UPDATE ON RAPID DIAGNOSTIC TESTING FOR COVID-19

Tan JH[1], Mohd Yusri MLA[1], Leong MY[1], and Lau YL[1].
[1]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Correspondence:
Yee Ling Lau
Department of Parasitology,
Faculty of Medicine,
University of Malaya,
50603 Kuala Lumpur, Malaysia
Email: lauyeeling@um.edu.my

Abstract

Coronavirus 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). To date, the disease has spread globally and caused 4 million deaths worldwide. SARS-CoV-2 spreads rapidly, leading to significant impacts on healthcare systems, social disruption and economic loss. To date, real time reverse transcription polymerase chain reaction remains the gold standard for diagnosis. However, it is costly and time consuming which result in delayed treatment and isolation of infected individuals. Hence, reliable and rapid diagnostic method is required for rapid detection of SARS-CoV-2. There have been a number of COVID-19 rapid diagnostic tests developed and evaluated widely for COVID-19 diagnosis, but a number of concerns related to these products have arisen. In this review, we provide an update on the available COVID-19 rapid diagnostic tests and discuss the feasibility and acceptability of these rapid tests for COVID-19 diagnosis. Continuous global improvement in diagnostic test is crucial for rapid detection of the infection to optimize patient management and prevent the spread of disease.

Keywords: COVID-19, Rapid Diagnostic Test, Loop-Mediated Isothermal Amplification, Recombinase Polymerase Amplification, CRISPR

Introduction

In late December 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) had made its first appearance in the city of Wuhan (Hubei Province, China) and the World Health Organization (WHO) announced the outbreak as global public emergency on 11th February 2020. This virus, belonging to the *Coronaviridae* family, causes the disease known as coronavirus disease 19 (COVID-19). The viral genome of SARS-CoV-2 consists of positive-sense single-stranded RNA with the size ranges from 26 to 32 kilobases. It has four main structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins which play important roles in the virus replication cycle (1, 2). As of 16th July 2021, there were more than 188 million COVID-19 confirmed cases, including 4 million deaths worldwide. Patients infected with COVID-19 may show common symptoms such as dry cough, fever and sore throat however, majority infected patients showed minor or no symptoms (asymptomatic). Infected patients may also develop a series of deadly complications including septic shock, severe pneumonia, organ failure and atypical Acute Respiratory Distress Syndrome (ARDS) especially the elderly, individuals with comorbidities and children (3-5).

Reverse transcription-polymerase chain reaction (RT-PCR) is the standard diagnostic method to detect the presence of the SARS-CoV-2 RNA viral genome from swab samples of the upper respiratory system. The method amplifies genes of the SARS-CoV-2 viral genome such as the ORF1b or ORF8, nucleocapsid (N), spike (S), envelope (E) or RNA-dependent RNA polymerase (RdRp) genes with specific primers. RT-PCR is highly sensitive and specific in COVID-19 diagnosis (6). However, it requires complex facilities, well-trained professionals, and is generally time-consuming, resulting in limited screening capability and delayed results (7). Serological tests such as enzyme linked immunosorbent assays (ELISAs) and chemiluminescent immunoassays (ChLIAs) detect the presence of antibodies against the viral antigens from blood, serum or plasma (8). These tests do not detect early infection as the body requires time to react to the antigenic viral invasion. Therefore, the method is more adequate to be used for study of seroprevalence and epidemiological surveillance of the infection.

Although the vaccine distribution is on-going actively worldwide, the number of COVID-19 cases is still increasing tremendously. Owing to this, rapid tests are required for detection of COVID-19 infected individuals for immediate isolation and proper management to reduce the morbidity and mortality caused by the disease. In this paper, we review and provide an update of the available COVID-19 rapid diagnostic tests as well as discuss the practicality of using these tests for rapid diagnosis of COVID-19.

