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Mini-Review

Comparison of Lateral Flow Immunoassays with PCR for the Detection of SARS-CoV-2 Virus

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Abstract

The COVID-19 pandemic has underscored the critical need for efficient and accurate diagnostic methods. This review compares Lateral Flow Immunoassays (LFIAs) and Polymerase Chain Reaction (PCR) tests for SARS-CoV-2 detection. LFIAs, which detect viral antigens or antibodies, offer rapid, cost-effective testing suitable for mass screening but are less sensitive than PCR. PCR, particularly Reverse Transcriptase PCR (RT-PCR), remains the gold standard due to its high sensitivity and specificity, albeit with longer turnaround times and higher costs. Digital Droplet PCR (ddPCR) is highlighted for its enhanced accuracy over conventional RT-PCR. This comparison evaluates these diagnostic methods' strengths, limitations, and practical applications in managing the COVID-19 pandemic.

Keywords: *COVID-19 Diagnostics, SARS-CoV-2 Detection, Lateral Flow Immunoassay (LFIA), Polymerase Chain Reaction (PCR), Rapid Antigen Test*

Introduction

The transition from an agrarian society to hunter-gatherer ones has facilitated the development of infectious illnesses in humans. Increased commerce between communities has increased contact between people and animals and made it easier for infections to spread between animals and people (Dobson and Carper, 1996). The earliest recorded pandemic was the Athenian plague of 430 B.C (Piret & Boivin, 2021) since then the world has recorded several other pandemic outbreaks including the black death of 1350 A.D responsible for one-third of global fatalities and the Spanish Flu of 1918 which resulted in approximately 50 million deaths (Piret & Boivin, 2021). Most recently, the COVID-19 pandemic that originated from Wuhan China at the end of 2019 has affected Over 20 million people, and so far, claimed the lives of about 194,000 in the UK (Wu et al., 2020; JHU CSSE). SARS-CoV-2 mostly affects the respiratory system, which can induce minor symptoms at first or develop into severe complications that can be fatal. It could show up as an acute lung infection, a coagulation disorder, or abdominal discomfort (Somborac Bačura et al., 2021). It is similar in structure to viruses in the Coronaviridae family and has a circular shape of approximately 60-140 nm, genetic material, and spike proteins on the surface Fig 1(a). After cell culture, the SARS-CoV-2 Transmission Electron Microscope (TEM) image seen in patient bronchoalveolar lavages is depicted in Fig 1(b). Proximity to an infected person, defined as being within 1.83m of them for a cumulative total of 15 minutes or more within 24 hours is largely the cause of the spread of the virus (Somborac Bačura et al., 2021).

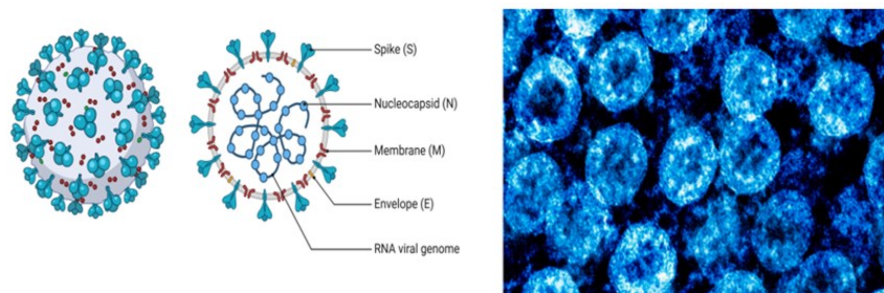

Figure 1. *(a) Schematic representation of the Covid-19 virus (b) Transmission Electron Microscope image of SARS-CoV-2.*

The COVID-19 pandemic's emergence has brought attention to how crucial mass testing is for facilitating disease management and control during pandemics. Comprehensive early testing, contact tracing, and patient isolation have all been successful in preventing the transmission of the SARS-CoV-2 virus in the initial stages of the outbreak in several countries (Lee and Lee, 2020; Shim et al., 2020). Early in the pandemic, RT-PCR of nose and throat swabs was utilised in the detection of the virus in symptomatic individuals, PCR tests are highly sensitive, can detect minute quantities of COVID genetic material, and are less likely to provide false-negative findings (Peto, 2021). However, there are significant challenges in creating testing capacity. To date, the turnaround time for issuing results from RT-PCR has been typically slow (after a day), though, the time of test of RT-PCR is usually a few hours (Peto, 2021). Therefore, most nations including the USA saw a pressing need for widespread, reliable, cost-effective, and quick diagnostic testing assays that could identify infected persons (Mahmoudinobar et al., 2021). The US National Institutes of Health (NIH) introduced the Rapid Acceleration of Diagnostics (RADx) Initiative in April 2020 to speed up the research, commercialization, and application of COVID-19 testing technologies. (Benda et al., 2021). Lateral flow immunoassays (LFIAs) are designed to detect COVID-19 antigens or antibodies produced in response to the Covid infection. Their relative simplicity of fabrication, use of small amount of sample volume, quick turnaround time and mass testing capabilities are only a few benefits of lateral flow immunoassays (Somborac Bačura et al., 2021; Smithgall et al., 2020). This review compares the detection of the virus using lateral flow immunoassays and PCR. The strengths, and weaknesses of each of the two diagnostic methods as well as the difficulties in applying them to the pandemic are assessed.

