"Diagnostic accuracy of Rapid Antigen Kit (RAT) and RT-PCR assay at a Tertiary Care Centre"

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Abstract:

Background: The Corona virus Disease-2019 (COVID-19) pandemics have led to significant morbidity, mortality in addition to unprecedented disruption of economic activities globally. Tests with high sensitivity and specificity are crucial for the identification and management of COVID-19 patients.

Aim: To validate the diagnostic accuracy of Rapid Antigen Detection Kit with Comparison to RT-PCR Assay.

Material and Methods: This was a cross sectional study which was carried in the Department of Microbiology, RMCH&RC for a period of 2 months i.e, in Jan to February2022. The performance of the STANDARD Q COVID-19 Ag Test for the detection of SARS-CoV-2 antigen was evaluated in comparison to RT-PCR KIT Tru PCR in 342 symptomatic patients who presented to health care facility at a tertiary care. Out of two samples taken from each patient, one sample was tested using the STANDARD Q COVID-19 antigen test and the other using RT-PCR (Tru PCR Kit).

Results: A total of 342 samples were included in our study. The Males were 209 (61.11%) and Females were 133 (38.8%). Mean age was found in 21-40 age group, and 51-60 years. Only 6.1% patients were admitted to ICU, 82.74% were IPD patients, 17.25% were OPD patients respectively. RT-PCR ct-value was found between 18-21 and 29-32 cycles. The sensitivity, specificity of the RAT was found to be 54.4%, 99.2% respectively.

Conclusions: Our study results show that the Rapid antigen test has a reasonable sensitivity, high specificity , RAT cannot replace the gold standard RT-PCR assays, they can helped us immensely in detecting and diagnosing COVID-19 at its early stage and also by large scale screening of communities residing in hot-spot areas with high incidence of disease..

Keywords: Rapid antigen, RT-PCR, Sensitivity, Specificity

Introduction

Corona virus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), has caused global health concerns since December 2019 [1, 2] India accounted for around 30 million cases and 0.4 million deaths of these numbers till June 2021 [3]. Timely and accurate diagnosis of COVID-19 is essential for limiting the spread and early clinical management of COVID-19 [4]. Real-Time quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is considered as the gold standard test for detection of Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) owing to its high sensitivity and specificity but the requirement of special equipment, long turnover time, high cost and need for skilled staff limit its use in the field settings [5,6]. A need for a rapid and less resource-intensive antigen detection assay was felt early in the course of this pandemic and multiple RAT were developed [7]. Despite having lower sensitivity and specificity than the conventional qRT-PCR, these tests still are an important tool for mitigation of COVID-19 Pandemic particularly in field/community settings [8, 9]. The World Health Organization (WHO) and several countries have released guidelines for the use of rapid antigen detection tests (RADTs) [10], [11], [12]. These tests can be performed without a trained expert or specialized instrument and interpreted within 30 min [12]. These rapid diagnostic tests are easy to perform, don't require specialized laboratory support and can easily be done at point of care. These benefits need to be balanced with the decrease in diagnostic accuracy and that needs data regarding the diagnostic accuracy of this Rapid Antigen Tests (RAT). The present study was conducted to add the purpose of estimating the diagnostic accuracy of one rapid antigen diagnostic kit in comparison to RT-PCR test.

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Material and Methods

This was a cross sectional study which was carried in the Department of Microbiology, RMCH&RC for a period of 2 months i.e, Jan 2022 to February 2022. The performance of the STANDARD Q COVID-19 Ag Test for the detection of SARS-CoV-2 antigen was evaluated in comparison to RT-PCR in 342 symptomatic patients who presented to health care facility in India. Where, one sample was tested using the STANDARD Q COVID-19 antigen test and the other using RT-PCR (Tru PCR Kit).

The required Data information along with the consent form was collected regarding the subject's age, gender,

clinical features and the primary reason for testing, which was referral form developed by Indian Council Of Medical Research (ICMR) for collection of RT-PCR samples [13-15].