COVID-19 rapid diagnostic testing

Antibody-based rapid diagnostic test (RDT)

Antibody-based rapid diagnostic test (RDT) serves as a simple and direct method in rapid detection of SARS-CoV-2. It gives immediate results with color lines that are visible to human naked eyes. The antibody-based RDT is a lateral flow device which consists of a shallow well or particular section on the strip for the placement of a few drops (~10-15 µl) of serum, plasma, or finger prick blood samples. After the sample is loaded, specific buffer is added to facilitate the flow of samples along the test strip. COVID-19 IgM and IgG antibodies from the samples form antigen-antibody complexes with colloidal gold labeled recombinant IgM and IgG antigens. Then, the labeled antigen-antibody complexes are captured by anti-human IgM and IgG antibodies which are immobilized in the indicator region of the test assay, forming a color line (9). RDT also includes a control line for the validation of test results. There are few advantages of RDT as it is relatively cheap and can be performed on a large scale, the test results are instant in which it can be achieved within 15-30 minutes. Other than that, the test is easy to perform and provides a simple interpretation of the result.

In Shen et al. (2020) (10), it was demonstrated that a SARS-CoV-2 colloidal gold immunochromatography assay has a relative fair sensitivity of 71.1% and high specificity of 96.2% from 150 suspected COVID-19 cases when compared with RT-PCR results. In addition, Rashid et al. (2020) (11) compared six rapid test antibodies kits and the sensitivity of the kits ranges from 72.7% to 100% while specificity ranges from 98.7% to 100%. Both studies showed increased sensitivity when tested on patients 9-14 days after onset of symptoms. Therefore, instead of detection of acute infection, the antibody-based RDT is more suitable for epidemiological surveillance of the disease.

Rapid antigen detection (RAD) tests

The COVID-19 rapid antigen detection (RAD) test acts as a point-of-care tool in the diagnosis of COVID-19. The method is able to be carried out in large-scale screening, affordable, and accurate. RADs are used to detect the presence of SARS-CoV-2 viral antigen from the nasopharyngeal or throat swab of a person. It employs the sandwich immunochromatographic assay to capture the viral antigen, usually the N protein which is found most abundantly in infected persons (12). Similar to antibody-based RDT, RAD assay is performed as a lateral flow device except the membrane of the strips is coated with antibodies targeting the antigen of interest instead of anti-human antibodies. The result of the RAD test can be obtained within 30 minutes (13).

Many of the commercial RAD tests were evaluated for their performance in detecting SARS-CoV-2 as the tests are used as an adjunct to overcome the shortfalls of RT-PCR (e.g. time-consuming, requires expensive instrument). Chaimayo et al. (2020) (14) showed that Standard™ Q COVID-19 Ag test (SD Biosensor®, Republic of Korea) has comparable sensitivity and specificity with RT-PCR as the test has a sensitivity and specificity of 98.33% and 98.73%, respectively. However, the test was estimated to have low positive predictive value (PPV) in the low COVID-19 prevalence area as few false positive results were detected from the evaluation.

Nevertheless, the performance of RADs vary with different Ct-value. Study by Jääskeläinen et al. (2021) (15) showed that Sofia (Quidel), Standard™ Q COVID-19 Ag test, and Panbio™ (Abbott) have sensitivity close to 100% when the Ct is <25. However, when the Ct-value is more than 30, sensitivity of the tests drops to 12%, 31% and 38%, respectively. Regardless of that, the researcher justified the use of RAD for COVID-19 rapid diagnosis despite having low sensitivity when Ct-value≥30 as 98% of the test samples have Ct-value<30.

