**JOURNAL OF ALGEBRAIC STATISTICS**
Volume 13, No. 3, 2022, p.5576-5582
https://publishoa.com
ISSN: 1309-3452

# A Critical Study on Effectiveness of Reverse Transcription-Polymerase Chain Reaction (RT-PCR) in Covid-19 Detection

Sudeesh Chouhan, Dr. Rajesh Keshavrao Deshmukh
Department of Computer Science and Engineering, Kalinga University, Naya Raipur, Chhattisgarh, India

**Abstract**
The reverse transcription-polymerase chain reaction (RT-PCR) is now widely considered the most reliable method for identifying the presence of SARS-CoV-2, the virus that causes COVID-19. RT-PCR identifies viral RNA in respiratory samples, such as swabs taken from the nose and throat, by amplifying certain genetic sequences. Although RT-PCR testing is effective, it encounters various obstacles such as the requirement for advanced laboratory infrastructure, skilled workers, and longer processing durations in comparison to fast antigen tests. Currently, COVID-19 is recognized as a worldwide public health crisis by the World Health Organization (WHO). Therefore, it is crucial to identify and prevent the spread of this disease in order to manage this emergency effectively. RT-PCR is a highly precise laboratory technique used to determine the presence of SARS-CoV-2 with great sensitivity. The method relies on the swift identification of SARS-CoV-2 by the qualitative detection of its genetic material. This assay identifies the specific locations of the primer and probe sets inside various sections of the SARS-CoV-2 genome.

**Keywords:** Diagnosis, COVID, Public health, Polymerase chain reaction, Respiratory illnesses

## I. Introduction

The new coronavirus SARS-CoV-2 emerged in late 2019, causing the global outbreak of COVID-19 and posing a huge challenge to public health systems around the world. As the virus swiftly spread across continents, causing widespread illness and death, the urgent need for reliable, fast, and accessible diagnostic testing became evident. For the pandemic to be contained, infected people to be isolated quickly, clinical management to be guided, and public health initiatives to be informed, accurate diagnostic methods are crucial. A wide variety of diagnostic tools, each with its own set of pros and cons, have been created and used during the pandemic. The molecular diagnostics community has rallied around the RT-PCR as the gold standard for COVID-19 testing. Respiratory specimens, including nasopharyngeal swabs, oropharyngeal swabs, and saliva, can have their viral RNA amplified using the very sensitive and specific real-time polymerase chain reaction (RT-PCR) method. Reverse transcriptase-polymerase chain reaction (RT-PCR) is based on the idea that certain viral gene sequences can be amplified when reverse transcriptase converts viral RNA to complementary DNA (cDNA). The presence of amplified products confirms a current infection with SARS-CoV-2. However, there are a few drawbacks to RT-PCR testing that make it less than ideal, particularly in contexts where resources are scarce. These include the need for expensive and complex laboratory equipment, highly skilled lab workers, and a long processing time.

A variety of new nucleic acid amplification tests (NAATs) have emerged to overcome these obstacles. There are more convenient, quicker, and portable methods for detecting viruses, such as transcription-mediated amplification (TMA) and loop-mediated isothermal amplification (LAMP). For instance, compared to conventional PCR, LAMP amplifies DNA at a constant temperature, therefore heat cycling equipment is unnecessary, and the process takes much less time. If you need findings quickly, these isothermal approaches are perfect for point-of-care testing or a decentralized diagnostic environment. Additionally, a new method for detecting viruses has been offered by developments in CRISPR-based diagnostics. The CRISPR-Cas systems, which were initially developed for gene editing, have recently been modified to identify viral RNA very specifically and sensitively. Promising instruments for extensive, rapid testing, CRISPR-based tests require minimum equipment to be done.