Lateral Flow Immunoassays

Lateral flow assays are diagnostic tools that determine if analytes like pollutants or pathogens are present or not in each sample. These analytes can be found in a wide range of matrices, including serum, saliva, urine, and tissue samples. The underlying principle is that a sample containing a target analyte flows through different zones of polymeric strips embedded with molecules that bind the analyte by capillary action and a positive test is confirmed by the appearance of coloured control and test lines (Koczula & Gallotta, 2016). Lateral flow immunoassay combines thin-layer chromatography and indirect immunochemical insert approach that allows the recognition of specific SARS-CoV-2 antigens or antibodies binding to them (Somborac Bačura et al., 2021b).

- **Antibody-based Lateral Flow Immunoassays**

An antibody is a blood protein that is produced in reaction to and neutralises a specific antigen. Antibody-based assays not only determine the exposure history of the patient but also their immune status. They detect SARS-CoV-2 antigen-specific antibodies in serum, plasma, or whole blood samples (Smithgall et al., 2020). There is a window period following the first viral infection before the immune system starts to produce antibodies. IgM antibodies are first formed during an immune response to a virus and indicate early infection (3–6 days), followed by a more robust IgG antibody, which is observed in the latter stages (10–18 days) or post-recovery hence the diagnosis of an acute or active COVID-19 infection cannot be made using the antibody-based approach. Age, gender, and the existence of associated conditions can all affect the antibody response level (Eftekhari et al., 2021).

In a lateral flow-based test for the quick detection of serological response to SARS-CoV-2, gold nanoparticles have been utilised as a colorimetric marker. The test makes use of gold nanoparticles bound to COVID-19-specific antigens. As shown in (Fig 2), the viral antigen and antibody would bind to the IgG or IgM present in the loaded samples (Alafeef & Pan, 2022). A 95% confidence interval is used to evaluate the performance features of these tests based on their clinical sensitivity and specificity. (Li et al., 2020) in their study obtained an overall testing sensitivity of 88.66% and a specificity of 90.63% from 397 PCR-confirmed COVID-19 patients. During Liverpool mass testing pilots in the UK, the Innova quick lateral flow COVID-19 test was applied. Having a low percentage of false positives, the total specificity obtained was 99.68% (Benda et al., 2021). In epidemiological research, antibody testing helps determine the population's prevalence of asymptomatic patients. However, there is evidence of antibody cross-reactivity amongst human coronaviruses (Jayamohan et al., 2021).

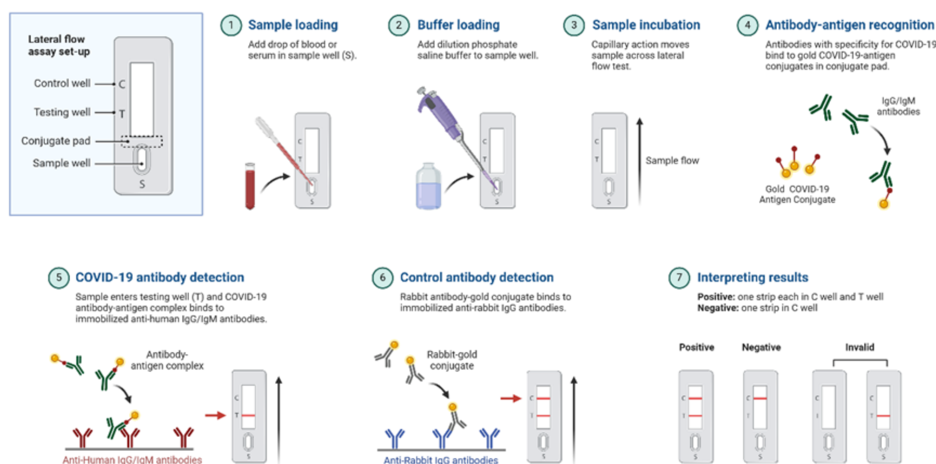

Figure 2: *Schematic representation of the SARS-CoV-2 Serological test. Recombinant antigens made specifically for the SARS-CoV-2 are immobilised on nitrocellulose paper for the test. Nanoparticles immobilised on conjugate pads conjugated with mouse anti-human IgM and IgG antibodies. A SARS-CoV-2-specific recombinant antigen will detect the antibodies in the patient sample. The test band will turn coloured on the test strip when SARS-CoV-2-specific IgG or IgM is present in the sample, indicating a positive test result.*

