



EDITORIAL

A perspective on the effectiveness of SARS-Cov-2 rapid testing kits

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ABSTRACT

Patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) are generally diagnosed by rapid antigen test (RAT) and reverse transcription-polymerase chain reaction (RT-PCR) tests. However, there is a conflict that the RT-PCR test is more effective than the RAT. The primary objective of this perspective is to compare and outline the effectiveness of SARS-CoV-2 rapid testing kits available in the market with RT-PCR tests. Recently published systematic reviews and meta-analysis reports containing comparative studies of RAT and RT-PCR were selected. The analysis results revealed that RAT kits provide remarkable specificity and sensitivity in the early stages of infection, particularly when the viral load is huge, compared to RT-PCR. In addition, a trustworthy substitute for nasopharyngeal sampling is the use of nasal specimens for antigen detection, which are patient-friendly and somewhat sensitive. RAT could be beneficial in the combat against the COVID-19 pandemic, but it has to be combined with appropriate monitoring of results that come back negative.

KEY WORDS: COVID-19, Rapid antigen tests, Reverse transcription-polymerase chain reaction

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new coronavirus, is the cause of COVID-19. SARS stands for severe acute respiratory syndrome.

[1] SARS-CoV-2 is an enclosed virus with a positive-sense, single-stranded RNA genome of 30 kb and various other coronaviruses (CoVs) (order Nidovirales, family *Coronaviridae*, subfamily *Coronavirinae*). SARS-CoV-2, along with SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), is classified as a betacoronavirus as shown in Figure 1.[2] There are six identified CoVs that generally cause disease in humans. Some, such as coronavirus 229E, OC43, NL63, and HKU1, produce only mild cold-like symptoms, but others, like SARS-CoV and MERS-CoV, cause life-threatening respiratory infections like pneumonia and even death.[3] There was a general consensus that CoVs mostly affected wildlife, such as birds and animals. The amazing potential of CoVs to overcome species boundaries and transfer between

humans has been repeatedly proved by the epidemics of SARS, MERS, and now COVID-19.[4] The sequence was connected to the virus that caused the SARS-CoV-1 (2003) pandemic (87.5% sequence similarity) and two bat-derived SARS-like CoV variants (bat-SL-CoVZXC21 and bat-SL-CoVZC45) that have been reported to infect humans.[5] SARS-CoV-2 is the official moniker for the 2019-nCoV. The simplot analysis also revealed that SARS-CoV-2 was more strongly linked with the BatCoV RaTG13 genome (96.3% similarity) than previously thought.[6] In the area containing the 3'-end of the open reading frame (ORF) 1a, the ORF 1b, and about half the spike region, the bat-SL-CoVZXC21 and bat-SL-CoVZC45 variants grouped differently than the trio formed by SARS-CoV-2 and BatCoV RaTG13.[7] In late 2019, unexpected pneumonia epidemics began in Wuhan, China (December 31, 2019);

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in early 2020, a novel coronavirus was discovered to be the cause of these illnesses (Paraskevis *et al.*, 2020).^[8] There have been 45,000 confirmed cases of pneumonia across 26 nations as of February 11, 2020. Almost all documented cases (96.8%) have come from China, with the province of Hubei alone being responsible for 75% of the cases reported (Coccolini *et al.*, 2020).^[9] Kerala, India, has the world's first confirmed case of infection with COVID-19. A 20-year-old woman from Thrissur, Kerala, who had been experiencing a dry cough and throat pain for the previous day, visited the emergency room on January 27, 2020 (Kailasa *et al.*, 2020).^[10] Early patient identification, isolation, and treatment are crucial for minimizing transmission amplification incidents and lowering the risk of subsequent infections between close contacts and healthcare professionals. There are so many diagnostic tests that are used to minimize local transmission and prevent an epidemic, as shown in Table 1. Since the identification of COVID-19 is a public health priority, rapid and accurate diagnostic tests need to be developed. reverse transcription-polymerase chain reaction (RT-PCR), reverse-transcription loop-mediated isothermal amplification, rapid testing kits, and enzyme-linked immunosorbent assay (ELISA) are all examples of molecular biology tools, while ELISA, RT-PCR, as well as lateral flow assays are all examples of nanotechnology-based analytical tools, all of which can be used for the quick identification of COVID-19.^[11] This review focuses more on rapid testing kits that can give a result within 15–30 min, depending on viral load and when samples were collected, utilizing swabs collected from the nasal passages and nasopharynx.

