INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Review Article

Detection of Corona Virus: A Review on Applications of RT-PCR in Detection of Corona Virus (Covid-19)

S. Agrawal*, B. Potdar, J. Shaikh, K. Pimpale, L. Kochage, N. Chougule

Department of Microbiology, Ashokrao Mane Institute of Pharmacy, Ambap-416112, India.

ARTICLE INFO

Received: 06 Nov 2023
Accepted: 08 Nov 2023
Published: 14 Nov 2023
Keywords:
Corona Virus, RT-PCR technique, Covid-19, Vaccines, Antibodies
DOI:
10.5281/zenodo.10124285

ABSTRACT

Reverse transcription polymerase chain reaction (RT-PCR) has emerged as the cornerstone of diagnostic efforts in the detection of corona viruses, particularly the formidable SARS-CoV-2 responsible for the COVID-19 pandemic. This review article provides a comprehensive overview of the diverse applications of RT-PCR in corona virus detection. The sensitivity and specificity of RT-PCR render it indispensable in early-stage diagnosis, enabling prompt isolation and treatment of infected individuals. Moreover, RT-PCR's ability to quantitatively analyze viral load allows for dynamic tracking of infection progression and the evaluation of therapeutic interventions. In addition to its clinical utility, RT-PCR plays a pivotal role in population-wide screening and surveillance programs, offering crucial insights for public health interventions. The adaptability of RT-PCR to detect specific genetic markers associated with viral variants is paramount in monitoring viral evolution and understanding its spread. Furthermore, this review highlights the pivotal role of RT-PCR in the development and evaluation of vaccines and antiviral drugs, providing invaluable data for vaccine efficacy studies and immune response assessments.

INTRODUCTION

COVID-19, declared a pandemic by the World Health Organization on March 11, 2020, has been a significant catastrophe for humanity [1]. It is an ailment affecting the respiratory and vascular systems, triggered by a virus known as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) belonging to the beta-coronavirus family. This virus is characterized by a single-stranded RNA enclosed in an envelope [2]. The virus is transmitted from animals to humans and between humans through respiratory droplets and physical contact [3]. When an infected person coughs, sneezes, or even talks, they emit both large droplets and small aerosols. The transmission through droplets is a significant pathway for infection. These droplets can infect a person in close proximity, while aerosols remain suspended

***Corresponding Author:** S. Agrawal
Address: *Department of Microbiology, Ashokrao Mane Institute of Pharmacy, Ambap-416112, India.*
Email : sahilagarwal1912@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

in the air and can lead to infection when inhaled by someone at a distance [4]. When a healthy individual comes into close contact with an infected person, their nasal, oral, and conjunctival mucosa are exposed to respiratory droplets containing the virus. Certain studies indicate that the virus can persist on various surfaces for several days [5-6]. Infection can be transmitted through contact by touching one's face, specifically the nose, mouth, and eyes, either directly after close contact with an infected person or through contact with contaminated objects (fomites) used by the infected individual [7]. The form of transmission where a person's own contaminated hands come into contact with other parts of their body, thereby introducing the contaminated material to those areas, is referred to as self-inoculation [8]. It has been observed that all respiratory infection viruses have the potential for transmission through self-inoculation. On average, an individual tends to touch their own face approximately 23 times in an hour, with their eyes being touched around 3 times per hour [9-10]. Prevention is more effective than treatment. In April 2020, the Government of India issued an advisory urging every individual to wear face masks due to the sudden surge in COVID-19 cases [11]. The Centers for Disease Control and Prevention (CDC) also recommended people to practice social distancing, maintain proper hand hygiene, and use face masks in order to mitigate the spread of the virus [12].

Correct and proper usage of a face mask by a healthy individual can substantially lower the risk of virus transmission. It acts as a barrier, preventing virus-containing droplets and aerosols from entering the nose and mouth. Additionally, it decreases the release of respiratory droplets and aerosols from an infected person when they cough, sneeze, or talk. Moreover, a face mask provides a safeguard against self-inoculation of the virus, as it reduces the likelihood of touching one's nose and mouth [13]. You're absolutely right. Wearing a face mask alone doesn't offer protection for the eyes against viral infection through respiratory droplets or self-inoculation. The conjunctival mucosa, being directly exposed to external pathogens, can potentially be the initial site of infection. Moreover, the presence of angiotensin-converting enzyme-2 (ACE-2) receptors on the conjunctival mucosa makes it a possible entry point for the virus [14]. The SARS-CoV-2 virus gains entry into host cells by binding to angiotensin-converting enzyme-2 (ACE-2) receptors. These receptors serve as the entry point for the virus, allowing it to infect and replicate within the host cells [15].

