Portable and visual assays for the detection of SARS-CoV-2

Yufan Zhang | Dan Ding

State Key Laboratory of Medicinal Chemical Biology, Frontiers Science Center for Cell Responses, Key Laboratory of Bioactive Materials, Ministry of Education, and College of Life Sciences, Nankai University, Tianjin, China

Correspondence
Dan Ding, State Key Laboratory of Medicinal Chemical Biology, Frontiers Science Center for Cell Responses, Key Laboratory of Bioactive Materials, Ministry of Education, and College of Life Sciences, Nankai University, Tianjin 300071, China. Email: dingd@nankai.edu.cn

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INTRODUCTION

A certain scale of transmission of unexplained viral pneumonia was observed in the population of Wuhan in Dec. 2019. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was officially named by the International Committee on Taxonomy of Viruses formally, and the disease was caused by coronavirus disease 2019 (COVID-19). Because the rapid spreading and highly infectious of SARS-CoV-2, it is considerable to supply quick screening, early isolation, and early treatment for patients suspected of COVID-19.

SARS-CoV-2 virus is a non-segmented, single-stranded RNA virus that belongs to the coronavirus family. The RNA genome contains a 5’ untranslated region (UTR), open reading frame 1ab (ORF1ab), spike gene (S gene), envelope gene (E gene), membrane gene (M gene), nucleocapsid gene (N gene), 3’ UTR and several unidentified unstructured open reading frames. Real-time quantitative PCR (qRT-PCR) is an approach that fluorescent groups are added into the PCR reaction system, and then detect the reaction process in real time by the accumulation of fluorescent signals, and finally the quantitative analysis is performed by the standard curves. qRT-PCR is the most generally used modality for the diagnosis of SARS-CoV-2 owing to its hypersensitivity and superb specificity. Currently, qRT-PCR usually uses throat swab or nose swab, while some studies use...
serum, fecal or ocular secretions to detect SARS-CoV-2.\textsuperscript{7–9}

As an RNA virus, SARS-CoV-2 needs to be reverse transcribed into cDNA, amplified by PCR, and quantified by Taqman probes.\textsuperscript{7} Results are generally available within 4–6 h. Although Taqman probes can reduce non-specific amplification, but if the binding sites have variants, the probe will not bind and false-negative results may appear. Besides, the targets for testing of SARS-CoV-2 have homologous gene fragments with SARS-CoV, therefore false-positive results for other coronavirus infections may occur. This can be done by improving the accuracy of nucleic acid test through synchronization detection of multiple target sequences, or by designing more specific primers and probes for the gene sequence of virus.

However, there have several problems with qRT-PCR assays in SARS-CoV-2 testing. First of all, as an RNA virus, both mutation and recombination of viruses can reduce the sensitivity of these assays. The detection results are greatly influenced by sample quality, experimental conditions and personnel operation.\textsuperscript{10} A patient with positive CT scan and negative qRT-PCR result has been reported in the clinic.\textsuperscript{11} Therefore, some researchers and clinicians believe that CT scan results should be combined with qRT-PCR results to assess infection, treatment, and prognosis.\textsuperscript{12–14}

qRT-PCR assays against SARS-CoV-2 show the importance of early identification, isolation, and treatment for SARS-CoV-2 at this stage. In the follow-up work of SARS-CoV-2 testing, the accuracy can be improved by standardizing specimen collection, combining multi-site and multi-specimen detection, strictly controlling the quality of reagent kits, and improving the quality of laboratory.

This mini-review presents the simple operation and visualization of results using portable devices for SARS-CoV-2 detection. The assays presented in this paper still generally on the basis of nucleic acid detection assay and antibody detection assay. We expect this paper to provide referential assays for quick and more precise diagnosis of COVID-19.

2  MOLECULAR VISUAL ASSAYS FOR DETECTION OF SARS-COV-2

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

Although qRT-PCR has been broadly applied for SARS-CoV-2 testing, the long reaction time and multiple heating-cooling cycles place high demands on the equipment and experimenter. qRT-PCR assays currently do not fully meet the challenges of asymptomatic infected populations and viral RNA conversion during convalescence. Therefore, there is a critically need for a more rapid, responsive, and straightforward method to diagnose infected individuals in different settings rapidly. LAMP enables efficient, quick, and highly responsive amplification of target sequences under 60–65°C isothermal conditions.\textsuperscript{15–17} LAMP shows rapid amplification in the presence of four to six different primers and a strand-displacement DNA polymerase (Bst-DNA polymerase) and can identify six different target sequences.\textsuperscript{18,19} In addition, LAMP can also be applied to point-of-care (POC) testing, contributing to advancement of field diagnostics, which are particularly important for controlling infectious diseases.\textsuperscript{18,20–22}