- **Antigen-based Lateral Flow Immunoassays**

An antigen is a toxin or other foreign matter that triggers the body's immune system, particularly the formation of antibodies. The virus's nucleocapsid, spikes, envelopes, or membrane proteins are targets for antigen detection. Viral antigens binding precisely to their matching antibodies may be recognised using techniques based on optical, magnetic, electrochemical, and surface plasmon resonance, among others (Jayamohan et al., 2021). Highly sensitive antibodies have been used in the LFIA design of most antigen tests. The lack of antibodies for the SARS-CoV-2 viral proteins is one of the difficulties in developing effective antigen testing. Since research has shown that the concentration of Nucleocapsid (N) protein can be greater than Spike (S) protein in samples prepared for antigen-based tests, the N protein has been the target of most of these antibodies. Another protein utilised to build LFIAs for antigen detection is the human angiotensin-converting enzyme-2 (hACE2), which is the cell entrance receptor of SARS-CoV-2 (Jayamohan et al., 2021;Mahmoudinobar et al., 2021).Quidel Corporation produced a lateral flow immunofluorescent sandwich assay technique for detecting the nucleocapsid protein antigen in direct anterior nasal swabs. Research carried out by (Chaimayo et al., 2020) on an antigen detection kit showed 98.33% sensitivity and 98.73%specificity on 60 RT-PCR-confirmed positive samples. Although the antigen tests have high specificity for the virus, they are less sensitive than PCR testing and cannot identify all the active cases.

Detection of SARS-COV 2 VIRUS WITH PCR

PCR is an enzyme-based technique for producing many copies of a gene, by separating the two strands of DNA carrying the gene segment, identifying its position using a primer, and then assembling and repeatedly replicating these copies using the enzyme, DNA polymerase (Ilkhani et al., 2021). Nucleic acid amplification-based tests determine the presence of an active infection with the target pathogen in a patient (Smithgall et al., 2020).

- **Reverse Transcriptase Polymerase Chain Reaction (RT- PCR)**

RT PCR is the gold standard technique for the detection of the Sars-CoV-2 virus since it can reach a high sensitivity. It has been the laboratory technique for diagnosing respiratory viral infections, such as influenza and respiratory syncytial virus (Eftekhari et al., 2021)(Alafeef & Pan, 2022). RT PCR entails the isolation of RNA from a sample and its subsequent reverse transcription into complementary DNA (cDNA) through which target sequences are amplified with a DNA polymerase. On January 11, 2020, the US Centers for Disease Control and Prevention released the SARS-CoV-2 genome sequence in its entirety, followed by the creation of the primers and probes (Alafeef & Pan, 2022). The virus comprises a positive single-stranded RNA genome of around 30,000 base pairs in length that codes for 27 proteins, including the ORF1b and ORF8 regions, RNA-dependent RNA polymerase and 4 structural proteins including surface glycoprotein, Matrix, envelop and nucleocapsid proteins which are targeted for detection (Böger et al., 2021).