RAPID ANTIGEN TESTING KITS (RAT)

RATs are a method for detecting COVID-19 that works by reacting to proteins on the surface of the virus called antigens. A RAT kit detects specific viral proteins. Point-of-care tests, such as COVID-19 rapid testing, are conveniently collected and assessed utilizing at-home kits, as well as at your neighborhood clinic, hospital, healthcare institution, or even at airports.^[12]

Mechanism of action of RAT kits

The specimen analyte is added to the sample well, and it contains SARS-CoV-2 antigen that has been suspended in assay buffer, Figure 2 shows the mechanistic process. After being absorbed by the sample pad, the analyte (antigen-containing; in green) starts diffusing through the reaction chamber and onto the conjugate pad. An antigen-antibody specific for SARS-CoV-2 is attached to a tag (often gold, latex-based substances, or a fluorophore) and then brought into close contact with the analyte. Diffusion carries the antigen-antibody combination through the nitrocellulose membrane. The test line is formed when the SARS-CoV-2 antigen-antibody complex comes into contact with a second SARS-CoV-2 antigen-antibody (which are distinct antigens) that is covalently attached to the device pad. A second, covalently attached antibody, selective for the primary SARS-CoV-2 antibody, is associated with the antigen after additional migration of excessive SARS-CoV-2 antibodies that have not yet bonded to the antigen. The control line is the product of a complex between two antibodies.^[13]

Advantages (RAT)

A RAT kit provides results in 15–30 min, does not need specialized equipment, and may provide a result on-site. RAT kits work well when a variety of tests are necessary, as is the case in regions where the infectious disease has spread. It does not require any major-specific training, and the individual can diagnose himself at any time. It's helpful for patients who have a detectable viral load and are at the possibility of transmitting the virus to others.^[14]

Limitations (RAT)

If a sample has a low viral antigen load, RAT kits may potentially produce false-negative findings. The likelihood of SARS-CoV-2 infection is not completely eliminated by a negative test result. The active stage of viral infection

Table 1: Comparison of RT-PCR and rapid antigen testing kits

RT-PCR	Rapid antigen testing kits	References
In other words, it is also called diagnostic, viral, molecular test, and nucleic amplification test, etc.	Rapid diagnostic test, Rapid test	[21]
Methods of collecting the sample:-Swab the nose or the throat, or spit.	Swab the nose or the throat, or spit.	[22]
Time required to observe the results:-The average wait time is between 2 and 3 days.	The average wait time is 15–13 min.	[23]
Should run another exam:-This is the most reliable test available for detecting COVID-19.	This exam has average reliability. If a test comes out negative, a PCR test should be done to be sure.	[24]
When this test shouldn't be used:-If you have tested positive for COVID-19 in the prior 90 days, you should not undergo further PCR testing.	In the absence of symptoms (e.g., before collecting or 3–4 days after contact), yet with a substantial possibility of a false negative result.	[16]
Reliability of Outcomes Extremely sensitive and specific.	High specificity with moderate sensitivity.	[25]

