ISSN 2515-8260 Volume 09, Issue 02, 2022

Assessment of validity of RT-PCR tests in the clinical practice

[1]Dr. Subodh Kumar, [2]Dr. Matin Ahmad Khan, [3]Dr. Mukunda Kumar, [4]Dr. Ruby Kumari

[1]Associate Professor, Department of Biochemistry, Laxmi Chandravanshi Medical College and Hospital, Bishrampur, Palamu, Jharkhand, India
[2]Professor, Department of Biochemistry, Laxmi Chandravanshi Medical College and Hospital, Bishrampur, Palamu, Palamu, Jharkhand, India
[3]Assistant Professor, Department of Biochemistry, Laxmi Chandravanshi Medical College and Hospital, Bishrampur, Palamu, Palamu, Jharkhand, India
[4]Tutor, Department of Biochemistry, Laxmi Chandravanshi Medical College and Hospital, Bishrampur, Palamu, Palamu, Jharkhand, India

Corresponding Author:
Dr. Subodh Kumar (somya2011.dsk@gmail.com)

Abstract

Outbreak of pandemic SARS-CoV2 in 2020 collapse the healthcare management system all around the world. People were scared, as invisible virus spreading its infection among the individual who were close contact with infected person via droplet nuclei or aerosol transmission. The only gold standard test for early detection of viral genome that time was RT-PCR tests which was due to its high sensitivity and specificity nature. During Covid period RT-PCR sensitivity was reported much less all around the world. Hence there is urgent need to find out the reason for less sensitivity or fallacy report of RT-PCR or developed alternative method for SARS-CoV-2 and its strain detection method in less time with High efficacy.

Keywords: RT-PCR Test Validity, COVID strains, Clinical practice, Covid appropriate behaviour

Introduction

Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR) is the gold standard test which play significant role in early detection of viral genome SARS-CoV-2 during pandemic. A positive test enables the clinicians and public health professionals to quickly isolate the patient and prevent spread of the disease. Though RT-PCR test is gold standard in detection of virus due to its high sensitivity as claimed by manufacturer even then Fallacy report of RT-PCR was observed during Covid pandemic period and its sensitivity was reported much less up to 50 to 70 percent worldwide, which attract attention of scientific community to find out the cause of low sensitivity of RT-PCR or developed an alternative method for detection of virus at minimum time with high accuracy and high sensitivity. Early studies from the United States [1] and China [2] reported that many cases were asymptomatic, based on the lack of symptoms at the time of testing. However, 75-100% of these people later developed symptoms. The present study mainly focused on lacune faced during pandemic

ISSN 2515-8260 Volume 09, Issue 02, 2022

Covid in diagnosis, sampling, screening and implementing COVID 19 guideline issued by WHO and National Centre for Disease Control (NCDC), Delhi.

Review of literature

Many of the RT-PCR assays have a 100% sensitivity in this analysis as reported by the manufacturers but its sensitivity was observed in real world clinical practice during detection of SARS-CoV-2 genome is only 50 to 70% [3, 4]. In actual use, the clinical sensitivity and specificity of many of these tests is lower in part because of issues surrounding sample collection, handling, and analysis. Much literature has been written about the issue of false negative RT-PCR tests in symptomatic, pre-symptomatic, and asymptomatic persons infected with the virus [5, 6]. Only few literatures has been published about the problem of false positive RT-PCR or other NAAT tests.

Table 1: General feature about Corona virus

Mode of Transmission of SARS CoV-2	Contact with infected person, droplet (Respiratory droplet >5-10 μm in diameter whereas droplets ≤5μm in diameter referred as droplet nuclei or aerosols), airborne, fomite (Respiratory secretions or droplets expelled by infected individuals can contaminate surfaces and objects, creating fomites (contaminated surfaces), fecal-oral, blood borne, mother-to-child, and animal-to-human transmission.
Incubation period of COVID-19	Is on average 5-6 days, but can be as long as 14 days.
Breaking chain of SARS-CoV-2	By means of testing, tracking and treatment, follow Covid appropriate behaviour (proper masking, Sanitization and physical distancing), Natural immunity, vaccination.
New variant/strain of Corona virus	**i) SARS-CoV-2 (Covid 19):** Responsible for 1st wave. **ii) Delta variant:** Responsible for 2nd wave. Having 18 mutation in spike protein. **iii) Omicron variant:** Designated as B.1.1.529, was first reported to WHO on 24 November 2021. Responsible for 3rd wave. Having more than 30 mutations in spike proteins. On the ACE2 receptor-the proteins that helps to create an entry point for the corona virus to infect immune cell. It has 10 mutations in comparison to the delta variants (only 2) Due to highly mutation, it is Immune to the vaccine induced-antibodies.