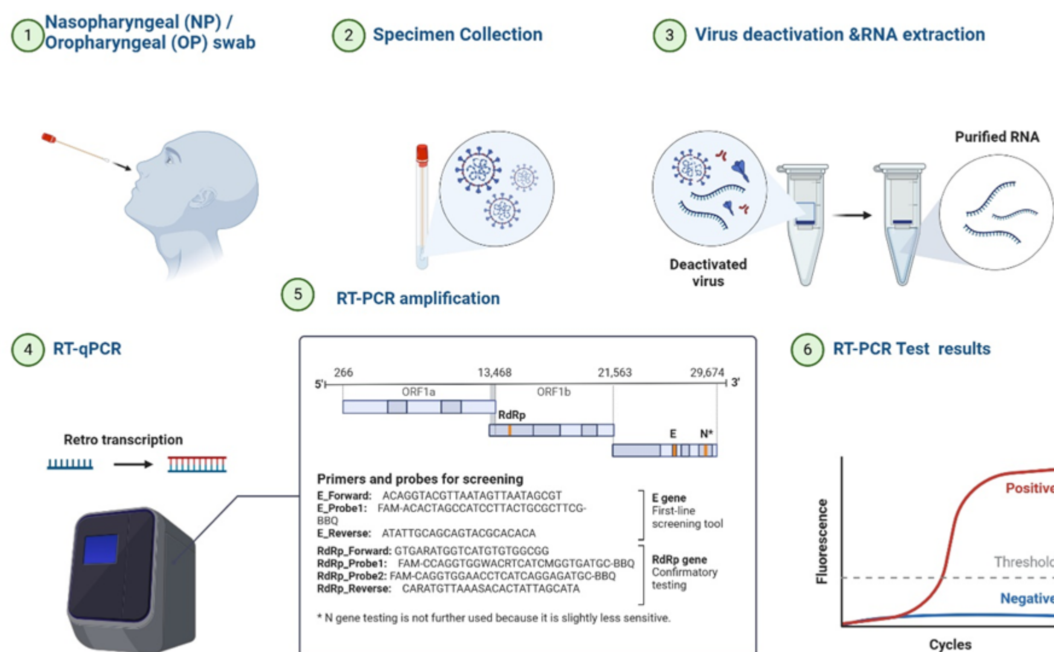

Figure 3. *RT-PCR-based diagnostic techniques' flowchart. 1) Collection of NP/OP swab samples from a suspected COVID-19 patient; 2) handling and storing collected swabs to maintain the integrity of the viral RNA; (3) thermal inactivation; (4) Viral RNA serves as a template to produce cDNA (5) RT-PCR amplification and real-time fluorescence signal detection; (6) analysis of amplification results and establishment of the positive threshold.*

The sample being analysed has a role on the specificity and sensitivity of RT PCR assays. The gold standard respiratory sample for SARS-CoV-2 rRT-PCR assays has been the nasopharyngeal swab (NPS) in Viral Transport Medium. However, the virus may now be found in more clinical collections including bronchoalveolar lavage, sputum, saliva, throat swabs, blood, and stool samples (Gupta et al., 2022). (Fig 3) portrays a schematic workflow of diagnosing the virus using RT PCR from sample collection through to thermal cycling to interpretation of results. In their study, (Winichakoon et al., 2020) reported a false-negative RT PCR result from nasopharyngeal/oropharyngeal samples that were later refuted when the virus was detected in the bronchoalveolar lavage (BAL) fluid collection. False-negative outcomes might be caused by preanalytical problems including insufficient respiratory sample collection, inappropriate sample handling, and analytical problems such variations in viral load or time of sample collection relative to disease progression (Jayamohan et al., 2021).

- **Digital Droplet PCR (ddPCR)**

ddPCR is a type of PCR where the sample is discretely partitioned at random, amplified to the end point, and counted by a droplet reader to ascertain the number of positive partitions. From there, the concentration is approximated by modelling as a Poisson distribution (Suo et al., 2020). This system leverages endpoint amplification to directly quantify viral load and is less sensitive to amplification inhibitors in the sample matrix. When compared to RT PCR on the same cohort, ddPCR reported higher values for sensitivity, specificity, Positive Predictive value (PPV), Negative Predictive Value, negative likelihood ratio (NLR), and accuracy, demonstrating greater precision in covid 19 identification (Suo et al., 2020). (Vasudevan et al., 2021) in their analysis showed ddPCR superior to quantitative (qPCR) for detecting SARS-CoV-2 due to its higher sensitivity and consistency when utilising crude lysate as the input rather than purified RNA. However, ddPCR is also susceptible to misestimation of viral RNA, which could lead to contradictory results (Kojabad et al., 2021).

Conclusion

RT PCR remains the gold standard for the detection of the SARS-CoV-2 virus as in other upper respiratory tract infections because of its high sensitivity and specificity. However, it rather has long turnaround times, can be costly, and needs skilled laboratory personnel. The sensitivity of RT PCR is also affected by the mutation and evolution of the virus. LFIAs are quite more affordable, faster, and easier to execute, though, regulatory bodies often require independent method validation of outcomes. RT PCR only detects patients currently shedding the virus and does not give a hint of past infection, unlike antibody-based tests that detect the patient's exposure history. Since antigen-based tests are completed in a short time, it takes at least 3 days for infected patients to produce antibodies (IgM), and at least 12 days for IgG to be produced, giving room for false negative results(Eftekhari et al., 2021). While molecular PCR tests are more sensitive and can identify all active instances, antigen tests are extremely specific for the virus and because of the high likelihood of a false-negative outcome, a negative antigen test result does not always rule out the infection. In summary, while LFIAs are suitable for point-of-care applications, which is necessary for mass screening, modern technologies should be integrated to increase their accuracy. Improvements in primers and probes are also needed for higher sensitivity and specificity of RT PCR. ddPCR shown to have more accuracy could supplement the current standard RT PCR to confirm diagnosis and reduce the risk of viral spread.

Conflict of Interest

The author declare no conflict of interest.

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