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

Loop mediated isothermal amplification (LAMP) is a molecular diagnosis method that was used in the detection of several diseases such as, malaria, tuberculosis and influenza viruses (16-18). It is a single-tube technique for the amplification of nucleic acid (DNA or RNA) (19). While PCR requires the change of temperature in each cycle for amplification of target sequence, LAMP is able to amplify the target sequence at fixed temperature within range of 60-65°C. Furthermore, LAMP is highly sensitive and specific as the assay consists of six primers which are two inner primers (FIP and BIP), two outer primers (F3 and B3), a forward loop primer (LF) and a backward loop primer (LB). These primers recognize eight distinct sites of the target sequence altogether and the employment of the loop primers accelerate the following rounds of amplification, hence increasing the detection efficiency (20, 21).

In a previous study, Huang et al. (2020) (22) designed four sets of primers (O117, S17, N1 and N15) that target ORF1ab gene, S gene, and N gene. The study showed that 2 copies of N gene and S gene can be amplified with RT-LAMP within 30 minutes using N1, N15, and S17 primers, respectively. In addition, the group combined the reverse transcription process and LAMP (RT-LAMP) into one single step with the use of WarmStart RTx Reverse Transcriptase (New England Biolabs, UK). This reduces the time to generate results. When coupled the reaction mix with a pH indicator, the results of RT-LAMP can be seen with naked eyes through the change in color of the amplified reaction mix (22, 23). Using the same approach, Zhang et al. (2020) (24) designed LAMP primers that were able to target two fragments of the SARS-CoV-2 genome, 5' region of the ORF1a gene and N gene. At the same time, they demonstrated that direct tissue or cell lysate may also be used as samples in RT-LAMP for the detection of SARS-CoV-2. This is considerably important as RNA purification step may not be needed for the results generation, allowing screening to be achieved at a shorter time and reducing the chances of getting carry-over contamination which frequently occurred during

handling of multiple genomic samples. However, further investigation needs to be done to validate the feasibility for using these types of samples in RT-LAMP.

Besides, a few studies have reported that RT-LAMP showed better sensitivity (>97%) when targeting ORF1ab SARS-CoV-2 gene, indicating a higher diagnostic accuracy than RT-PCR (25, 26). Though RT-LAMP is relatively sensitive compared to RT-PCR, this method may produce false positive results as it is prone to carry-over contamination. It can be reduced with good practice of sample handling, master mix preparation and sample addition in separate laboratory space, as well as scheduled time for the decontamination of pipettes and equipment. Addition of mineral oil is also recommended to minimize risk of contamination to the reaction mix (27).

Recombinase Polymerase Amplification (RPA)

Recombinase polymerase amplification (RPA) assay is shown to have high sensitivity and specificity in the diagnosis of diseases (28, 29). The mechanism of RPA starts when the recombinase protein uvsX from T4 binds to the primers with the presence of ATP and crowding agent to form a recombinase-primer complex. The recombinase-primer complex scans the homologous sequence and the primer will promote strand invasion at the cognate site. The recombinase will then disassemble, and a strand displacing DNA polymerase will bind to 3' end of the primer to elongate with the presence of dNTPs (30).

Lau et al. (2021) (31) designed primers targeting SARS-CoV-2 N gene for reverse transcription-RPA (RT-RPA) to detect SARS-CoV-2. SYBR Green I was added to the cap of the tube during preparation of reaction mixture for colorimetric detection as well as to avoid possible aerosol contamination. The developed RT-RPA can be performed in less than 25 minutes at 37°C. In addition, the assay was shown to have a limit of detection (LoD) of 5 RNA copies. Both clinical sensitivity and specificity of the assay were 98% and 100%, respectively which are comparable to RT-PCR in COVID-19 diagnosis. However, the assay requires an isothermal fluorescence reader for result interpretation.

In a previous study, Song et al. (2017) (32) mentioned that RPA combined with LAMP (RAMP) takes advantage of RPA's high inhibitor tolerance as well as overcoming RPA's proclivity for producing false amplicons. The method begins with RPA amplification at 37°C using outer LAMP primers F3 and B3 following by second round of amplification using the other 4 LAMP primers at 65°C. With the change in temperature in RAMP assay, false signals can be omitted as it does not undergo long RPA amplification. Furthermore, when tested with purified and crude samples, the sensitivity of RAMP is 10- and 100-fold better than LAMP owing to the nested-like principle of the assay. Besides, RAMP can simultaneously detect multiple DNA and RNA targets without undue demands of sample volumes.