HOWARD TEMIN'S DISCOVERY OF REVERSE TRANSCRIPTASE

From his early days as a student, Howard Temin demonstrated a keen interest in biology. This fascination was honed during his participation in a specialized biology program at the Jackson Laboratory in Bar Harbor, Maine, during his summers [16]. After graduating from Swarthmore College in 1955, Howard Temin served as a counselor for a group of students, one of whom was David Baltimore, who was on the brink of starting at Swarthmore. Temin later pursued graduate studies at the California Institute of Technology (Caltech) and became a member of Renato Dulbecco's laboratory. During this time, he collaborated closely with Harry Rubin, a postdoctoral fellow in the same research group. Caltech was a hub for pioneers in molecular biology, including notable figures from the phage group like Max Delbrück and Matthew Meselson. [17]. Renato Dulbecco, who later shared the 1975 Nobel Prize with Temin and Baltimore, made significant contributions to the field of quantitative animal virology. He achieved this by pioneering the development of the first plaque assays for animal viruses, such as poliovirus. Additionally, Dulbecco played a pivotal role in demonstrating the oncogenic transformation of tissue culture cells

following infection with DNA tumor viruses. [18-19].

DISCOVERY OF REVERSE TRANSCRIPTASE BY DAVID BALTIMORE

David Baltimore's interest in animal virology took root during his time in graduate school. He made a pivotal move from MIT to the Rockefeller University to study under the mentorship of Richard Franklin. Franklin was a trailblazer in investigating genome replication of picornaviruses, which are small, positive-sense RNA viruses, including notable examples like poliovirus and mengovirus. These viruses were particularly suitable for study in the era before molecular cloning because they replicate in the cytoplasm and induce a shutdown of host protein and RNA synthesis. Additionally, their RNA synthesis remains unaffected by actinomycin D, making it easy to experimentally halt host RNA synthesis and detect viral RNA synthesis shortly after infection. Baltimore focused on studying viral RNA and protein synthesis by concentrating on cytoplasmic fractions and employing pulse-labeling with radioactive precursors for RNAs or proteins. Through this rigorous approach, Baltimore successfully identified cytoplasmic RNA polymerase activity in virus-infected cells [20], As a postdoctoral fellow in James Darnell's laboratory at MIT, Baltimore furthered this research and showed that the RNA polymerase activity could indeed be identified in in vitro reactions [21].

Early Detection

RT-PCR, or Reverse Transcription Polymerase Chain Reaction, is a crucial diagnostic tool for detecting the virus in the early stages of infection, sometimes even before symptoms manifest. This early detection is pivotal for promptly isolating infected individuals and implementing measures to halt further transmission.

Diagnosis of SARS-CoV-2 Infection

The current diagnostic methods for identifying individuals infected with SARS-CoV-2 primarily employ two distinct approaches [22].

Indeed, direct detection methods like culture, protein detection, and RT-PCR techniques are widely employed during the current pandemic for identifying the virus or its components. Immunological tests gauge the host's immune response, while whole-genome sequencing aids in the identification of variants. These techniques play a vital role in addressing the ongoing pandemic and formulating an effective response to the virus [23].

Direct virus detection methods, such as culture, protein/component detection, and RT-PCR techniques, can be costly when used for understanding disease transmission and severity. In contrast, immunological assays like ELISAs (Enzyme-Linked Immunosorbent Assays), CLIAS (Chemiluminescent Immunoassays), and LFAs (Lateral Flow Assays) are frequently employed in studying virus epidemiology and factors contributing to viral spread. These immunological assays provide a more cost-effective alternative for large-scale epidemiological studies [24].

Virus detection encompasses the direct identification of the virus or its components, which can be achieved through methods like culture, protein/component detection, or RT-PCR techniques. Virus neutralization tests are used to identify neutralizing antibodies, which are valuable for assay validation and research purposes. ELISA assays have been found to exhibit a strong correlation between antibody titers and the presence of virus-neutralizing antibodies. This makes ELISA a valuable tool in studying immune responses to viruses [25].

Certainly, in addition to the mentioned assays, numerous other diagnostic tests and assays have been developed. For more detailed information, it's recommended to refer to summaries and

resources available in relevant literature or publications [26].