RT-LAMP is highly specific because four specific primers are used (forward primer [F3], backward primer [B3], forward internal primer [FIP], and backward internal primer [BIP]). FIP and BIP bind to four various regions of the target gene fragments and the addition of loop primers and cluster primers can shorten the duration of the LAMP reaction.\textsuperscript{23} In brief, the primers, reverse transcriptase, Bst-DNA polymerase, and pH indicator are blended with the extracted RNA of samples and heated at 60–65°C for about 60 minutes.\textsuperscript{24} When the pH value decreases, a color change visible to the naked eye occurs. This rapid DNA amplification without thermal cycling is more rapid than qRT-PCR.

Ganguli et al. reported a rapid (< 40 min) detection of COVID-19 directly from a nasopharyngeal swab in virus transport medium (VTM) based on the RT-LAMP method with 50 copies/μL and 0% false results, using a POC device including a portable handheld reader and cartridge (Figure 1A).\textsuperscript{25} This method did not use an RNA extraction kit and allowed for rapid detection of diagnostics without laboratory-grade testing equipment. This POC device was easy to make on a 3D printer and its optical inspection could be done with a smartphone. Lu et al. reported a real-time fluorescence and visualization RT-LAMP assay based on its N genes using the mismatch-tolerant technique. This method had high specificity, sensitivity, and good repeatability with 118.6 copies per 25 μL reaction. And compared with the traditional qRT-PCR, the results matched 92.9%. 40 min was the optimal time point for visual detection with its red color changing to a yellowish color enabled visualization of the results.\textsuperscript{26} Compared to the accustomed LAMP method, the mismatch-tolerant technique increases the concentration of high-fidelity DNA polymerase, making it more suitable for highly mutated viruses and shortening reaction time by 10–15 min.\textsuperscript{21} The assay can be heated at 63°C in an incubator (water bath or heating block) for 40 min in a conventional PCR machine and detected visually using pH-sensitive indicator dyes. Cresol Red is used as a pH indicator dye with a transparent color changing between negative (red) and positive (yellow).\textsuperscript{27} Similarly, Nawattananapiboon et al. also used RT-LAMP assay for SARS-CoV-2 testing according to the RdRp gene’s conserved region with 25 copies per reaction and accuracy rate of 99.86%.\textsuperscript{28} Yan et al. proposed several specificity primers to the ORF1ab (20 copies per
reaction) and S gene (200 copies per reaction) and monitor the amplification by real-time turbidity and colorimetric detection based on the RT-LAMP assay. Positive results could be observed with the naked eyes based upon the orange color changing to a greenish color, while negative results were still orange (Figure 1B). And the sensitivity and specificity were all 100%. Lau et al. reported that LAMP (one copy per reaction) amplification products were detected by observing the color change of the reaction tubes after the addition of Hydroxynaphthol blue (HNB) after heating at 65°C. The negative result remained royal purple, while the positive result changed into blue with result accuracy rate of 100%. These studies show that the RT-LAMP assay is rapid, manageable, and productive, therefore promises to be a pragmatic program for monitoring a large population during COVID-19 epidemics.

2.2 Clustered regularly interspaced short palindromic repeats (CRISPR)-based assays

Cas13 and Cas12a are used to Specific High Sensitivity Enzymatic Reporter Unlocking (SHERLOCK) system and the DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) system, respectively. Under CRISPR RNA (crRNA) guidance, Cas13 recognizes and binds to the target sequence to outline a nuclease-inactive ribonucleoprotein complex (RNP). RNP indiscriminately cleaves any surrounding single-stranded RNAs (ssRNAs) when unites to matching RNAs. Activated Cas13 cleaves fluorescent RNA that is quenchable, causing a quantifiable signal revealing the target sequence. The extracted DNA or RNA of the samples amplified by recombinase polymerase amplification (RPA) or RT-RPA reagent in order to increase the sensitivity. RPA is combined with T7 transcript to alter DNA into RNA for following Cas13 testing, a method known as “SHERLOCK”. Recently, this technique was used for SARS-CoV-2 detection and was further advanced as “SHINE” for the detection of specimens without gene extraction.