With this, El-Tholoth et al. (2020) (7) developed a method known as Penn-RAMP which targets SARS-CoV-2 ORF1ab gene. The method involved RPA which was first run for 15-20 minutes at 37°C to amplify the viral targets, followed by isothermal LAMP for 40 minutes at 63°C. The method was able to detect the gene with 100% sensitivity at 7 virions per reaction.

CRISPR-based assay

The CRISPR (clustered regularly interspaced short palindromic repeats)/Cas systems such as CRISPR/Cas12a and CRISPR/Cas13a are effective for detecting viral genomes and they are able to bind and cleave DNA as well as RNA, respectively (33). In a previous study, Gootenberg et al. (2018) (34) developed SHERLOCK (specific high-sensitivity enzymatic reporter unlocking), which is a multiplex and portable nucleic acid detection platform that uses Cas13, Cas12a, and Csm6 for effective detection of human viruses. When the CRISPR/Cas12a system is used, Cas12a cleaves double-stranded DNA bound by a probe tagged with a fluorescent reporter and the hydroxyl group, and generates a readable signal (35).

With a similar approach, Broughton et al. (2020) (36) developed SARS-CoV-2 DETECTR for the rapid detection of SARS-CoV-2. The group designed LAMP primers targeting the E and N genes of SARS-CoV-2 and used Cas12 for detection. The result was interpreted as positive only when both the E and N genes were detected. The assay was able to complete within 30 minutes and has a detection limit of 10 copies/µl input. Furthermore, the reaction can also be interpreted with lateral flow strips if no fluorescence reader is available.

Several other CRISPR-based diagnostic were also developed for SARS-CoV-2 rapid detection such as CRISPR-COVID by Hou et al. (2020) (37) which showed almost single-copy sensitivity within 40 minutes, and Lucia et al. (2020) (38) in which the CRISPR/Cas12a system developed was able to detect he SARS-CoV-2 RNA with LoD10 copies/µl. Interestingly, primers targeting the ORF1ab region have the best performance in CRISPR-based diagnostic method. Overall, CRISPR/Cas-based assays provide a low-cost alternative for diagnosing COVID-19 with high sensitivity and specificity for detection of SARS-CoV-2 (38).

Biosensor test

Biosensors offer an excellent option to clinical diagnosis, real-time detection, and continuous monitoring (39, 40). Localized surface plasmon resonance (LSPR) biosensing device is one of the biosensing techniques that may be used to detect a variety of clinically relevant assays (41). When coupling occurs at the surface of plasmonic materials, LSPR is a strong photon-driven coherent oscillation of surface conduction electrons that can be controlled (42). As a result, LSPR is an excellent choice for detecting micro- and nanoscale analytes in real time and without using labels (43). SARS was diagnosed using an SPR-based biosensor with coronaviral surface antigen (SCVme) attached onto a gold substrate. The SPR chip detected the anti-SCVme antibodies at a lower detection limit of 200 ng/mL within

10 minutes (44). In 2020, PathSensors, Inc announces the development of Cellular Analysis and Notification of Antigen Risks and Yields (CANARY) biosensor to detect SARS-CoV-2, and it can generate results within 3-5 minutes (45).

Discussion

Majority of the rapid tests performed greatly for SARS-CoV-2 detection especially LAMP, RPA and CRISPR/Cas system due to the assays' high sensitivity and specificity when compared to RT-PCR. Nonetheless, several shortfalls of the rapid tests need to be highlighted and discussed to identify the feasibility of the tests for use in COVID-19 rapid diagnosis.