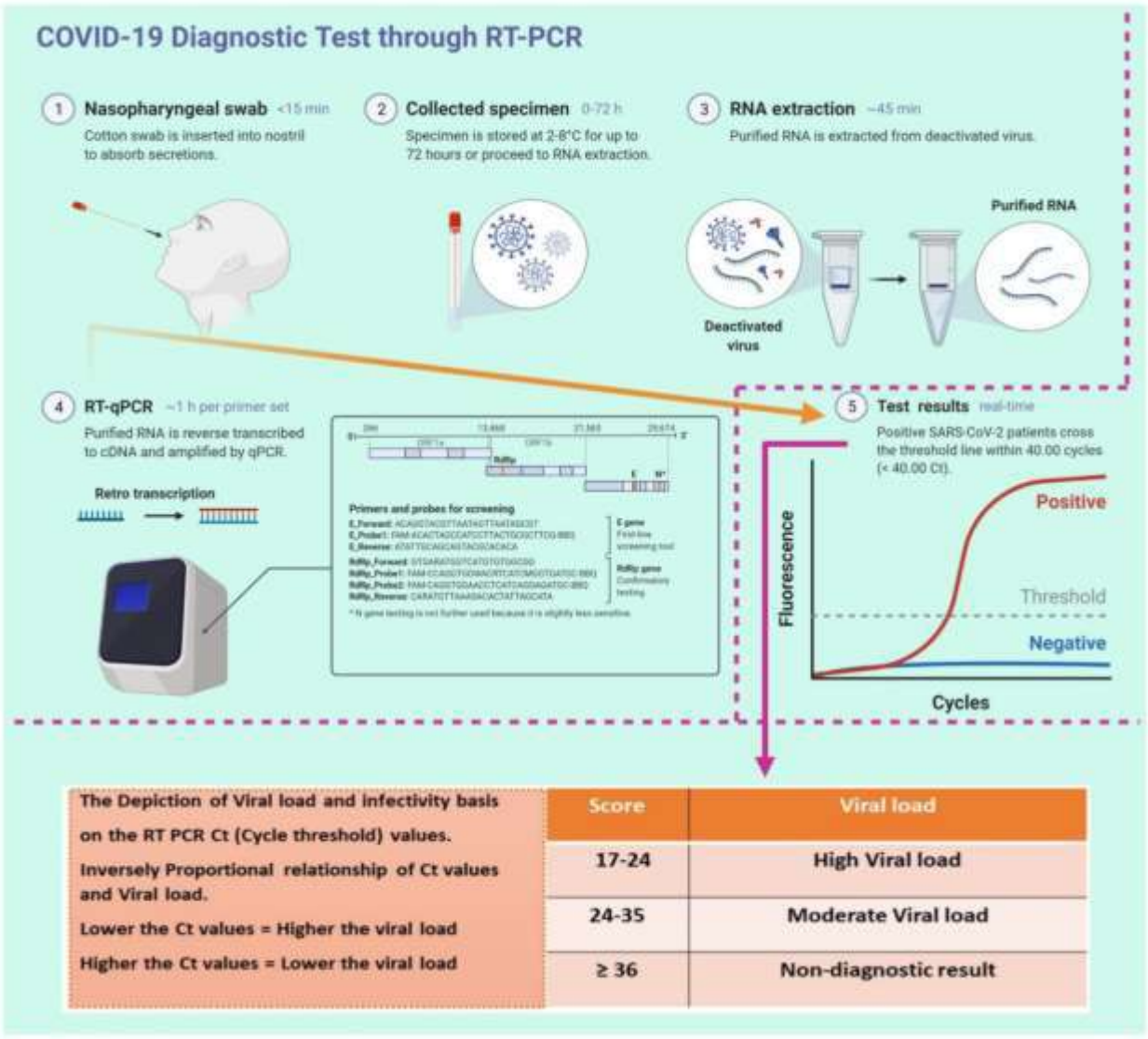

Figure 1: Covid-19 Diagnostic Test through RT-PCR

Diagnostics for COVID-19

Depending on factors like age, immune responses, and any underlying health conditions, infection with SARS-CoV-2 can result in highly varied responses across individuals. This spectrum ranges from asymptomatic cases to individuals displaying a wide array of symptoms. Young and healthy individuals often experience no or mild symptoms, yet they can unknowingly spread the virus, leading to covert infections. On the other hand, severe cases of COVID-19 can lead to hospitalization, with some patients requiring assistance from mechanical ventilation. Unfortunately, in certain instances, the disease can be fatal [27-28].

Diagnostic Confirmation

RT-PCR (Reverse Transcription Polymerase Chain Reaction) is frequently employed to confirm suspected cases of COVID-19, particularly when rapid tests produce inconclusive or negative results. It is considered a highly reliable method for detecting the presence of the SARS-CoV-2 virus in a sample.

Preanalytical Issues Affecting the Diagnosis of COVID-19:-

Indeed, it is widely recognized that pre-analytical factors play a significant role in influencing the accuracy and reliability of laboratory testing. These factors encompass various steps and conditions before the actual analysis of a sample takes place, and they are crucial in ensuring the validity of test results [29]. An accurate overview of the pre-analytical factors that can significantly impact the accuracy of laboratory testing. These factors include issues such as improper collection of biological material, inadequate sample storage or transportation, pipetting errors, contamination, and sample mismatch. Among these, sample contamination is particularly critical, as even tiny amounts of foreign nucleic acids can compromise the accuracy of RT-PCR assays. Additionally, using correct procedures for collecting nasopharyngeal specimens is crucial. This involves inserting the swab deep into the nostril parallel to the palate to reach the target area, allowing for secretion absorption, and promptly placing the swab into a sterile tube. Following these procedures diligently is vital for obtaining reliable test results [30]. Failure to adhere to the recommended pre-analytical practices can lead to various diagnostic errors. These errors can range from inaccurate test results to false positives or negatives, potentially leading to incorrect diagnoses and subsequent treatment decisions. It underscores the critical importance of strict adherence to established protocols in sample collection, handling, and storage for accurate and reliable laboratory testing [31].