Fozouni et al. reported the diagnosis of SARS-CoV-2 straight from nasal swab RNA using CRISPR-Cas13a beyond gene amplification, and the results could be observed by a smartphone camera. Moreover, the method could achieve a sensitivity of ~100 copies/µL in 30 min (Figure 1C). Improved sensitivity and specificity by combining crRNAs targeting SARS-CoV-2 and direct quantification of viral loads. The smartphone-integrated assay enables rapid, low-cost POC screening of SARS-CoV-2. To demonstrate this approach’s simplicity and portability, the authors measured fluorescence in a small unit that
contains low-budget laser illumination and optical collection. With the high sensitiveness and wireless connectivity of the cellphone camera, make it a potential tool for POC disease diagnosis in low-resource areas.\(^4^0,^4^1\) Broughton et al. reported a DETECTR-based approach (10 copies per reaction) through extracted RNA from nasopharyngeal swabs in universal transport medium (UTM) to detect predefined coronavirus sequences by Cas12 for the diagnosis of SARS-CoV-2. And positive predictive value was 95% while negative predictive value was 100%.\(^4^2\) Ding et al. reported an innovative system called “All-In-One-Dual CRISPR-Cas12a (AIOD-CRISPR)” for convenient, quick, and ultrasensitive optical testing that can be used at home or in clinics (Figure 2A). These researchers validated the clinical feasibility of this diagnostic method using COVID-19 clinical swabs. Besides, they achieved POC molecular diagnostics for COVID-19 using a simple hand warmer as an incubator, which allows visualization of the results under LED lights within a sensitivity of ~5 copies/μL in 20 minutes.\(^4^3\) CRISPR-Cas-based nucleic acid assays usually require pre-amplification and several manipulations, which undeniably embroils the detection procedure and may enhance the possibility of contamination due to the transport of amplification products. In AIOD-CRISPR analysis, the constituent parts of amplification and detection assays were all set in a single pot,\(^4^3\) which completely avoided isolating pre-amplification of samples\(^3^2\) or manual segregation of Cas enzymes.\(^4^4\) Furthermore, this method shows its high specificity in detection with no cross-talk with other sequences, which may be due to the specific single bases of CRISPR-Cas12a.\(^4^5,^4^6\)

### 2.3 RT-LAMP-CRISPR

Chen et al. developed an optical detection approach based on LAMP and CRISPR/Cas12a technologies with a contamination-free process for SARS-CoV-2 testing, and the whole process could be finished with 20 copies RNA in 40 min (100% positive and negative agreement) (Figure 2B). After the RNA was extracted from respiratory pharyngeal swabs, RT-LAMP reagent was added to the test tube. Subsequent addition of mineral oil to cover the RT-LAMP reagent prevented volatilization of the various solution. The CRISPR/Cas12a reagent was directly pre-added to the inside of the tube cap and placed under isothermal conditions to complete the RT-LAMP reaction. The tube was then shaken to allow the pre-added reagent to influx into the tube, instead the tube was re-opened and reagent was added, which
avoided potential aerosol impureness from opening the cap of the tube. Then put the tube into the 3D printer and the fluorescence signal could be observed directly by only using a smartphone. The entire amplification and detection procedure could be achieved in less than 40 min. This method prevents cross-reactivity with other common viruses and had a high sensitivity for the determination of SARS-CoV-2.47

Similarly, Wang et al. reported a one-pot SARS-CoV-2 visual testing system called “opvCRISPR” by coordinating RT-LAMP and CRISPR-Cas12a systems in a simple activation system with 5 copies.48 The method involved explicitly adding the SARS-CoV-2 RNA template extracted from swabs to a tube with RT-LAMP reagent at the bottom, and after amplification, then blended with CRISPR/Cas12a reaction reagent on the lid. The minimum configuration of equipment required for this solution includes pipettes, reagent tubes, heating blocks and blue light lamps. All equipment could be stored in a 60–50 cm² carrying case. Cleavage of CRISPR-Cas12a allowed the LAMP amplifier to trigger a second round of signal amplification, resulting in a 10-fold increase in detection sensitivity. This proposed method was based on fluorescence detection, which was easier to distinguish than the naked eye colorimetric method.49

3 OTHER PORTABLE AND VISUAL ASSAYS FOR DETECTION OF SARS-COV-2

3.1 Lateral flow assay (FLA)

From the organism’s humoral immune response to the pathogen, some particular proteins of the pathogen, such as the S and N proteins (from SARS-CoV-2) can stimulate the immune system to cause an antibody response. The earliest antibodies to appear are IgM and later IgA antibodies, followed by IgG antibodies. When IgG antibodies appear, the concentration will continue to increase, while IgM will continue to decrease until it disappears, and IgG antibodies will be present for a more extended period. Therefore, specific IgM and IgG antibodies are a pair that appears in an orderly and the changes of the two antibodies are related.