**JOURNAL OF ALGEBRAIC STATISTICS**
Volume 13, No. 3, 2022, p.5576-5582
https://publishoa.com
ISSN: 1309-3452

Because of their rapid and inexpensive results, antigen-based testing has also become popular, alongside molecular diagnostics. Virus surface proteins like spike and nucleocapsid proteins can be detected by these techniques, which use antibodies that are immobilized on a test strip. One of the most popular methods is the lateral flow immunoassay (LFA), which looks like a home pregnancy test and can give you results in about 15 to 30 minutes. Mass screening and frequent testing situations, including in schools, workplaces, and travel settings, where the rapid identification of contagious persons is paramount, are ideal for antigen tests. In those with low viral loads or in the early or late phases of an illness, antigen testing tends to be less sensitive than molecular tests. Because of this restriction, more sensitive molecular testing may be required to verify negative antigen test results.

In order to understand the immunological response to SARS-CoV-2 and conduct epidemiological studies, serological testing is vital. These tests identify antibodies that are created in response to infection. Through the detection of immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA) antibodies in the blood, these tests are able to identify prior illnesses. It is usual practice in serology to quantify antibody levels using enzyme-linked immunosorbent assays (ELISAs) or chemiluminescent immunoassays (CLIAs). The time it takes for antibodies to form (usually 1-3 weeks post-infection), making serological tests unsuitable for diagnosing acute infections. However, they are very useful for gauging the level of population exposure, finding seroprevalence, and evaluating vaccine-induced immunity. The length and intensity of immunity after spontaneous infection or vaccination can be better understood with the use of serological data, which in turn can guide public health policy decisions and actions.

Efforts to make these diagnostic tools more accessible and efficient have been continuing alongside the fast development and deployment of these tools. Validating and quality controlling tests to guarantee their accuracy and dependability is a big problem. Diagnostic tests have been made more accessible by emergency use authorization (EUA) procedures put in place by regulatory bodies like the U.S. Food and Drug Administration (FDA), however these tests must undergo thorough validation studies to prove how well they work in actual situations. Coming up with diagnostic tools that can detect new strains of SARS-CoV-2 is another obstacle. The specificity and sensitivity of molecular and antigen tests can be impacted by variants with mutations in important genomic regions; thus, testing protocols must be continuously monitored and adjusted as needed.

Especially in low- and middle-income countries (LMICs), the problem of equitable access to diagnostic testing is critical. A scalable, cost-effective diagnostic system that may be implemented in varied healthcare settings is necessary due to the variance in testing capacity around the world. Researchers are looking for new ways to increase the capacity and efficiency of testing, such as developing more user-friendly testing instruments and developing multiplex assays that can identify many respiratory pathogens concurrently. In addition, for public health systems to effectively monitor and respond to diseases, diagnostic data must be integrated. By allowing for the reporting and analysis of test findings in real-time, digital health technology such as electronic health records and mobile apps can help detect outbreaks and implement targeted interventions more quickly.

Combating COVID-19 will require a multipronged strategy, as the variety of diagnostic tools available for SARS-CoV-2 detection demonstrates. In contrast to antigen-based tests, which give quick and inexpensive screening alternatives, molecular diagnostics, such as real-time polymerase chain reaction (RT-PCR) and isothermal amplification approaches, have excellent sensitivity and specificity for identifying current infections. The immune response and the spread of viruses can be better understood with the help of serological tests. Because of the inherent limitations of any given diagnostic tool, it is essential to develop individualized plans that make use of relevant tests in order to achieve desired outcomes. In order to tackle the ever-changing difficulties presented by SARS-CoV-2, make sure that worldwide efforts to manage the pandemic are effective, and be ready for future infectious disease threats, there must be continuous innovation and collaboration in the diagnostics sector. The significance of reliable and easily available diagnostic testing is paramount as we delve deeper into the intricacies of COVID-19. We can detect, confine, and eventually defeat this formidable viral enemy with its help; it is the bedrock of efficient disease management and public health approach.