Quantitative Analysis

RT-PCR not only identifies the presence of the virus but can also quantify the viral load. This information is crucial for gaining insights into the development of the infection and for assessing the efficacy of treatments. Monitoring changes in viral load over time can be a valuable tool in managing and tailoring treatment strategies for individuals with COVID-19.

Screening and Surveillance

RT-PCR plays a pivotal role in large-scale screening programs and in monitoring the prevalence of the virus within a population. This data is of utmost importance for public health interventions and for informing policy decisions. It helps authorities gauge the spread of the virus, identify hotspots, and implement targeted measures to mitigate transmission. Additionally, it provides valuable information for planning and adjusting healthcare resources and responses to the ongoing pandemic.

Time Dependency and False-Negative Results

The kinetics of viral load, or the pattern of how the amount of virus changes over time, is a variable factor influenced by several elements. This includes an individual's epidemiological history, their immune response, and any treatments or medications they may be receiving. These factors collectively contribute to the unique progression of viral load in different individuals, making it an important consideration in understanding and managing the course of infection [32]. It is another factor that can contribute to false-negative results.

A false-negative case of SARS-CoV-2 infection occurs when an individual, initially suspected of being infected, receives a negative result from the RT-PCR test, but subsequently tests positive on a follow-up test. This phenomenon garnered significant attention, particularly in hospitalized patients during the early stages of the COVID-19 pandemic, especially when clinical and radiographic findings conflicted with negative test results. These false-negative results can often be attributed to the dynamic nature of viral load over time. Viral RNA is typically undetectable during the first two weeks after infection, a period when the patient is usually asymptomatic, as well as two to four weeks after the onset of clinical symptoms. Furthermore, viral RNA load tends to decrease rapidly in the second and third weeks following symptom onset. This underscores the importance

of timing in testing for the virus, as there is a limited window during which it is detectable. Tests conducted outside this window may yield false-negative results [33].

In a literature review and pooled analysis, Kucirka et al. [34] analyzed the rate of false-negative RT-PCR performed on nasopharyngeal swabs of symptomatic patients, with respect to the timing of symptom onset. The probability of a false-negative result decreased from 100% on day 1 to 67% (CI, 27–94%) on day 4. On the day with onset of symptoms the probability of a false-negative rate was 38% and then decreased to 20% 3 days after symptom onset), while, during the asymptomatic stage (1–4 days), the false-negative rate ranged from 100 to 94%.

Overall, the sensitivity of RT-PCR testing is therefore severely limited. The sensitivity in identifying SARS-CoV-2 bearing individuals by RT-PCR test ranges from 44% to 80% [35], as being significantly influenced by viral shedding and by the time of sample collection when compared to the onset of the infection, as already observed for other corona viruses [36]. Exactly, due to the dynamics of viral load and the timing of testing, it's highly likely that a significant number of mild, asymptomatic, or individuals in the pre-symptomatic phase of COVID-19 may not be identified through current testing efforts. This is a critical consideration in the broader context of understanding and managing the spread of the virus. It emphasizes the need for a multi-faceted approach to control and monitor the pandemic, which includes not only testing but also measures like contact tracing, vaccination, and public health interventions [37].

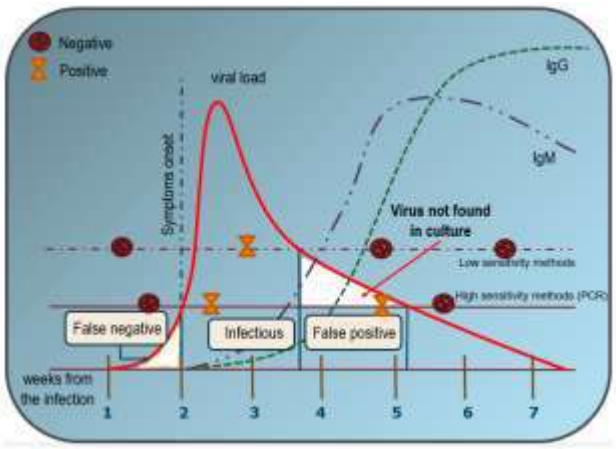

Figure 2: Analytical Performance of Covid-19 Virus Detection with RT-PCR Test

Variant Detection

RT-PCR can be customized to target specific genetic markers associated with different variants of the virus. This capability is vital for monitoring the evolution of the virus and gaining insights into its dissemination. By identifying specific genetic signatures unique to various viral strains, scientists can track the prevalence and distribution of different variants, which is instrumental in shaping public health responses and vaccine development strategies.

Research and Vaccine Development

RT-PCR plays a fundamental role in the research and development of vaccines and antiviral drugs. It enables scientists to evaluate vaccine efficacy by measuring viral load reduction in vaccinated individuals. Additionally, it aids in understanding the immune response triggered by vaccines. This information is crucial for advancing vaccine development efforts and ensuring their effectiveness in controlling the spread of the virus. Similarly, in the development of antiviral drugs, RT-PCR is used to assess the drug's ability to inhibit viral replication, providing valuable data for drug testing and refinement.

