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# Diagnostic Accuracy of Rapid Antigen Testing (RAT) Compared to Polymerase Chain Reaction (PCR) for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Diagnosis: Laboratory Validation

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## ABSTRACT

***Objective:*** To evaluate the diagnostic accuracy of rapid antigen detection test for detection of SARS-CoV-2 infection using real-time polymerase chain reaction as a gold standard.

***Study design:*** Cross-sectional validation study.

***Place and Duration of Study:*** Department of Virology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from Jul to Dec 2021.

***Methodology:*** A total of 330 individuals were enrolled in this study. The sample size was calculated using the WHO calculator. It represents the total number of patients presenting to the Hospital during study period. Paired nasopharyngeal swabs of patients fulfilling the inclusion criteria were collected. The samples were simultaneously tested by both a rapid antigen detection test by iChroma, Korea, based on immunochromatography (ICT) and polymerase chain reaction. For polymerase chain reaction, automated extraction was done on Genru, USA, and amplification was performed using Argene kit on Ampli lab thermal cycler, Italy.

***Results:*** A total of 330 patients were tested by RAT and PCR for the detection of SARS-CoV-2. RAT was positive in 75(22.7%) patients and negative in 255(77.3%) patients, and PCR was positive in 52(15.8%) patients and negative in 278(84.2%) patients. RAT test had a sensitivity of 80.77% and specificity of 88.13%, positive predictive value 56%, negative predictive value 96.08%, and diagnostic accuracy 86.97% in comparison with PCR.

***Conclusions:*** This study showed that RAT has good sensitivity and specificity but was much less as calculated by SARS-CoV-2 PCR. However, this test can be applied for screening in small hospital setups and situations for mass screening, where PCR facility is not available.

**Keywords:** Rapid Antigen Detection Test, SARS-CoV-2, RT-PCR.

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## INTRODUCTION

In December 2019, the appearance of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) signified the introduction of an extremely pathogenic coronavirus into the human population. After the first incidence in Wuhan, China, the extremely contagious virus, which is respiratory in nature, spread throughout the world.[1,2] On March 11, 2020, the World Health Organization (WHO) voiced concerns about the gravity of COVID-19 and classified it as a pandemic.[3] Most of the patients remained asymptomatic or developed mild symptoms; however, some patients with COVID-19 acute illness progressed to critical illness, especially in elderly patients or those with comorbidities. The clinical manifestation of COVID-19 was generally characterized by fever, cough, body aches, difficulty in breathing, loss of smell, and taste. The incubation period is typically 2-14 days, with most cases showing symptoms around 4-5 days after exposure.[4,5]

Accurate and timely detection of infection played a pivotal role in the management of patients.[6,7] Reverse transcriptase-polymerase chain reaction (RT-PCR) is considered the gold standard for diagnosing COVID-19. Several factors that limit the use of this technique include specialized, costly equipment, high testing costs, and skilled staff.[8] The best course of action for controlling regional SARS-CoV-2 outbreaks is to incorporate an approach for prompt detection and separation of infected cases.

Rapid immunoassays are quick method for detecting SARS-CoV-2 antigen in upper respiratory tract specimens in addition to the molecular detection of SARS-CoV-2 RNA.[9] Rapid Antigen Detection Test (RAT) is a point-of-care testing technique which can

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have a significant role in screening of suspected patients. According to WHO, these rapid tests could be used as a screening tool in community-based and specialized settings where PCR cannot be performed due to several reasons, especially in resource limiting settings. In a study conducted in Bahrain, reported prevalence of COVID-19 infection was 17.5% with sensitivity and specificity of 82.1% and 99.1% respectively.[10]

To implement RAT in our population, this study was planned to determine the diagnostic accuracy of iChroma RAT for detecting SARS-CoV-2 in comparison with RT-PCR. The purpose of this study is to evaluate the sensitivity and specificity of this kit. It has reduced cost and turnover time as compared to PCR. If the diagnostic accuracy of this kit is found to be comparable with RT-PCR then it can be used for early diagnosis and management as it will help in early detection and screening of suspected Covid-19 patients.

## METHODOLOGY

After taking approval from Institutional Review Board (FC-VIR12-13/READ-IRB/21/1805), a cross-sectional study was conducted at Department of Virology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from Jul to Dec 2021. Non-probability consecutive sampling technique was used. A questionnaire was used to collect demographic and personal data of each subject and confidentiality of patient was ensured. Sample size was calculated using WHO calculator. The sample size was calculated to be 330 with confidence level 95%, precision 10% and prevalence of 17.5% [7] in a previous study.

**Inclusion Criteria:** All individuals of both gender and age > 12 years with respiratory tract infection (Cough, sore throat and flu) reporting to AFIP, Rawalpindi with suspicion of COVID-19 were included in this study. The incubation period is typically 2-14 days, with most cases showing symptoms around 4-5 days after exposure.