RT-PCR: Reverse transcription-polymerase chain reaction

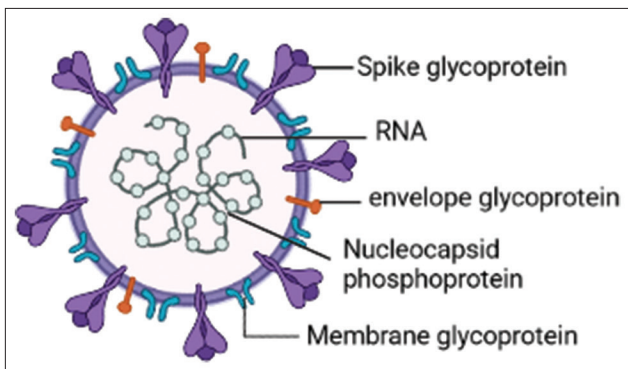


Figure 1: Severe acute respiratory syndrome coronavirus 2

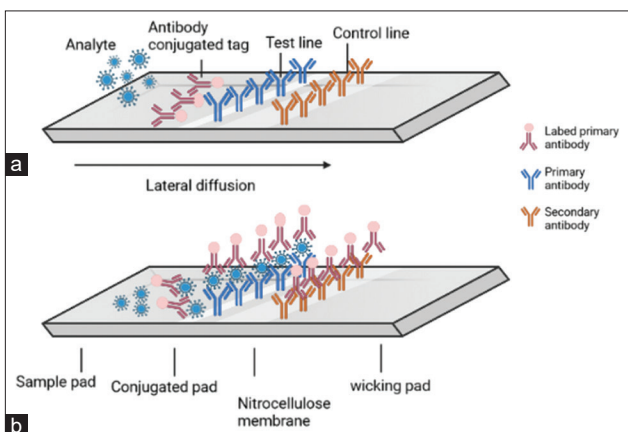


Figure 2: (a) Rapid antigen testing kit (mechanism of action): Analyte entrance and processing. (b) Rapid antigen testing kit (mechanism of action): Diffusion of antigen-antibodies through nitrocellulose membrane

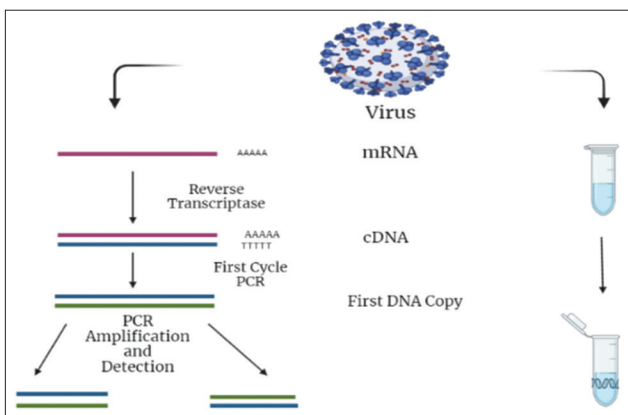


Figure 3: Reverse transcriptase polymerase chain reaction (mechanism of action)

affects the test’s sensitivity. Molecular RT-PCR testing is required to confirm positive results.^[15]

RT-PCR

One of the most rapidly established laboratory diagnostic methods in this COVID-19 epidemic was the RT-PCR identification of the viral nucleic acid test.^[16] Respiratory

secretions were analyzed using RT-PCR, which successfully confirmed the presence of viruses within 2 h. SARS-CoV-2 infection was suspected in all cases due to (1) common cold-like symptoms such as fever, cough, and dyspnea, or (2) proximity to a person infected with the closely related COVID-19.^[17]

Mechanism of RT-PCR

Real-time RT-PCR was used to identify SARS-CoV-2 in respiratory specimens by amplifying segments of the ORF 1ab and nucleocapsid protein (NP) genes using Shanghai Huirui Biotechnology Co., Ltd. kits. The conditions for the amplifications were 50°C for 15 min, 95°C for 3 min, and then 45 cycles of 95°C for 15 s and 60°C for 30 s. When particular real-time RT-PCR results for both targets (ORF 1ab and NP) are positive, the case may be regarded to have been verified in the lab [Figure 3]. The test was considered positive if the cycle threshold (Ct) value was <37, and it was considered negative if the Ct value was 40 or above. Ct values between 37 and under 40 were considered medium loads and required retesting for verification.^[18]