**JOURNAL OF ALGEBRAIC STATISTICS**
Volume 13, No. 3, 2022, p.5576-5582
https://publishoa.com
ISSN: 1309-3452

## II. Review Of Literature

Sekar, Priyadharshini et al., (2021) The emergence of Coronavirus Disease 2019 (COVID-19) was initially documented in December 2019 in Wuhan, China. In a matter of weeks, the disease had disseminated to other areas of China and then to many locations across the globe. As of March 17, 2021, COVID-19 has impacted 221 countries and territories globally, resulting in a total of 121,290,697 confirmed cases and 2,682,554 deaths. Precise identification of diseases (such as the SARS-Cov-2 virus and its variations) and the subsequent isolation of patients are currently crucial measures in limiting the spread of the disease. Insufficient time during the epidemic prevented thorough validation of the diagnostic assays. Occasionally, real-time reverse transcription polymerase chain reaction (rRT-PCR) testing for SARS-CoV-2 may fail to detect infected persons due to errors or inefficiencies in the sampling process, a low ability to detect the virus, and the virus's epidemiology. The interpretation of rRT-PCR test results should be done in conjunction with clinical examination and Computed Tomography (CT), especially in those who are suspected to have symptoms or have a history of contact with confirmed COVID-19 cases. Given the limitations noted earlier, the current situation requires fast and on-site tests for identifying SARS-CoV-2 in distant areas. Currently, there is no dependable antigen testing kit that is available for purchase. The individuals who are infected display diminished levels of antibodies against SARS-CoV-2 during the initial stages of the virus. Furthermore, novel methods such as Digital RT-PCR and isothermal RNA amplification with electrochemical biosensors are now under development to offer precise and sensitive detection of SARS-Cov-2 antigens. The recently identified variant, SARS-CoV-2 VUI 202012/01, is unlikely to affect diagnostic results since most PCR techniques globally utilize two or more dependable gene targets (such as RdRp, E, and N) in addition to the S gene.

Sethi, Shneh & Chakraborty, Trinad. (2021) The emergence of COVID-19, a viral disease caused by SARS-CoV-2, originated in Wuhan, China. COVID-19 was initially categorized by the World Health Organization as a public health emergency and then designated as a global pandemic. COVID-19 can manifest in three separate forms: severe acute respiratory distress syndrome with a potentially lethal consequence, mild respiratory disease (pneumonia with eventual recovery), and asymptomatic infection. All three disease kinds possess the capability to transfer the illness to individuals who are in good condition. Currently, the only laboratory method available to validate the presence of viral RNA in patient specimens is real-time reverse transcription polymerase chain reaction (RT-PCR). These assays are specifically developed to identify and detect at least two SARS-CoV-2 RNA gene targets, enabling the identification of the virus. Commercial RT-PCR techniques utilize different gene targets from the viral genome in their testing systems. Moreover, there are variations in the choice of primers for the identical gene area of SARS-CoV-2. Currently, it is uncertain whether the outcomes obtained from various RT-PCR tests may be considered similar in identifying the full range of COVID-19 symptoms. This paper has two main objectives: firstly, to provide a brief overview of the findings from these reports; and secondly, to highlight the several obstacles and defects that may affect the diagnostic accuracy of RT-PCR testing for SARS-CoV-2.

Olalekan, Adesola et al., (2020) Proper execution of the test, interpretation of results, and reporting necessitates sufficient understanding of real-time Reverse Transcriptase-Polymerase Chain Reaction (rRT-PCR). In this concise review, the concepts, methods, and current state of rRT-PCR assays for COVID-19 pandemic control are outlined. We updated the landscape of rRT-PCR protocols and described the principles of rRT-PCR. We also elucidated the process control involved in the pre-analytical, analytical, and post-analytical stages of COVID-19 testing. This was done through a narrative review. Currently, the gold standard for verifying a COVID-19 diagnosis using SARS-CoV-2 RNA detection, rRT-PCR is the way to go. This method involves converting RNA to cDNA and then amplifying target genes in real time with sequence specific TaqMan® probes. There is evidence to suggest that in countries affected by the pandemic, there are various rRT-PCR methods that can be used for validation and emergency use permission (EUA). These techniques differ in the quantity and type of target genes within the SARS-CoV-2 genome. These procedures can detect a total of one to three target genes, which include ORF1a, ORF1b, RdRp, Nucleoplasid protein, spike glycoprotein, and envelope protein. rRT-PCR is still the gold standard for COVID-19 illness confirmation, surveillance, and management in all nations hit by the current pandemic. Research into the creation of alternative testing methods