The test Procedure followed for the RTPCR and RAT:

A. **For RTPCR:** The patient was made to sit comfortably. A nasopharyngeal/ or pharyngeal swab for RT-PCR was collected first under proper aseptic procedures and as per the recommended procedure by a trained laboratory technician [16, 17]. The swab was sealed in viral transport medium, labeled and stored in a cold chain (2-8oC) for transportation to the laboratory. A volume of 200 µL was collected from each Viral Transport Medium (VTM) and processed further for Ribonucleic Acid (RNA) extraction [18].

B. **For RAT:** A second nasopharyngeal sample was collected by the same technician and the sample was processed for RAT. The test was done as per the manufacturer's guidelines mentioned in the kit. The nasopharyngeal /Or pharyngeal swab was put in the buffer medium provided with the kit. The swab was kept in the buffer for 15 seconds. After this the swab was withdrawn while squeezing the sides of buffer tube. The rapid card was then kept on level surface and three drops from the buffer mixture were put in sample well. The results were read after 15 minutes and the same was communicated to the subject. The RAT was done using STANDARD Q COVID-19 (SD Biosensor kit).

The samples were transported under proper precautions and were processed on same day of collection. Sample for RT-PCR was taken before RAT so that the technician working in the RT-PCR lab was not aware of the result on RATs, which came's prior to it.

Results

There were a total of 342 samples included in our study. The most common symptom was Fever and Loss of smell. The ratio of males was more than compared to the Females. With Males being 209 (61.11%) and Females were 133 (38.8%). Mean age was found to be 21-40 age group, and 51-60 years. Only 6.1% patients were admitted to ICU, 82.74% were IPD patients, 17.25% were OPD patients respectively.

Table no1: Gender wise distribution of patients from the study

Gender	Number of Isolates	Percentage
Male	209	61.11%
Female	133	38.8%

We have compared the Ct values of the positive samples on RT-PCR on the basis of their symptoms and their results on Rapid tests. Similarly samples positive of RAT had a lower CT value than those negative on RAT.

RT-PCR ct-value was found between 18-21 and 29-32 cycles. The sensitivity, specificity of the RAT was found to be 54.4%, 99.2% respectively.

Discussion

Timely and accurate testing for SARS-CoV-2 is crucial if we are to limit the spread of the virus. RT-PCR remains the gold standard for diagnosis, but it is laborious and time-consuming. RAT tests are easy to handle, are inexpensive, and provide results in a short time [19]; there were a total of 342 samples included in our study. The most common symptom was Fever and Loss of smell. The ratio of males was more than compared to the Females. With Males being 209 (61.11%) and Females were 133 (38.8%). Mean age was found to be 21-40 age group, and 51-60 years. This study was supported by other also where Males were more in number than the Females. [20, 21] Only 6.1% patients were admitted to ICU, 82.74% were IPD patients, 17.25% were OPD patients respectively. RT-PCR ct-value was found between 18-21 and 29-32 cycles, this was in accordance with the other studies also. [22, 23] The sensitivity, specificity of the RAT was found to be 54.4%, 99.2% respectively. There are other studies also done where the authors estimated that the STANDARD Q rapid test had a very high specificity. This is comparable to multiple previous studies that also found the specificity to be more than 98% [24-26]. It infers that rapid antigen kits have very less likelihood to give false positive results and a subject with a positive test should be considered positive for SARS-CoV-2. All test kits have to apply for validation before actual use and the regulatory authorities in India have kept minimum acceptance criteria of 50% sensitivity and 95% specificity for point of care tests which are used in a field setting without laboratory support [27] In our study samples positive of RAT had a lower CT value than those negative on RAT which means samples positive on RAT are more likely to have lower Ct values. This was also supported by the other author where the lower Ct values means higher viral loads which in turn increase the probability of a positive RAT [28,29]..

Conclusion

Despite having lower sensitivity and specificity than the conventional qRT-PCR, RAT tests are an important tool for diagnosis of COVID-19 pandemic particularly in field/community settings.

As RT-PCR requires special equipment, long turnover time, high cost and need for skilled staff limit its use in the field settings so RAT is preferably used and can helped us in detecting and diagnosing COVID-19 at its early stage

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