Limits of Current RT-PCR Tests

Reports have indicated that RT-PCR tests for SARS-CoV-2 may have varying levels of diagnostic accuracy. Factors such as proper primer

and probe design, technical proficiency in conducting the test, and the absence of third-party validation can all contribute to potential inaccuracies. It's essential to ensure that these tests are rigorously developed, validated, and performed by trained professionals to obtain reliable results in diagnosing COVID-19 cases [38]. The Emergency Use Authorization (EUA) and Instructions for Use (IFU) documents provide guidelines for currently approved virology tests for SARS-CoV-2. However, it's worth noting that these tests can vary significantly in their design and methodology. This lack of standardization can present challenges in interpreting and comparing results across different testing platforms and laboratories. Therefore, it's crucial for healthcare professionals to be well-informed about the specific characteristics and performance metrics of the tests they use [39].

The data from the FDA on Emergency Use Authorization (EUA) SARS-CoV-2 virology tests does indeed indicate a notable variation in the limits of detection (LoDs) among different assays. Tests with higher LoDs are more likely to miss detecting infected individuals, which in turn increases the risk of false-negative results. This highlights the significance of understanding the sensitivity and specificity of different testing methods, as well as the potential impact on diagnostic accuracy. It underscores the importance of selecting and utilizing tests with appropriate sensitivity for the specific clinical context and population being tested [40].

Various primer-probe sets have been created, and they exhibit high specificity in differentiating samples that are positive for SARS-CoV-2 from those positive for other respiratory viruses. Moreover, these assays demonstrate high sensitivity, meaning they are effective at accurately detecting the presence of SARS-CoV-2 in samples. This balance of high specificity and sensitivity is crucial in ensuring accurate and reliable diagnostic results [41].

Variations in primer concentrations and DNA probe lengths, which may not adhere strictly to FDA guidelines, could potentially lead to false positive results in SARS-CoV-2 detection assays. It underscores the importance of adhering to standardized protocols and ensuring that testing procedures are meticulously followed to maintain the accuracy and reliability of results. This also highlights the need for thorough validation and quality control measures in diagnostic testing processes [42].

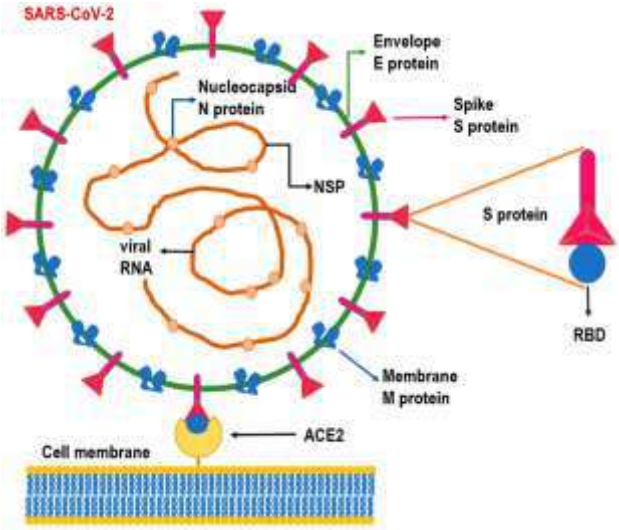

Figure 3: Structure of Corona Virus

MATERIALS AND METHODS

RNA isolation from clinical samples. Clinical samples, including nasopharyngeal aspirates (NPAs) (n = 131) and stool specimens (n = 5), were provided by the Department of Microbiology, The University of Hong Kong (HKU). In addition, tracheal dispersion fluid and lung biopsies from index patient A, who was described previously, were also collected at three time points. Sample collection was conducted from 1 April to 28 April 2003 in local hospitals by a previously described procedure. Collected samples were stored at 4°C prior to RNA extraction, and viral RNAs were extracted within a few days of arrival. Briefly, total RNA extraction

from clinical samples was performed with the SV Total RNA isolation system and the SV96 Total RNA isolation system (Promega, Madison, Wis.), with the following modifications to the manufacturer's protocol. Five hundred microliters of an NPA or stool sample in viral transport medium (containing, per litre, 2 g of sodium bicarbonate, 5 g of bovine serum albumin, 200 µg of vancomycin, 18 µg of amikacin, and 160 U of nystatin in Earle's balanced salt solution) was mixed with an equal volume of SV RNA lysis buffer containing 100 µl of pig kidney epithelial (PK-15) cells (ATCC CCL-33; 5.0 × 105 cell/ml) in complete minimum essential medium with Earle's salt (Invitrogen) as an internal control. The mixture was transferred to the wells of an SV96 binding plate or an individual binding tube for the SV RNA isolation system. After being washed with 500 µl of SV RNA wash solution prior to the elution step, the plate or tube was spun at 3,000 × g for 30 s to remove any residual wash solution. The RNA was then eluted with 50 µl of nuclease-free water and was collected in a clean 96-well PCR plate or 1.5-ml micro centrifuge tube by spinning of the plate or tube at 3,000 × g for 1 min. The eluted RNA was then concentrated by incubation on ice for 15 min in the presence of 5 µl of 3 M sodium acetate and 200 µl of 95% ethanol. After centrifugation at 3,000 × g at 4°C for 15 min, the RNA pellet was washed with 200 µl of 75% ethanol and dissolved with 12 µl of nuclease-free water. Extracted RNAs were immediately reverse transcribed to first-strand cDNAs [43-44].