is greatly encouraged, and it is imperative that all rRT-PCR protocols be validated before being used for COVID-19 testing.

Timsit, Edouard et al., (2010) A commercial RT-PCR kit for the detection of Bovine respiratory syncytial virus (BRSV) has recently been offered for sale. The results of this kit cannot be used for diagnostic purposes without comparing them to other regularly used procedures. Thus, this study set out to compare the efficiency of this kit to that of the more traditional direct fluorescent antibody test (FAT). The sensitivity and specificity of the kit were tested using twenty strains of BRSV and fourteen heterologous bovine viruses. By testing dilution series of a BRSV strain, we were able to estimate the kit's effectiveness and detection limit. Using 94 clinical samples from calves exhibiting clinical symptoms of respiratory disease, including lung tissues (n = 55), transtracheal aspiration samples (n = 20), and nasal swab samples (n = 19), the comparison was made between the real-time RT-PCR kit and FAT. The real-time RT-PCR method was able to identify every single BRSV strain that was tested. There was no evidence of cross-reaction with any of the fourteen different bovine viruses. With a detection limit of 0.1 TCID (50) (half of the tissue culture infectious dose), the real-time RT-PCR achieved a 99.3% efficiency rate. For 65 out of 94 clinical samples, the results from FAT and real-time RT-PCR were in agreement. The greater sensitivity of real-time RT-PCR was demonstrated by the 29 remaining clinical samples that tested positive by real-time RT-PCR but negative by FAT. Finally, this study's evaluation of the kit found that it was sensitive, specific, and had a low detection threshold. In addition, sensitivity for BRSV detection in clinical samples can be enhanced by using this kit instead of FAT.

## III. Diagnostic Techniques For The Detection Of Sarscov-2

A significant number of instances of severe febrile respiratory sickness, often referred to as atypical pneumonia, were reported in the city of Guangdong in China, and they quickly spread over the entirety of Asia. A virus known as SARS-CoV was responsible for this occurrence. The World Health Organization (WHO) later referred to the illness as severe acute respiratory syndrome (SARS). The signs and symptoms of this illness were comparable to those of other respiratory illnesses, as is the case with virtually all viral diseases on the planet. The diagnosis and identification of the infector are the first and most critical tasks that need to be completed in order to prevent and manage this pandemic. This requires a method that is both more sensitive and trustworthy. Tissue culture isolation, antibody detection, and reverse transcription-polymerase chain reaction (RT-PCR) are the three approaches that are utilized in the process of illness diagnosis. Among these, reverse transcription-polymerase chain reaction (RT-PCR) is regarded as the most effective method for detecting SARS-CoV-2.

## IV. Real Time Rt-Pcr Test Method

Real-time reverse transcription-polymerase chain reaction (RT-PCR) is a quantitative laboratory test that is capable of detecting specific genetic material in disease-causing bacteria, such as viruses, using in-vitro detection. Due to the fact that it is both highly specific and quick, this technique is now considered to be the gold standard for the detection of some viruses, notably SARS-CoV-2. Real-time polymerase chain reaction (RT-PCR) is a molecular technique that involves the amplification of particular DNA from a wide range of sources, including saliva, hair, nose swabs, and blood. Owing to the fact that the SARS-CoV-2 virus uses RNA rather than DNA as its genetic material. Therefore, in order to transform RNA into DNA, scientists must use an enzyme called reverse transcriptase. This process is referred to as reverse transcription. The DNA that is obtained through this procedure is referred to as cloned DNA or cDNA, and it is used to create cDNA libraries. After some time has passed, the process of amplification of DNA begins, as depicted in figure 1, which ultimately leads to the identification of a specific gene. In both real-time RT-PCR and RT-PCR, the procedures described above are identical in every way.