Viral load decreases over time after the first week of symptoms, so the efficacy of the test depends on the timing of sample collection.50,51 The detection of IgG and IgM counts on the invalid’s seroconversion capacity and usually manifests itself within one to two weeks after the inception of symptoms, but it may be longer.52,53 The sensitivity of PCR detection will be significantly reduced at this time.50,54 In previous studies, it was found that when SARS-CoV-2 was detected by immunoassay, IgG ratio increased over time from one week after the development of symptoms,55–58 while the susceptibility of the method peaked at first and then decreased.51,58 And in early studies, it was found in the development of COVID-19, that IgM may appear earlier than IgG, so simultaneous measurement of both IgM and IgG may enhance the diagnostic accuracy.57

Lateral flow assay (LFA) is a fast and ease of use method that does not need to complex professional training before use.59 For resource-challenged testing environments or hospital POC environments, LFA offers the advantage of ease of use and affordability. Grant et al. reported a half-strip LFA in SARS-CoV-2 testing.60 The half-strip LFA consisted of a nitrocellulose analytical membrane and a wicking pad, and the sample and conjugate can be pre-mixed in a 96-well plate. And the limit of detection was 0.65 ng/mL. Van Elslande et al. showed that IgG antibody testing is highly relevant not less than eighteen days after the onset of first symptoms or in symptomless individuals. Although there is no apparent proof that estimating IgA or IgM is equally effective, their results advise that measuring IgM or IgA may result in biologic false-positive results without significantly improving diagnostic performance. The sensitivity of all LFAs for detecting IgG antibodies 14–25 days after symptom onset was 92.1%, while the sensitivity of the IgG ELISA was only 89.5%.61 Furthermore, on 2–3 weeks following the initial syndromes, the sensitivity of LFAs for detecting IgG antibodies was found to be >92%, while the sensitivity of ELISA for detecting IgG was 89.5%.

A dual optical/chemiluminescent immunosensor based on LFA was proposed by Roda et al. that was aimed to detect the IgA in serum and saliva (Figure 2C).52 Dual colorimetric and chemiluminescent assays enable low-cost and ultrasensitive measurement of IgA for SARS-CoV-2. The signal color of nanogold-labeled anti-human IgA can be measured by a simple mobile phone camera-based device. In previous work by this group, the N protein was highly immunogenic and abundantly expressed in SARS-CoV-2 infection.63,64 In serum or saliva samples, the IgA was caught by N antigen and stained with nanogold-labeled anti-IgA in the formation of a colored band on the test line. Previous reports have found that IgM- and IgG-based identification alone or IgM/IgG ratio quantification lack sensitivity and are prone to identification error in SARS-CoV-2 infection.50,65 Therefore, a significant advantage of the targeted IgA assay complemented the IgG assay is that saliva can be used for analysis instead of blood. For POC testing, saliva collection is preferable to blood collection.66 In summary, rapid, specific assays for serum and salivary IgA can provide early, promising, time-dependent information on the infection.
3.2 Nanoplasmonic sensor

To date, the diagnosis of patients with early infection has been peculiar to detecting viral nucleic acids or antigens in human nasopharyngeal or oropharyngeal swabs. Ding et al. reported a direct ocular detection that dispenses with pretreatment of samples. Virus levels as low as 370 vp/mL could be detected. Measurements can be displayed on the microplate reader and smartphone-connected portable device within 15 minutes using a specific nano-plasmonic sensor based on the S protein.67 Surface plasmon resonance (SPR) sensing is a prospective unlabeled and one-step method applied to the rapid detection of virus particles.68,69 On the surface of the SPR sensor chip, when the antibody captures the virus particles, the change in wavelength or intensity of the plasmon resonance caused by virus particles can be determined by the optical sensing system.70,71 Moitra et al. reported that based on the target sequences of SARS-CoV-2, designed thiol-modified antisense oligonucleotide (ASO) capped AuNPs alternative aggregate and show changes in their SPR (Figure 2D). The addition of RNase H cleaves RNA strands from RNA-DNA hybrids, resulting in visually visible precipitation in solution, enabling rapid visualization of SARS-CoV-2 in 10 min with a limit of detection of 0.18ng/μL.72 Karami et al. invented a colorimetric method for detection PCR products of SARS-CoV-2 (Figure 3A). The method is according to a monocomponent gathering of gold nanoparticle-core (AuNP-core SNAs) of the linker, for which a palindromic linker was designed for the E gene. In conventional PCR reactions, the linker can be used as a probe for SARS-CoV-2 RNA, and the cleavage of the palindromic sequences during amplification prevents the formation of monocomponent gathers of SNAs. Therefore, viral samples show the different color of red (positive) and purple (negative) respectively in the colorimetric test after PCR. This assay is tantamount to the RT-PCR assay in the light of sensitivity.73