Table 2: Causes of False Positive SARS-CoV-2 RT-PCR Results [7-10]

i) ii)	Contamination during sampling (an infected worker or surface; Aerosolization of virus during Collection [9]
iii)	Primer and Probe contamination
iv)	Extraction (e.g., Aerosolization in contaminated hood)
v)	PCR amplification
vi)	Production of Lab Reagent (e.g., manufactures of the positive control may have contaminated other reagents produced in the same facility, contamination of other consumables.
vii)	Contamination of the equipment by high viral titre specimen (e.g., sample carryover) [10]
viii)	Cross-reaction with other viruses (e.g., another coronavirus)
ix)	Sample mix-ups
x)	Software problems
xi)	Data entry or transmission errors
xii)	Nonspecific reaction [9]
xiii)	Variation in parameters around the LOD (Limit of Detection) definition of an indeterminate results [10]

Table 3: Modern Techniques and Test used in COVID detection

Real Time RT-qPCR	A method of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome detection based on measuring the amplification of RdRP, E, N or S gene fragments using fluorescent probes. Uses respiratory tract sample
Multiplex real-time PCR diagnostic kit	The Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used real time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (c DNA) and nucleic acid amplification technology.
NAATs (Nucleic acid amplification test)	Detect viral genetic material (nucleic acid). Uses respiratory tract sample
Next-generation sequencing (NGS)	These technologies allow for sequencing of DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing, and as such revolutionised the study of genomics and molecular biology.
Isothermal nucleic acid amplification assays	These assays use various nucleic acid amplification reactions that are conducted at a constant temperature. They generate large amounts of cDNA that can be detected by colorimetric or turbidimetric approaches.
Antigen Rapid diagnostic test	Detect spike protein /nucleocapsid protein present on surface of the virus. Uses respiratory tract sample
Serological test	Detect human antibodies (IgM and/or IgG) against the SARS-CoV-2 virus, which is produced by the humoral immune response. Uses blood as a sample

Modern Techniques and Test used in COVID Detection: Real Time RT-qPCR, Nucleic acid amplification test (NAATs), Multiplex real-time PCR diagnostic kit, Next generation sequencing (NGS), Isothermal nucleic acid amplification assays, Antigen test and serological test (Table 3). The most widely utilized Nucleic Acid Amplification Test (NAAT) to detect SARS-CoV-2 RNA is the reverse transcriptase-polymerase chain reaction (RT-PCR) test, manufactured by many companies targeting one or more genomic regions of the virus. Next-generation sequencing (NGS) was instrumental in the identification and assembly of the SARS-CoV-2 genome [11] and can also be used for high-throughput testing to identify mutations in the viral genome. As only a small amount of sequencing is required to detect amplified viral complementary DNA (cDNA), thousands of patient samples can be tested in a single sequencing run, which facilitates very large-scale testing. Each sample is labelled with a unique molecular barcode that can be identified in the output library. Such barcoding can occur during initial reverse transcription, cDNA amplification or library preparation. The samples are then pooled and undergo multiplexed sequencing, an advance specific to large-scale testing [12] Emerging findings indicate that NGS can distinguish between different SARS-CoV-2 variant strains and can also detect other respiratory viruses (such as influenza viruses) and synthetic RNA internal controls [13]. Rapid RT-PCR tests appear to perform comparably to standard laboratory-based NAAT, but rapid isothermal tests may be less sensitive. The WHO, and an international consortium of experts have addressed these issues and have produced a checklist for laboratories to reduce possible causes of false-positive RT-PCR results and how to handle equivocal results [14]. Timely and reliable laboratory diagnosis is crucial for clinical care and to inform public health responses in the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic [15]. Indeed, the sensitivity of tests for detecting SARS-CoV-2 can be as dependent on the time and site sampled as it is on the technical performance of the assay. Low specificity is particularly problematic for large-scale testing schemes, as it can result in overwhelming absolute numbers of false positives. Among nucleic acid tests, the polymerase chain reaction (PCR) method is considered as the 'gold standard' for the detection of some viruses and is characterized by rapid detection, high sensitivity and specificity. As such, real-time reverse transcriptase-PCR (RT-PCR) is of great