**JOURNAL OF ALGEBRAIC STATISTICS**
Volume 13, No. 3, 2022, p.5576-5582
https://publishoa.com
ISSN: 1309-3452

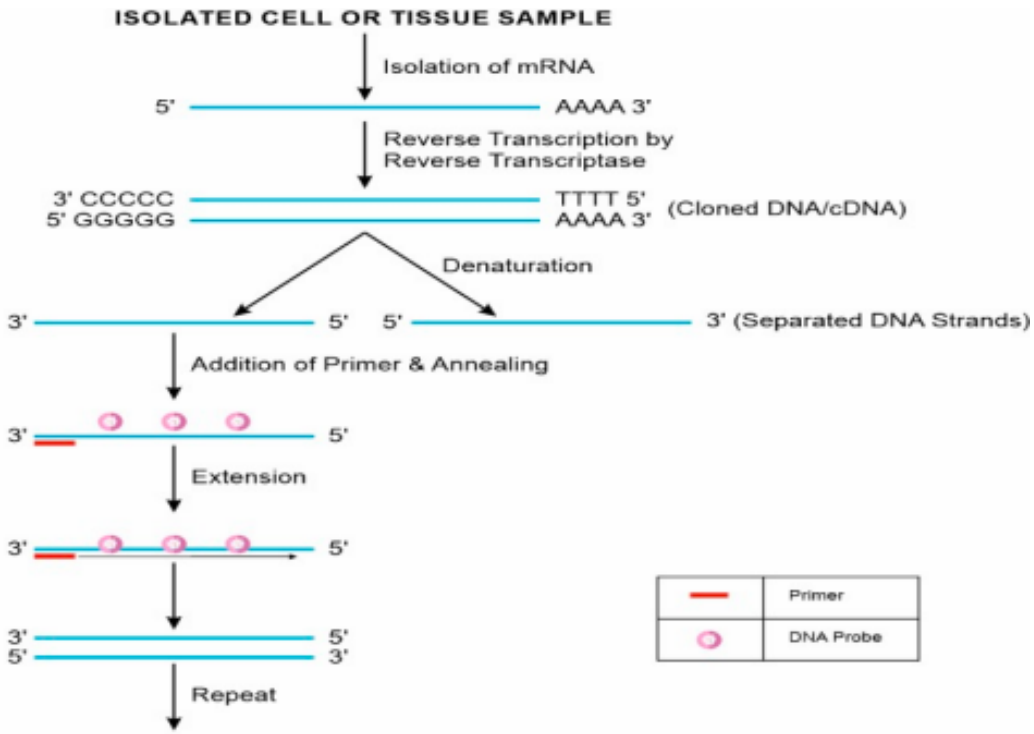


**Figure 1: Schematic Representation of real-time RT-PCR**

Probes or primers that are specifically designed to bind to DNA are utilized as fluorescent reporters in real-time RT-PCR. Through the use of these probes, it is feasible to identify the amplification of DNA at any moment, but in the case of RT-PCR, this will only be achieved at the conclusion of the process. The two types of real-time RT-PCR that are most widely used are quantitative and semi-quantitative variations. This category is further separated into non-specific dye and specific probes types. Both of these groups are presented below. Figure 2 provides a representative illustration of the comprehensive classification of real-time RT-PCR.

