exhibit rapid results with high sensitivity and specificity. They are also capable of indicating whether a person has been infected with SARS-CoV-2 even if no symptoms were ever present, a highly desirable attribute when transmission is possible from asymptomatic carriers.

Disadvantages of Serological Tests. Serological tests depend on the response of the infected individual's immune system. For each disease, this differs. Thus far, the tests designed for SARS-CoV-2 have been developed using the disease progression timeline of SARS-CoV. If tests are used too early in infection, a false positive could be obtained, as sufficient immune response may not yet have been mounted.

■ CONCLUSION AND FUTURE OUTLOOK

Less than a month after the first reported case in Wuhan, China, SARS-CoV-2 was designated a world health emergency, and now—9 months later—over 850 000 deaths have been attributed to the virus. Differing approaches taken by countries to contain SARS-CoV-2 have shown various levels of success. While the response on how to best contain the virus has varied

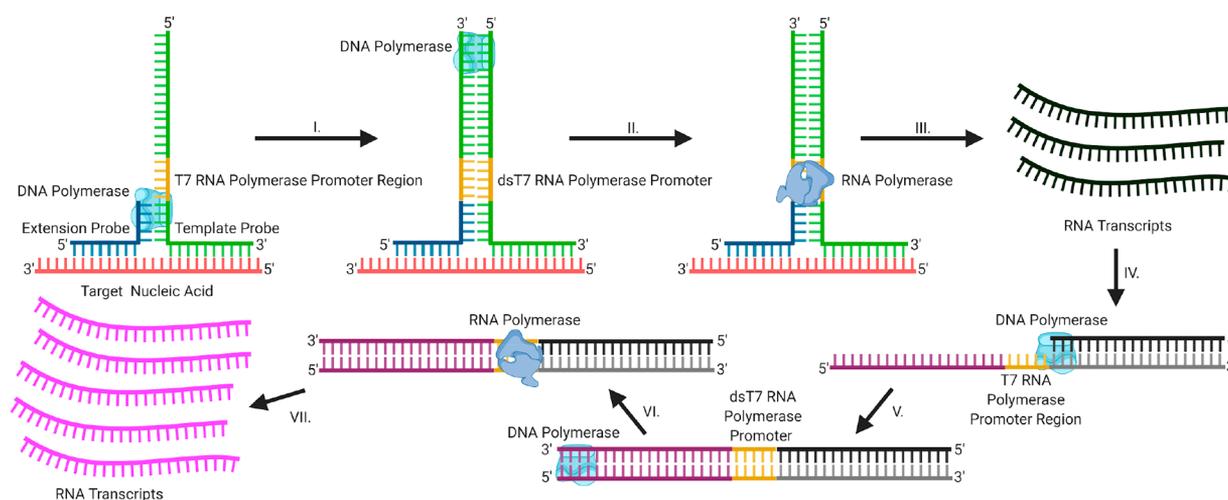


Figure 10. Signal mediated amplification of RNA technology (SMART). (I) An extension probe and a template probe bind to the target DNA, allowing DNA polymerase to extend the extension probe. (II) A newly synthesized double-stranded T7 (dsT7) RNA polymerase promoter region is formed, allowing for RNA polymerase to start transcription.⁹³ (III) RNA polymerase transcribes multiple RNA transcripts containing the detectable signal. (IV) For greater sensitivity, a third probe can be added with a region complementary to step III RNA transcripts, allowing for the RNA transcripts to bind. (V) DNA polymerase extends the RNA transcript, creating a dsT7 RNA polymerase promoter. (VI) RNA polymerase transcribes multiple RNA transcripts, amplifying the detectable signal.

around the world (strict immediate lockdowns, stay at home orders, mandated usage of masks in public, ban of international travel, access to testing resources, etc.¹⁰⁷), a common theme between countries that have a smaller case count is the speed with which they responded. A correlation exists between countries that immediately implemented tactics to stop the spread of SARS-CoV-2 and the total number of cases reported.¹⁰⁸

Having the ability to detect such diseases quickly is imperative to helping mitigate the spread of devastating pathogens. The ability to respond swiftly to changing diagnostic demand is especially critical in the current environment. Reopenings present unique challenges, in that the spread of infections must be monitored while individuals resume normal daily activities. Low-cost isothermal technologies such as those described in this Review provide a uniquely well-suited means of performing large-scale testing, as well as distributed testing, including by end-users (e.g., CLIA-waived testing in the United States).

Multiple arenas of interaction exist that possess a unique combination of both (1) high economic and social benefits associated with reopening and (2) increased risk of disease spread. These include universities, airports, and essential workplaces in which human contact is difficult, at present, to avoid, such as retail locations and food processing sites. The use of rapid, frequent, distributed tests to determine infectiousness in these arenas provides a powerful potential means of realizing these benefits while simultaneously mitigating associated risks of viral transmission.

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Notes

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