ELISA Immunoassay

The ELISA method for detecting IgM and IgG antibodies has demonstrated high specificity and sensitivity in identifying COVID-19 thus far. ELISA, short for enzyme-linked immunosorbent assay, involves binding enzymes to specific antibodies to identify different proteins and pathogens within the system. This system is versatile, capable of detecting either the COVID-19 viral antigen or the antibodies produced against COVID-19 within the host body. It utilizes micro titer plates with 96 wells, where the antibody is immobilized on the surface. The sample containing the specific analyte (such as virus, proteins, or antigens) is introduced, allowing the fixed antibody-analyte conjugate to be recognized by an enzyme-tagged antibody in the presence of a specific substrate. This interaction generates a visible indication, such as color, fluorescence, or luminescence, allowing for easy identification [45]. For COVID-19 antibody detection, a similar process is employed. Companies like Bio-Rad and Euroimmun US Inc have already created ELISA-based techniques for diagnosing COVID-19 infection by detecting IgG and IgM antibodies [46-47].

CONCLUSION

In conclusion, this review underscores the paramount importance of Reverse Transcription Polymerase Chain Reaction (RT-PCR) in the detection and management of corona viruses, particularly in the context of the unprecedented COVID-19 pandemic. RT-PCR has proven to be a highly sensitive and specific diagnostic tool, enabling early identification and isolation of infected individuals. Its quantitative capabilities have furthered our understanding of infection dynamics and facilitated timely therapeutic interventions.

Beyond clinical applications, RT-PCR has played a pivotal role in population-wide screening and surveillance efforts, providing crucial data for public health decision-making. Its adaptability for variant detection has been instrumental in tracking viral evolution and assessing the impact of emerging strains. Additionally, RT-PCR's contribution to vaccine development and evaluation cannot be overstated, providing critical insights into immune response and vaccine efficacy.

However, it is essential to acknowledge the challenges associated with RT-PCR, including the need for skilled personnel, adequate resources, and potential issues related to false negatives or positives. Moreover, as new technologies and approaches continue to emerge, ongoing research and development are vital for refining and expanding our diagnostic arsenal.

In light of the ongoing battle against COVID-19 and the potential for future viral threats, a comprehensive understanding of RT-PCR's capabilities and limitations remains crucial. Continued investment in research, infrastructure, and training will ensure that RT-PCR continues to be a cornerstone in our global efforts to detect and control corona viruses.

REFERENCES

1. World Health Organization (WHO). WHO Director-General's opening remarks at the media briefing on COVID-19. 2020 Mar 11 [internet]. Geneva: [cited 2020 Oct 30]. Available from:https://www.who.int/director-general/speeches/detail/who-director-general-s-opening- remarks-at-the-media-briefing-on-covid-19---11-march-2020
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382:727-733. doi: https://doi.org/10.1056/NEJMoa2001017
3. Liu J, Liao X, Qian S, Yuan J, Wang F, Liu Y et al. Community transmission of severe acute respiratory syndrome coronavirus 2, Shenzhen, China, 2020. Emerg Infect Dis. 2020 Jun;26(6):1320-1323. doi: https://doi.org/10.3201/eid2606.20023.
4. Zhang R, Li Y, Zhang AL, Wang Y and Molina MJ. Identifying airborne transmission as the dominant route for the spread of COVID-19. PNAS. 2020 June 30;117(26)14857-14863.doi: https://doi.org/10.1073/pnas.2009637117
5. Doremalen NV, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med. 2020:382:1564-1567. doi: https://doi.org/10.1056/NEJMc2004973
6. Chin AWH, Chu JTS, Perera MRA, Hui KPY, Yen HL, Chan MCW, et al. Stability of SARS-CoV-2 in different environmental conditions. Lancet. 2020;1:E10. doi: https://doi.org/10.1016/S2666-5247(20)30003-3.
7. Ong SW, Tan YK, Chia PY, Lee TH, Ng OT, Wong MSY, et al. Air, surface. environmental and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARSCOV-2) from a symptomatic patient. JAMA. 2020 Apr 28;323(16):1610-1612. doi: https://doi.org/10.1001/jama.2020.3227
8. Nicas M and Best D. A study quantifying the hand-to-face contact rate and its potential application to predicting respiratory tract infection. J Occup Environ Hygiene. 2008;5:347-352.doi: https://doi.org/10.1080/15459620802003896
9. Macias AE, Torre A, Moreno-Espinosa S, Leal PE, Bourlon MT and Ruiz-Palacios GM. Controlling the novel A (H1N1) influenza virus: don't touch your face! Journal of Hospital Infection. 2009 Nov 1:73(3):280-281.doi: https://doi.org/10.1016/j.jhin.2009.06.017
10. Kwok YLA, Gralton J and McLaws M. Face touching: A frequent habit that has implications for hand hygiene. Am J of Infection Control. 2015 Feb 01:43(2):112-114.doi: https://doi.org/10.1016/j.ajic.2014.10.015
11. COVID-19: Government issues advisory asking people to wear 'homemade face covers. The New Indian Express. 2020 April 04