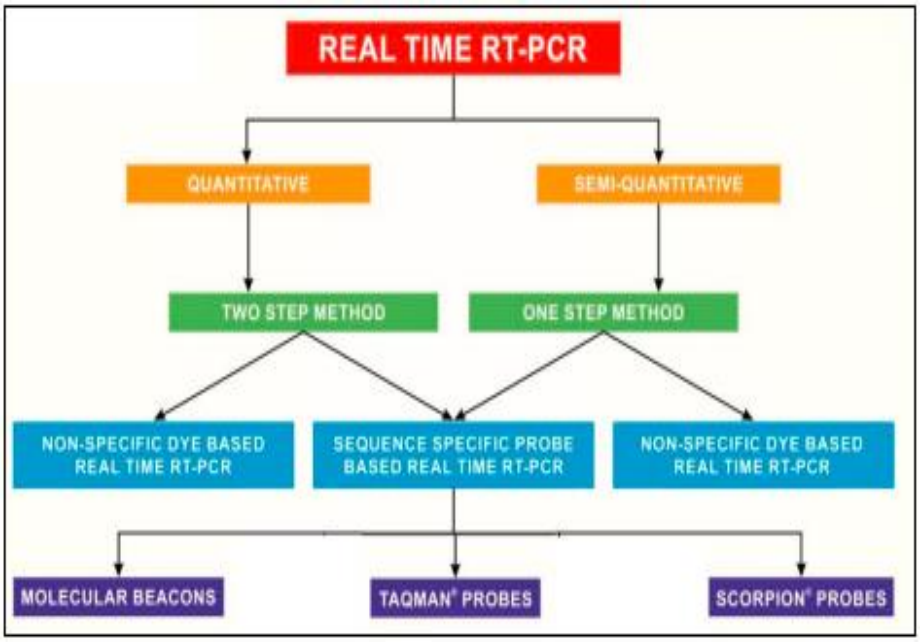


**Figure 2: Classification of Real Time RT-PCR**

The dye-based real-time PCR is also referred to as SYBR green-based quantitative PCR. This is due to the fact that SYBR green is the DNA binding dye that is utilized the most frequently for real-time RT-PCR. When

excited, SYBR green emits light after it has bound with the double-stranded DNA of the products of the polymerase chain reaction (PCR). Increases in fluorescence intensity occur whenever the results of the PCR process accumulate.

## V. Conclusion

The emergence of the SARS-CoV-2 virus, which has caused the COVID-19 pandemic, has made it imperative to quickly create and implement accurate diagnostic techniques in order to effectively control its transmission. Out of these tools, real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) has become widely accepted as the most reliable method because of its exceptional ability to accurately detect viral RNA with high sensitivity and specificity. The early detection capability of RT-PCR, even at low levels of viral presence, has played a vital role in promptly isolating and treating infected persons, thus effectively reducing the spread of the virus. Although there are obstacles such as the requirement for specialized equipment, skilled workers, and certain logistical bottlenecks, RT-PCR remains essential in the worldwide endeavor to fight against COVID-19. The continuous progress of RT-PCR technology, together with its strategic incorporation into wider testing frameworks, will be crucial in maintaining its efficacy and availability. In order to effectively monitor the progression of the pandemic, it is crucial to retain strong RT-PCR testing capabilities. This will enable us to continuously track the disease, manage new variants, and be prepared for any future public health risks.