**Exclusion Criteria:** All recovered and diagnosed cases of SARS-CoV-2 infection by RT- PCR and patients diagnosed with diseases other than SARS-CoV-2 were also excluded.

Demographic data was collected regarding age, gender, and occupation using a preformed questionnaire. Nasopharyngeal swabs (NPS) of patients satisfying the inclusion criteria were taken wearing personal protective equipment. The patient was advised to tilt his head at 45o.The swab was inserted into the nostrils with 2-3 rotation of 180o and wait for 3-5 secs. The swab was withdrawn from nostrils and inserted into tube containing 2-3 ml viral transport medium (VTM). These samples were transported to Virology Department, AFIP, maintaining cold chain (2-6°C). Once received in the laboratory the samples dealt inside Biosafety Cabinet class 2. After initial processing the samples subjected to PCR and iChroma RAT test within 4 hours of sample collection. In PCR, Automated Extraction was done on Genru Automated system (China) and amplification was carried out using Argene kit (Biomerieux, France) and Amplilab Thermal Cycler (Adaltis, Italy). PCR amplification kit having 2 targets i.e N gene and RNA Dependent RNA Polymerase gene (RdRp) for detection of SARS-CoV-2 genome was used. All the samples with Cycle threshold value less than 34 were considered as positive and with 35 and more CT value were considered as negative. Test on iChroma RAT was done using manufactures protocol. The samples were vortexed and 75 uL of sample was loaded onto the cartridge with the help of pipette. After 15 mins incubation, this cartridge was read by iChroma reading device.

The data was analysed using the statistical analysis program Statistical Package for the social sciences (SPSS) version 23.00. For quantitative variables such as age and duration of disease, the Mean±SD was reported. Qualitative variables like gender, age groups, occupation, and signs and symptoms were analyzed by calculating frequency and percentage. To determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the iChroma RAT kit with PCR as the gold standard, a 2 x 2 table was created. Stratification was performed for qualitative variables to examine their impact on SARS-CoV-2 infection. Post-stratification diagnostic accuracy was applied, and a $p$-value <0.05 was considered statistically significant.

## RESULTS

A total of 330 patients were included in this study. The minimum age was 12 years and maximum age was 70 years with mean 40.87±17.18 years. The minimum duration of disease was 3 days and maximum duration of disease was 18 days (incubation period (time from exposure to the virus to the onset of symptoms) is typically 2-14 days, with most cases showing symptoms around 4-5 days after exposure)

with mean 10.34±4.73 days. There were 179(54.2%) male patients and 151(45.8%) female patients. The age of 154(46.7%) patients were < 40 years and 176(53.3%) patients were ≥ 40 years. Patients with various occupation were included in this study, 121(36.7%) patients were Govt servant, 105 (31.8%) patients had private job and 104(31.5%) patients were unemployed. Individuals with various occupations were analysed for disease trend. Cough was the most common symptom found in 103(31.2%) patients, followed by fever in 89(27.0%) patients, flu in 85(25.8%), shortness of breath in 9(2.7%), sore throat in 7(2.1%), myalgia in 10(3%), loss of smell in 12(3.6%) and loss of taste was found in 15(4.5%) patients (Table-I).

**Table-I: Distribution of symptoms (n=330)**

| Symptoms | Frequency (%) |
|---|---|
| Fever | 89(27.0%) |
| Cough | 103(31.2%) |
| Flu | 85(25.8%) |
| Shortness of Breath | 9(2.7%) |
| Sore throat | 7(2.0%) |
| Myalgia | 10(3.0%) |
| Loss of smell | 12(3.6%) |
| Loss of taste | 15(4.5%) |

RAT test was used for the detection of acute SARS-CoV-2 infection, it was found positive in 75(22.7 %) patients while in 255(77.3%) patients it was negative. PCR test was used as a gold standard. Out of total 330 patients, SARS-CoV-2 PCR was positive in 52(15.8 %) patients and was negative in 278(84.2%) patients. Sensitivity of RAT test was calculated as 80.77%, specificity as 88.13%, positive predicted value as 56.00%, negative predicted value as 96.08% and diagnostic accuracy as 86.97% (Table-II). By stratification of duration of disease (days), the diagnostic accuracy of < 10 days was 85.71% and the diagnostic accuracy of ≥ 10 days was 75.00%. The diagnostic accuracy was found as 88.89% in males while in females it was 72.00%.

## DISCUSSION

The study is a validation approach to evaluate the diagnostic performance of iChroma™ RAT for detecting SARS-CoV-2 infection in all suspected indoor and outdoor patients at all Armed Forces Hospitals in Rawalpindi. The findings of the study revealed that RAT was positive in 75(22.7 %) patients and negative in 255(77.3%) patients, and PCR test was positive in 52(15.8%) and negative in 278(84.2%) patients. The RAT test used for the detection of SARS-CoV-2 infection had a sensitivity of 80.77%, a specificity 88.13%, a positive predictive value 56.00%, a negative predictive value 96.08%, and a diagnostic accuracy 86.97%. By stratification of age, the diagnostic accuracy of the age group (< 40 years) was 92.00%, and the age group (≥ 40 years) was 70.37%. The diagnostic accuracy of RAT was 88.89% in males and 72.00% in females as compared to PCR.