[internet]. New Delhi: [cited 2020 Oct 30]. Available from: https://www.newindianexpress.com/nation/2020/apr/04/covid-19-government-issues-advisory-asking-people-to-wear-homemade-face-covers-2125687.amp

12. Centers for Disease Control and Prevention (CDC). How to Protect Yourself and Others. Updated 2020 Nov 4. [internet]. Atlanta: [cited 2020 Nov 30]. Available from https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/prevention.html
13. Chen Y, Qin G, Chen J. Xu J. Feng D, Wu X, et al. Comparison of Face-Touching Behaviors Before and During the Coronavirus Disease 2019 Pandemic. JAMA Netw Open. 2020;3(7):e2016924. doi: https://doi.org/10.1001/jamanetworkopen.2020.16924
14. Sun CB, Wang YY, Liu G and Liu Z. Role of the Eye in Transmitting Human Coronavirus: What We Know and What We Do Not Know. Front Public Health 2020 Apr 24:8:155. doi: https://doi.org/10.3389/fpubh.2020.00155
15. Ni W, Yang X, Yang D, Bao J, Li R, Xiao Y, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care. 2020 Jul 13;24:422. doi: https://doi.org/10.1186/s13054-020-03120-0
16. Temin HM. 1976. Howard M. Temin-biographical. In Le Prix Nobel en 1975, ed. W Odelberg Stockholm: Nobel Found. http://www.nobelprize.org/nobel prizes/medicine/laureates/1975/ temin-bio.html
17. Stent G. 2007. Phage and the Origins of Molecular Biology. Cold Spring Harbor, NY: Cold Spring Harbor Lah. Press
18. Dulbecco R, Vogt M. 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med. 99:167-82
19. Dulbecco R. 1964. Transformation of cells in vitro by DNA-containing viruses. JAMA 190:721-26
20. Baltimore D, Eggers HJ, Franklin RM, Tamm 1. 1963. Poliovirus-induced RNA polymerase and the effects of virus-specific inhibitors on its production. PNAS 49:843-49
21. Baltimore D. 1964. In vitro synthesis of viral RNA by the poliovirus RNA polymerase. PNAS 51:450-56
22. Vandenberg, O: Martiny, D; Rochas. O van Belkum, A; Kozlakidis, Z. Considerations for diagnostic COVID-19 tests. Nat. Rev. Microbiol.2020, 14, 1-13.
23. Gerbalenya, A E.; Baker, SC.; Baric, R.S., de Groot, R.J. Drosten, C.; Gulyaeva, A.A.; Haagman, BL; Lauber, C.;Lentowich, A.M.; Neuman, B.W., et al. The species Severe acute respiratory syndrome- related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. Nat. Microbiol. 2020, 5.
24. Yan, Y. Chang, L., Wang, L. Laboratory testing of SARS-CoV. MERS-CoV, and SARS-CoV-2 (2019-CoV): Current status challenges, and countermeasures. Rev. Mod. Viral 2020, 30, e2106.
25. Padoan, A., Bonfante, F: Pagliari, M.; Bortolami, A.; Negrini, D.; Zuin, S.; Bozzato, D: Cosma, C., Sciacovelli, L.; Plebani, M. Analytical and clinical performances of five immunoassays for the detection of SARS-CoV-2 antibodies in comparison with neutralization activity. ElioMedicine 2020, 62, 103101.
26. Sheridan, C. Coronavirus and the race to distribute reliable diagnostics. Nat. Esotecional, 2020. 38, 382-384.
27. Oran D.P., Topol E.J. Prevalence of Asymptomatic SARS-CoV-2 Infection: A Narrative Review. Ann. Intern. Med. 2020;173:362–367. doi: 10.7326/M20-3012.