**References:**

1. A. Das et al., "Removal of Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) Inhibitors Associated with Cloacal Swab Samples and Tissues for Improved Diagnosis of Avian Influenza Virus by RT-PCR," *Journal of Veterinary Diagnostic Investigation*, vol. 21, no. 6, pp. 771-778, 2009, doi: 10.1177/104063870902100603.
2. A. Olalekan et al., "Understanding the Use of Real Time Reverse Transcriptase-Polymerase Chain Reaction (rRT-PCR) For COVID-19 Diagnosis," *Pan African Journal of Life Sciences*, vol. 4, no. 2, pp. 86-97, 2020, doi: 10.36108/pajols/0202/40(0270).
3. D. Li et al., "False-Negative Results of Real-Time Reverse-Transcriptase Polymerase Chain Reaction for Severe Acute Respiratory Syndrome Coronavirus 2: Role of Deep-Learning-Based CT Diagnosis and Insights from Two Cases," *Korean Journal of Radiology*, vol. 21, no. 4, 2020, doi: 10.3348/kjr.2020.0146.
4. D. Rani, H. Bajaj, and R. Singh, "SARS-COV-2 (COVID-19) and role of real time Reverse Transcription Polymerase Chain Reaction (RT-PCR) in its diagnosis," *Research Journal of Pharmacy and Technology*, vol. 14, no. 6, pp. 3437-3440, 2021, doi: 10.52711/0974-360X.2021.00598.
5. D. Rio, "Reverse Transcription-Polymerase Chain Reaction," *Cold Spring Harbor Protocols*, vol. 2014, no. 11, 2014, doi: 10.1101/pdb.prot080887.
6. E. Timsit, C. Maingourd, E. Dréan, et al., "Evaluation of a Commercial Real-Time Reverse Transcription Polymerase Chain Reaction Kit for the Diagnosis of Bovine Respiratory Syncytial Virus Infection," *Journal of Veterinary Diagnostic Investigation*, vol. 22, no. 2, pp. 238-241, 2010, doi: 10.1177/104063871002200211.
7. J. Bridge, "Reverse transcription-polymerase chain reaction molecular testing of cytology specimens: Pre-analytic and analytic factors: RT-PCR Testing of Cytology Specimens," *Cancer Cytopathology*, vol. 125, no. 1, 2016, doi: 10.1002/cncy.21762.
8. J.-C. Tu et al., "COVID-19 Diagnosed by Real-Time Reverse Transcriptase-Polymerase Chain Reaction in Nasopharyngeal Specimens of Suspected Cases in a Tertiary Care Center: A Descriptive Cross-sectional Study," *Journal of Nepal Medical Association*, vol. 59, no. 237, pp. 464-467, 2021, doi: 10.31729/jnma.5383.
9. J.-H. Hwang et al., "Robust real-time reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus neutralizing carryover contamination," *Journal of Clinical Microbiology*, vol. 54, no. 1, 2015, doi: 10.1128/JCM.01944-15.
10. K. Saeed and N. Ahmad-Saeed, "Real-Time Polymerase Chain Reaction: Applications in Diagnostic Microbiology," *Int J Med Students*, vol. 1, no. 1, pp. 28-36, 2013, doi: 10.5195/ijms.2013.22.
11. P. Sekar, G. Menezes, P. Shivappa, B. George, and A. Hossain, "Considerations in Real-time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) for the Detection of SARS-CoV-2 from Nasopharyngeal

Swabs," *Journal of Pharmaceutical Research International*, vol. 33, no. 17, pp. 68-78, 2021, doi: 10.9734/JPRI/2021/v33i1731309.

12. R.-J. Lu et al., "Real-time reverse transcription-polymerase chain reaction assay panel for the detection of severe acute respiratory syndrome coronavirus 2 and its variants," *Chinese Medical Journal*, vol. 134, no. 17, pp. 2048-2053, 2021, doi: 10.1097/CM9.0000000000001687.
13. S. Reid et al., "Performance of Real-Time Reverse Transcription Polymerase Chain Reaction for the Detection of Foot-and-Mouth Disease Virus during Field Outbreaks in the United Kingdom in 2007," *Journal of Veterinary Diagnostic Investigation*, vol. 21, no. 3, pp. 321-330, 2009, doi: 10.1177/104063870902100303.
14. S. Sethi and T. Chakraborty, "Molecular (real-time reverse transcription polymerase chain reaction) diagnosis of SARS-CoV-2 infections: complexity and challenges," *Journal of Laboratory Medicine*, vol. 45, no. 3, pp. 135-142, 2021, doi: 10.1515/labmed-2020-0135.