**Table-II: PCR and RAT for detection of SARS-CoV-2 infection (n = 330)**

| PCR | RAT | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | TP= 42 | FN = 10 | 52 |
| Negative | FP= 33 | TN= 245 | 278 |
| Total | 75 | 255 | 330 |

Sensitivity = 80.77%
Specificity = 88.13%
Positive Predictive Value = 56.00%
Negative Predictive Value = = 96.08%
Diagnostic accuracy = 86.97%
Likelihood ratio = 6.80

The timely detection of infected individuals has been crucial for managing the viral infection.[11] During the start of the pandemic in 2020, huge testing was initiated to minimize the spread by early recognition of infected individuals.[12] Mushtaq *et al*., studied the Anti-SARS-CoV-2 IgG antibody test, which was done using the chemiluminescence method. RAT tests have effectively decreased the time it takes to obtain test results, enabling prompt clinical intervention and implementation of preventive measures. However, it is important to acknowledge that these tests may have decreased specificity in comparison to RT-PCR tests.[13] Yamayoshi *et al*., reported that all RATs tested failed to detect viral antigens in several specimens from which the virus was isolated. The current RATs will likely miss some COVID-19 patients who are shedding infectious SARS-CoV-2.[14]

This study was designed to evaluate RAT, a cost-effective and rapid diagnostic tool, can be conveniently conducted at the point of care. A similar study was conducted by Khalid *et al*. The study explained that the implementation and execution of RAT testing were carried out smoothly without encountering any challenges. Although the sensitivities of various RAT tests range from 24% to 93%, the documented sensitivities of Standard RAT primarily fall within the range of 68% to 90%.[15]

A study by Fenollar *et al*., on the field evaluation of a rapid antigen test for COVID-19 diagnosis. In the study involving 412 patients, it was found that 43

individuals (10.4%) tested positive for COVID-19 using both RT-PCR and RAT, while 358 individuals (86.9%) tested negative with both methods. When compared to RT-PCR as the reference, the rapid antigen detection (RAT) exhibited an overall specificity of 100% (95% confidence interval [CI] 98.7 - 100%), indicating that RAT accurately identified all negative cases. The sensitivity of RAT was 79.6% (95% CI 67.0 - 88.8%), suggesting that it effectively detected COVID-19 positive cases.[16] When considering an estimated prevalence of 5% and 10%, the negative predictive value of RAT tests was observed by Sochy *et al*., to be 99% (95% CI 97.4 - 99.6%) and 97.9% (95% CI 95.9 - 98.9%) overall, respectively.[17] A study conducted by Albert *et al*., compared the diagnostic accuracy of RAT with PCR in diagnosing SARS-CoV-2. The review included 24 studies with a total of 14,188 patients. The overall pooled sensitivity of RAT for diagnosing SARS-CoV-2 was 0.68 (95% confidence interval [CI], 0.59 - 0.76), indicating that RAT correctly identified approximately 68% of positive cases.[18] Additionally, the overall pooled specificity of RAT was also reported by Lee *et al*., to be 0.99 (95% CI, 0.99 - 1.00), indicating that RAT accurately identified negative cases in 99% of instances.[19]

Considering these findings, the present study, which aimed to validate the performance of a SARS-CoV-2 rapid antigen test against PCR, demonstrates that RAT exhibits comparatively lower sensitivity, specificity, and predictive values than the molecular gold standard. Despite these limitations, the test retains practical utility in selected contexts. Its rapid turnaround time and operational simplicity make it a viable screening tool in high-throughput or time-sensitive environments such as airports, border control points, emergency departments, and resource-constrained facilities where PCR testing is not readily accessible. Accordingly, while RAT cannot replace PCR for definitive diagnosis, it offers meaningful value as an adjunct diagnostic modality in settings where immediate decision-making is essential or laboratory capacity is limited.

## LIMITATIONS OF STUDY

Our study had certain limitations. Firstly, it relied on data from a single centre, which may restrict the generalizability of the findings to the broader population. Secondly, the duration of the study was relatively short, potentially impacting the results.

## CONCLUSION

This study concluded that RAT had relatively lesser sensitivity, specificity, positive predictive value, and negative predictive value as compared to PCR. However, it can be used as a screening tool or as a diagnostic modality in special situations like airports, border control, emergency services, and resource-constrained facilities where a PCR facility is not available.

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**Discolure:**

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**Authors' Contribution**

Following authors have made substantial contributions to the manuscript as under:

HH & MAR: Data acquisition, data analysis, critical review, approval of the final version to be published.

EG & SKN: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

FA & TG: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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