RDT that detects antibodies greatly depends on the seroconversion of the infected patients. Asymptomatic patients have lower IgG and IgM titres against SARS-CoV-2 compared to symptomatic patients (46-48). Therefore, these individuals could be reported as false negative when tested with antibody-based RDT (49). A review on prevalence of asymptomatic SARS-CoV-2 infection by Oran and Topol (2020) (50) found that asymptomatic patients account for approximately 40-45% and many studies have shown possible transmission of the disease through asymptomatic patients (51-53). Therefore, antibody-based RDTs may not be able to identify infected individuals from asymptomatic populations.

RADs have been widely used for rapid diagnosis of COVID-19 and were said to be the 'game changer' against COVID-19 in several countries (e.g. India and Africa) due to the large number of cases. The minimum performance requirements of RAD set by the WHO is ≥80% sensitivity and ≥97% specificity when compared to RT-PCR. Although it is well known that RAD has generally lower sensitivity than RT-PCR, we believe that short turn-around-time of the test assay is more important to identify infected individuals from a large population. However, RAD tests shall be performed by experienced healthcare personnel. This is due to incorrect way of sample collection may lead to false negative or inconclusive results in the tests (54). Furthermore, sample collection from nasopharyngeal or throat may expose one to viral particles as high viral loads were reported in these areas of SARS-CoV-2 infected individuals (55).

As mentioned, RPA, RT-LAMP and CRISPR/Cas systems are highly sensitive (low limit of detection) and specificity is usually 100%. The characteristics that RPA, RT-LAMP and CRISPR have in common which make the tests adequate for COVID-19 point-of-care detection are: 1) the tests are relatively low cost and do not require expensive equipment; 2) colorimetric detection and lateral flow device can be used which ease the interpretation of result; and most importantly 3) short turn-around-time (within an hour or less). Among these three tests, RT-LAMP was shown to be able to detect SARS-CoV-2 from RNA-spiked crude cell lysates, hence reducing the time to process the sample and allow quick release of results (24). In another study, Alves et al. (2021) (56) performed RT-LAMP in clinical samples without any pre-treatment or RNA extraction and it was shown that three out of five samples turned out to be positive. It is noteworthy that crude clinical samples may contain interferences to the tests such as viral transport medium and enzymes, resulting in reduced sensitivity. Hence, purified RNA samples are still preferable over crude clinical samples to avoid false negative results.

Though these rapid molecular tests have performance which is comparable to RT-PCR, they are not without imperfection. An issue arises with the use of molecular diagnostic is the emergence of SARS-CoV-2 variants. Several SARS-CoV-2 variants such as B.1.1.7 (Alpha), B.1.351 (Beta), B.1.6172 (Delta), and P.1 (Gamma) have been reported in several countries and all mutants involved amino acids substitutions in the S gene including the new Lambda variant (57). Hence, the performance of these molecular tests including RT-PCR, which target the S gene may be affected, resulting in reduced sensitivity (58). To compensate for this, double gene tests that amplify two different genes are required.

Among the rapid tests that have been reviewed, RT-LAMP targeting two different genes that incorporate lateral flow device or colorimetric detection is highly recommended for rapid diagnosis of COVID-19 as the test is sensitive, specific, and relatively inexpensive, can be performed in large scale, and has short turn-around-time. This is especially required for immediate patient care and to identify infected individuals from a large population, especially asymptomatic individuals as they are likely the ones who carry the virus around and transmit the infection by not knowing they have contracted the disease. Nonetheless, no diagnostic methods are without flaws. Therefore, careful investigations of the test results, timely testing, contact tracing, patient history, medical monitoring, and proper data management are needed to curb the disease. In depth research needs to be done to investigate the reliability of these rapid tests for case confirmation purposes. Until then, close contacts of COVID-19 patient or persons with COVID-19 symptoms with a negative rapid test should take a confirmatory test using RT-PCR.

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Competing interests

The authors declare that there is no conflict of interest.

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