ISSN 2515-8260 Volume 09, Issue 02, 2022

interest today for the detection of SARS-CoV-2 due to its benefits as a specific and simple qualitative assay [16]. An important issue with the real-time RT-PCR test is the risk of eliciting false-negative and false-positive results. It is reported that many 'suspected' cases with typical clinical characteristics of COVID-19 and identical specific computed tomography (CT) images were not diagnosed [17]. Thus, a negative result does not exclude the possibility of COVID-19 infection and should not be used as the only criterion for treatment or patient management decisions. It seems that combination of real-time RT-PCR and clinical features facilitates management of SARS-CoV-2 outbreak. Several factors have been proposed to be associated with the inconsistency of real-time RT-PCR [18]. Here the attempt had been made to discuss various challenges regarding the detection of SARS-CoV-2 by real-time RT-PCR. It is expected that this could provide beneficial information for the comprehension of the limitations of the obtained results and to improve diagnosis approaches and control of the disease. Finally, the Centres for Disease Control and Prevention (CDC) has designed a SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel to minimize the chance of false-positive results. In accordance, the negative template control (NTC) sample should be negative, showing no fluorescence growth curves that cross the threshold line. The occurrence of false positive with one or more of the primer and probe NTC reactions is indicative of sample contamination. Importantly, the internal control should be included to help identify the specimen containing substances that may interfere with the extraction of nucleic acid and PCR amplification. Because of the several risks to patients in the event of a false-positive result, all clinical laboratories using this test must follow the standard confirmatory testing and reporting guidelines based on their proper public health authorities.

Discussion & Results

Before testing the sample of infected person, internal as well as external quality control must be maintained to reduce the chances of contamination. General feature about Corona virus, Cause of False Positive SARS-CoV-2 RT-PCR Results and various screening method for Covid detection were shown in Table 1,2 & 3 respectively [19-22] The overall accuracy of a RT-PCR test is based upon its sensitivity representing the ability to detect infected individuals and the specificity, which is the percentage of uninfected people who test negative. In practice, variables such as sampling and processing errors decrease real-world sensitivity or True Positive rate (TPR) [23] Low specificity is particularly problematic for large-scale testing schemes, as it can result in overwhelming absolute numbers of false positives.

Expert opinion

The results of real-time RT-PCR tests must be cautiously interpreted. In the case of real-time RT-PCR negative result with clinical features suspicion for COVID-19, especially when only upper respiratory tract samples were tested, multiple sample types in different time points, including from the lower respiratory tract, if possible, should be tested. Importantly, combination of real-time RT-PCR and clinical features especially CT image could facilitate disease management. Proper sampling procedures, good laboratory practice standard, and using high-quality extraction and real-time RT-PCR kit could improve the approach and reduce inaccurate results. ICMR has discussed the issue of correlating COVID-19 disease severity with Ct values and accordingly deciding on patient management protocol, with a panel of esteemed laboratory experts and suggest that it is not recommended to rely on numerical Ct values for determining infectiousness of COVID-19 patients and deciding patient management protocols [21].

ISSN 2515-8260 Volume 09, Issue 02, 2022

Conclusion

On the basis of various research articles from indexed Journal, Google search and past experiences during Covid pandemic we conclude that there is urgent need to explore the ideal specific method for screening and monitoring of various samples over a long period of time and validate it. Natural Immunity is more powerful than vaccination in term of antibodies stability and generation of anamnestic response. Infected respiratory droplets /Aerosol, fomites, wind velocity, humidity, temperature, geographical distribution also play significant role in managing fallacy positive SARS CoV-2 RT-PCR report, when it is conducted in contamination free environment. Last but not the least we have to developed more specific alternative method for detection of COVID and its strain within short period of time, which must be less expensive and also it should be mandatory for all people for wear mask whenever needed to minimise the infection.