[PMC free article] [PubMed] [CrossRef] [Google Scholar]

28. Richardson S., Hirsch J.S., Narasimhan M., Crawford J.M., McGinn T., Davidson K.W., The Northwell COVID-19 Research Consortium. Barnaby D.P., Becker L.B., Chelico J.D., et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. JAMA. 2020;323:2052–2059. doi: 10.1001/jama.2020.6775. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
29. Lippi, G.; Simundic, A.M.; Plebani, M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin. Chem. Lab. Med. 2020, 58, 1070–1076.
30. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19.
31. Irving, S.A.; Vandermause, M.F.; Shay, D.K.; Belongia, E.A. Comparison of nasal and nasopharyngeal swabs for influenza detection in adults. Clin. Med. Res. 2012, 10, 215–218.
32. Kim, J.Y.; Ko, J.H.; Kim, Y.; Kim, Y.J.; Kim, J.M.; Chung, Y.S.; Kim, H.M.; Han, M.G.; Kim, S.Y.; Chin, B.S. Viral Load Kinetics of SARS-CoV-2 Infection in First Two Patients in Korea. J. Korean Med. Sci. 2020, 35, e86.
33. Sethuraman, N.; Jeremiah, S.S.; Ryo, A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA 2020, 323, 2249–2251.
34. Kucirka, L.M.; Lauer, S.A.; Laeyendecker, O.; Boon, D.; Lessler, J. Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure. Ann. Intern. Med. 2020, 173, 262–267.
35. Poon, L.L.; Chan, K.H.; Wong, O.K.; Yam, W.C.; Yuen, K.Y.; Guan, Y.; Lo, Y.M.; Peiris, J.S. Early diagnosis of SARS coronavirus infection by real time RT-PCR. J. Clin. Virol. 2003, 28, 233–238.
36. de Sousa, R.; Reusken, C.; Koopmans, M. MERS coronavirus: Data gaps for laboratory preparedness. J. Clin. Virol. 2014, 59, 4–11.
37. Kobayashi, T.; Jung, S.M.; Linton, N.M.; Kinoshita, R.; Hayashi, K.; Miyama, T.; Anzai, A.; Yang, Y.; Yuan, B.; Akhmetzhanov, A.R.; et al. Communicating the Risk of Death from Novel Coronavirus Disease (COVID-19). J. Clin. Med. 2020, 9, 580.
38. Loeffelholz, M.J.; Tang, Y.W. Laboratory diagnosis of emerging human coronavirus infections-The state of the art. Emerg Microbes Infect. 2020, 9, 747-756.
39. MacKay, M.J., Hooker, AC Afshinnekoa, E., Salit, M., Kelly, J.; Feldstein, J.V., Haft, N., Schenkel, D. Nambi, S., Cai, Y., et al. The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing. Nat. Biotechnol. 2020, 38, 1021-1024.
40. Amaout, R. Lee, R.A., Lee, G.R., Callahan, C. Yen, C.F. Senith, K.P, Arora, R., Kirby, JE. SARS-CoV2 Testing: The Limit of Detection Matters. bioRxiv 2020.
41. Nalla, A.K., Caste, A.M.; Huang, M.W.; Perchetti, G.A.; Sampoleo, R., Shrestha, L.; Wei, Y.; Zhu, H.; Jerome, K.R.; Greninger, A.L Comparative Performance of SARS-CoV-2 Detection Assays Using Seven Different Primer-Probe Sets and One Assay Kit. J. Clin. Microbial. 2020, 58, 00557-20.
42. Lee, S.H. Testing for SARS-CoV-2 in cellular components by routine nested RT-PCR followed by DNA sequencing, Int. J. Geriatr. Rehabil. 2020, 2, 69-96.
43. Drosten, C., S. Günther, W. Presier, S. Werf, H. Brodt, S. Becker, et al. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. 348:1967-1976. [PubMed] [Google Scholar]

44. Poon, L., O. Wong, W. Luk, K. Yuen, J. Peiris, and Y. Guan. 2003. Rapid diagnosis of a coronavirus associated with severe acute respiratory syndrome (SARS). Clin. Chem. 49:953-955. [PMC free article] [PubMed] [Google Scholar]
45. Dutta G., Kim S., Park S., Yang H. Washing-free heterogeneous immunosensor using proximity-dependent electron mediation between an enzyme label and an electrode. Anal. Chem. 2014;86:4589. doi: 10.1021/ac5006487. [PubMed] [CrossRef] [Google Scholar]
46. Dutta G., Park S., Singh A., Seo J., Kim S., Yang H. Low-interference washing-free electrochemical immunosensor using glycerol-3-phosphate dehydrogenase as an enzyme label. Anal. Chem. 2015;87:3574. doi: 10.1021/ac504485a. [PubMed] [CrossRef] [Google Scholar]
47. Li Z., Yi Y., Luo X., Xiong N., Liu Y., Li S., Chen L., Ye F. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J. Med. Virol. 2020;92:1518–1524. doi: 10.1002/jmv.25727. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

HOW TO CITE: S. Agrawal*, B. Potdar, J. Shaikh, K. Pimpale, L. Kochage, N. Chougule, Detection of Corona Virus: A Review on Applications of RT-PCR in Detection of Corona Virus (Covid-19), Int. J. in Pharm. Sci., 2023, Vol 1, Issue 11, 260-271. https://doi.org/10.5281/zenodo.10124285