Low-cost nanoplasmonic sensors were fabricated by previously reported methods for large-scale fabrication with high uniformity and reproducibility.74,75 Nanotechnology-based colorimetric detection technology has a broad-spectrum in application of biosensor design due to its simplicity, intuitiveness, and no complex instrumentation required.76–78 AuNPs have gained much concentration in the area of colorimetric-based biosensing applications as a result of their remarkable optical properties, high extinction coefficient, and intrinsic photostability.79
3.3 | Electrochemical detection

Electrochemical biosensors offer an aspirational scheme for clinical diagnosis with their high sensitivity performance, low-cost, ease of use, and ruggedness features. With the development of microfabrication and intelligence of electrochemical instruments, electrochemical biosensors are supposed to have important applications in diagnosis clinically and POC detection. In the domain of nucleic acid biosensors, super sandwich-type electrochemical biosensors have great potentials in diagnosis.

Paper as a substrate material has the benefits of inexpensive and portability, and the paper can be securely incinerated after use, which make it more appropriate for epidemic disease detection. The electrochemical paper analysis device for COVID-19 testing consists of a working ePAD, counter ePAD, and closing ePAD, each with its own function. The S protein including receptor-binding domain (RBD) is used to secure the SARS-CoV-2 antibody entering the test section of the working ePAD. Monitor electrochemical response using square-wave voltammetry (SWV) technique, which decreases when immune complexes are formed. Yakoh et al. demonstrated a paper-based electrochemical method for the detection of immunoglobulin with 100% sensitivity and 90% specificity (Figure 3B). Using mobile devices as testing tools, data acquisition can be seamlessly interfaced through telemedicine platforms for epidemiological reporting and sharing test results with physicians. Zhao et al. reported an ultrasensitive electrochemical detection technique for targeting RNA of SARS-CoV-2 using calixarene-functionalized graphene oxide (Figure 3C). And predictive agreement was 85.5% with 200 copies/mL. Their team developed technology is a new plug-and-play diagnostic system.

4 | CONCLUSION AND FUTURE PERSPECTIVES

At present, there are two types of new coronavirus pneumonia detection reagents approved for marketing, nucleic acid and antibody detection. Nucleic acid testing is the primary clinical diagnostic tool for COVID-19, and its average testing time is 2–3 h. However, the following problems exist in (a) the assay need professionals to perform, (b) the long testing time, (c) the need for specialized instruments to analyze the results, (d) 14% of patients discharged from the hospital with two negative nucleic acid tests have “re-positive” results. Serum antibody testing provides another dimension of viral infection. Some LFA platform-based colorimetric/fluorescent optical assays for targeting immunoglobulins (IgG, IgM, or IgA) generated by SARS-CoV-2 throughout pathogenesis are available, and their results complement those for viral RNA detection. However, there is a window period for antibody testing, and some patients with weak humoral immunity have low levels of antibody production, which can lead to false negative antibody tests. Therefore, a combination of both tests is still needed.

Nowadays, the diagnosis of SARS-CoV-2 can be accomplished in a portable and fast way on the smartphone. The smartphone can edit the fluorescence photos, analyzes the fluorescence intensity of the images, and reports the qualitative or semi-quantitative test results. And the testing results can be transferred wirelessly to a remote server along with GPS coordinates to the physician. This is essential to enable simple, fast, and intelligent disease diagnosis and tracking.

As the COVID-19 epidemic continues to spread globally, national epidemic preparedness remains on edge. At this stage, we still need precise prevention and control, and the key to precise prevention and control is precise diagnosis. We expect that future tests will be easier to perform, more accurate, and visible to the naked eye, providing a more appropriate diagnostic tool for the clinical diagnosis and supervision of COVID-19 and large-scale population epidemiological surveys.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Dan Ding https://orcid.org/0000-0003-1873-6510

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AUTHOR BIOGRAPHIES

Yufan Zhang received her Master’s degree from the Tianjin Medical University in 2020. She is currently a PhD fellow under the supervision of Prof. Dan Ding in the State Key Laboratory of Medicinal Chemical Biology in Nankai University. Her current research focuses on the smart/functional nanomaterials in diagnosis and treatment of cardiovascular diseases.

Dan Ding received his PhD degree from the Department of Polymer Science and Engineering in Nanjing University in 2010. After a Postdoctoral training in the National University of Singapore, he joined Nankai University, where he is currently a Professor in State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Bioactive Materials, Ministry of Education, and College of Life Science. He also conducted his work in Hong Kong University of Science and Technology as a visiting scholar. His current research focuses on the design and synthesis of smart/functional molecular imaging probes and exploration of their biomedical applications.