References

1. Kimball A, Hatfield KM, Arons M, James A, Taylor J, Spicer K, *et al.* Asymptomatic and presymptomatic SARS-CoV-2 infections in residents of a long-term care skilled nursing facility-King County, Washington, MMWR Surveill Summ. 2020 March;69(13):377.
2. Wang Y, Liu Y, Liu L, Wang X, Luo N, Ling L. Clinical outcome of 55 asymptomatic cases at the time of hospital admission infected with SARS-Coronavirus-2 in Shenzhen, China. J Infect Dis. 2020;221(11):1770-1774.
3. Liu, R. *et al.* Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. Clin. Chim. Acta 505, 2020, 172-175.
4. Wikramaratna P, Paton R, Ghafari M, Lourenco J. Estimating false-negative detection rate of SARS-CoV-2 by RT-PCR. Euro Surveill. 2020;25:2000568.
5. Kucirka LM, Lauer SA, 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 Int Med. 2020;173:262-267.
6. Woloshin S, Patel N, Kesselheim AS. False negative tests for SARS-CoV-2 infection-challenges and implications. N Engl J Med. 2020, 383:e38.
7. Cohen AN, Kessel B, Milgroom MG. Diagnosing SARS-CoV-2 infection: the danger of over-reliance on positive test results. False positive test results impact clinical and policy decisions (including Supplemental Material). Available at: https://www.medrxiv.org/content/10.1101/2020.04.26.20080911v4.full.pdf. Accessed December 11, 2020.
8. Mayers C, Baker K. Impact of false-positives and false-negatives in the UK's COVID-19 RT-PCR testing programme. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/895843/S0519_Impact_of_false_positives_and_negatives.pdf. Accessed December 11, 2020.
9. Skittral JP, Wilson M, Smielewska AA, *et al.* Specificity and positive predictive value of SARS-CV-2 nucleic acid amplification testing in a low-prevalence setting. Clin Microbiol Infect, 2020. 10.1016/j.cmi.2020.10.003
10. Katz AP, Civantos FJ, Sargi Z, *et al.* False-positive reverse transcriptase polymerase chain reaction screening for SARS-CoV-2 in the setting of urgent head and neck surgery and otolaryngologic emergencies during the pandemic: clinical implications. Head Neck. 2020;42:1621-1628.
11. Zhou P, *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat

origin. Nature. 2020;579:270-273.
12. Schmid-Burgk J. *et al.* LAMP-Seq: population-scale COVID-19 diagnostics using combinatorial barcoding, 2020. Preprint at bioRxiv https://doi.org/10.1101/2020.04.06.025635.
13. Bloom JS, *et al.* Swab-Seq: a high-throughput platform for massively scaled up SARS-CoV-2 testing. Preprint at medRxiv https://doi.org/10.1101/2020.08.04.20167874 (2021).
14. Huggett JF, Benes V, Bustin SA, et al. Cautionary note on contamination of reagents used for molecular detection of SARS-CoV-2. Clin Chem. 2020;66:1369-1372.
15. Reusken CB, Broberg EK, Haagmans B, Meijer A, Corman VM, Papa A, *et al.* On Behalf Of Evd-LabNet And Erli-Net. Laboratory readiness and response for novel coronavirus (2019-nCoV) in expert laboratories in 30 EU/EEA countries, January 2020. Euro Surveill. 2020; Feb;25(6):2000082.
16. Wan Z, Zhang Y, He Z, *et al.* A melting curve-based multiplex RT-qPCR assay for simultaneous detection of four human coronaviruses. Int J Mol Sci. 2016 Nov;17(11):E1880.
17. Wang Y, Kang H, Liu X, *et al.* Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. J Med Virol., 2020 Feb. [Epub ahead of print] DOI:10.1002/jmv.25721.
18. Xi M, Wei Q, Qihua F, *et al.* Understanding the influence factors in viral nucleic acid test of 2019 novel coronavirus (2019-nCoV). Chin J Lab Med. 2020;43:E002-E002.
19. World Health Organization. Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. http://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020 (Accessed on February 12, 2020).
20. CDC. Coronavirus disease 2019 (COVID-19). Testing overview; 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html. Accessed December 11, 2020.
21. ICMR; Evidence Based Advisory on Correlation of COVID-19 Disease Severity with Ct Values of the Real Time RT-PCR Test dated 05/08/2020 pg.1-2
22. Centers for Disease Control and Prevention. Coronavirus disease 2019 (COVID-19). 2020 interim case definition, approved August 5, 2020. Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance_Case_Definition-2020.1. Accessed December 11, 2020.
23. Arnaout R, *et al.* SARS-CoV2 testing: the limit of detection matters. Preprint at bioRxiv https://doi.org/10.1101/2020.06.02.131144 (2020)