Advantages (RT-PCR)

Due to its superior sensitivity and specificity, RT-PCR has replaced older diagnostic procedures as the standard for SARS-CoV-2 diagnosis. This test is moderately accurate. Negative test results should be confirmed with a PCR test. This test is highly accurate and considered the gold standard in COVID-19 testing.^[19]

Limitations (RT-PCR)

RT-PCR is a lengthy process that needs specialized equipment, employees, and laboratories. RT-PCR mostly takes 48–72 h to get results.^[20]

Recent advanced techniques for SARS-CoV-2 rapid testing kits

Reliable antigen tests are constantly required for the quick and simple identification of people with a severe SARS-CoV-2 infection. Kruttgen *et al.* examined both the sensitivity and specificity of the SARS-CoV-2 RAT using 75 swabs from patients who had previously tested positive by SARS-CoV-2 PCR and 75 swabs from patients who had previously tested negative by SARS-CoV-2 PCR. A specificity of 96% was found. The assay’s sensitivity was 100%, 95%, 44.8%, and 22.2%, respectively, for samples with Ct of 25, 25–30, 30–35, and ≥35. The PCR test has superior sensitivity and specificity to the antigen assay. The antigen test, however, can be a rapid and simple option for separating SARS-CoV-2-infectious people from non-contagious people.^[26] In this research, Mak *et al.* compare the three currently available rapid antigen detection (RAD) kits’ clinical and analytical sensitivity. The three kits under

consideration were the COVID-19 Ag Respi-Strip from Coris Bioconcept in Belgium, the NADAL COVID-19 Ag test from Nal Von Minden GmbH in Germany, and the Standard Q COVID-19 Ag from SD Biosensor in Korea. The three RAT kits ranged in sensitivity from 102 to 105% less sensitive than RT-PCR, according to the limit of detection data. The clinical effectiveness of RAD tests for identifying COVID-19 patient specimens varied from 22.9% to 71.4%.^[27] In this research, Eshghifar *et al.* detected the performance of seven rapid testing kits for the detection of the SARS-CoV-2 virus. Only one sample from the five SARS-CoV-2 specimens that were verified positive by RT-PCR had a positive response from one of the seven antigen testing kits that were reviewed. The specimen with the favorable outcome had a Ct value of 15. All five positive specimens were negative for all other examined fast tests. RATs are not accurate for testing asymptomatic individuals, who frequently have low viral loads, as this comparison research demonstrates that they are less accurate than RT-PCR assays.^[28] Jian *et al.* compared the RAT kit with an RT-PCR assay for developing variants was conducted at large-scale population testing in Taiwan. As a result, 70 (3.33%) of the 2096 specimens subjected to the quick antigen test had positive results, whereas 2026 (96.7%) had negative results. The outcomes of the RT-PCR were contrasted with this clinical performance. The fast antigen test had a sensitivity of 76.39% (95% confidence interval (CI) 64.91–85.60%) and a specificity of 99.26% (95% CI 98.78–99.58%), with a high sensitivity in people with Ct values below ≤ 24 . This quick SARS-CoV-2 antigen detection assay for fast screening, together with RT-PCR as a two-time confirmatory screening method, can help avoid widespread transmission during COVID-19 situations due to its quick turnaround times and cheaper costs.^[29]

CONCLUSION

Antigen tests are helpful due to their short turnaround time, ease of performance and interpretation, and low cost. In the early stages of a virus infection, while the amount of virus has not yet accumulated in substantial proportions, RAT might not be as precise as RT-PCR assays. Those in close touch with someone infected with COVID-19 may benefit from regular antigen testing, even if the RAT may be less accurate than the RT-PCR test. Mass screenings using RAT are often employed in containment zones and healthcare facilities to promptly diagnose the SARS-CoV-2 infection. Although RT-PCR tests tend to be more specific and accurate, they are also more challenging to execute, take longer to provide results from, and are more expensive.

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