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Research Article

Comparative Evaluation of Rapid Antigen Detection Assay and RT-PCR for SARS-CoV-2 Diagnosis in a Tertiary Care Setting

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A B S T R A C T

Introduction: COVID-19, caused by SARS-CoV-2, underscored the need for fast, reliable diagnostics. While RT-PCR remains the gold standard, it is time-consuming and requires specialised resources, delaying decisions. Rapid antigen detection tests (RADTs) offer quicker results and ease of use, especially in resource-limited settings, though they have lower sensitivity than RT-PCR, particularly in asymptomatic cases. This study aimed to compare the diagnostic performance of RADTs and RT-PCR in a tertiary care setting.

Materials and Methods: This analysis was carried out at PJMC (Phulo Jhano Medical College Hospital, Jharkhand) retrospectively. Department of Microbiology, Jharkhand. Nasopharyngeal and oropharyngeal swabs from adult and paediatric patients were collected for RT-qPCR and RADTs. For RT-qPCR, RNA was extracted and amplified using standard protocols, and results were considered positive if Ct values pertaining to the N and E genes were ≤ 35. For RADTs, testing followed the manufacturer's guidelines, with results interpreted within 30 minutes. Predictive values, specificity and sensitivity were computed, and agreement with RT-qPCR was assessed using Cohen's Kappa coefficient.

Results: Out of 180 samples, the RADT showed a sensitivity of 76.19% and specificity of 100%. Sensitivity was greater in patients with symptoms (80.95%) as opposed to those without symptoms (57.3%). The overall accuracy was 86.11%, with strong agreement observed among symptomatic cases (Kappa = 0.84).

Conclusion: The RADT demonstrated excellent specificity and good sensitivity, particularly in symptomatic patients, making it a valuable tool for rapid diagnosis in clinical settings. However, its lower sensitivity among asymptomatic patients underscores the need for confirmatory RT-PCR testing, especially in cases of known exposure.

Keywords: SARS-CoV-2, COVID-19, RT-PCR, RADTs

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that caused the COVID-19 pandemic has had a major impact on healthcare systems around the world, underscoring the essential need for quick, accurate, and easily available diagnostic techniques. For effective care, prompt isolation of infected persons, control of the virus's propagation and precise identification of SARS-CoV-2 are essential. The most common outcome of a SARS-CoV-2 infection is a moderate or asymptomatic sickness; serious pneumonia is less common. When the illness worsens, it can lead to severe breathing difficulties (acute respiratory distress syndrome) which has a mortality incidence of roughly 6% on average, with rates varying from 2 to 14.4%.[1–3] Because of its great sensitivity and specificity, the real-time polymerase chain reaction-reverse transcription (RT-PCR) is currently regarded as the gold standard for confirming COVID-19. However, RT-PCR is resource-intensive, requires specialised equipment and trained personnel, and often involves longer turnaround times, which can delay clinical decision-making.[4]

Rapid antigen detection assays (RADTs) have emerged as a practical alternative, offering the advantages of speed, ease of use, and cost-effectiveness, particularly in resource-limited settings and point-of-care testing scenarios. These assays detect viral antigens within minutes, making them a valuable tool for rapid clinical judgments and widespread screening. Still, the performance of RADTs varies significantly depending on viral load, specimen quality, and the stage of infection, often resulting in lower sensitivity compared to RT-PCR.[5–7]

The most common method for identifying SARS-CoV-2 infection is the real-time polymerase chain reaction-reverse transcription (RT-PCR) assay, which takes at least four hours to complete and experienced personnel to perform. This emphasises how important it is to have quick and precise diagnostic tests in order to speed up efforts to control illness and make pre-operative screening of invasive operations easier.[8] If the accuracy of lateral flow immunoassays, that employ monoclonal antibodies to target SARS-CoV-2 antigens is found to be on par with RT-PCR tests, they may be used as supplementary screening methods.[7,8]

The ICMR mandates that point-of-care tests (POCT) outside laboratories must have a minimum sensitivity of 50% and specificity of 95%.[6] Although a threshold of 96.52% and a specificity of 99.68% is claimed for the Basic QRAT kit,[7] studies report variable sensitivity (17.5% to 89%) and specificity (92.4% to 100%) worldwide[6,7]. In India, the sensitivity of rapid antigen tests ranges from 37.5% to 71.9%, with specificity between 99.3% and 100%.[4,6,7]

Despite their widespread use, there is ongoing debate regarding the diagnostic accuracy of RADTs, particularly in different clinical settings. Comparative evaluations of RADTs and RT-PCR are essential to determine the suitability of these rapid tests as reliable diagnostic tools in various healthcare contexts. This study aimed to evaluate the sensitivity, accuracy, and overall clinical value of fast antigen detection tests in comparison to RT-PCR in a hospital context.

Materials and Methods

This cross-sectional study was carried out retrospectively at the Department of Microbiology, PJMC (Phulo Jhano Medical College Hospital, Jharkhand). One hundred and eighty samples from patients with ages ranging from 5 years to 75 years who visited our hospital's flu clinic were included in the study. Patients gave their oral consent for oropharyngeal and nasopharyngeal swabs to be taken.

Specimen Collection

The three swab specimens were taken concurrently from each patient. In the real-time RT-qPCR assay, one nasal and one oropharyngeal biopsy were mixed together in a 3 ml virus transport medium (VTM) tube (Hi-Media). As directed by the manufacturer, a different nasopharyngeal swab was used for the fast antigen detection test and put in the buffer tube that was provided in the COVID-19 RAT kit (STANDARD Q).

STANDARD Q COVID-19 Ag Test for Rapid Detection of SARS-CoV-2 Antigens

For the qualitative identification of antigens from SARS-CoV-2 in human nasopharyngeal samples, the fast Ag Test is a chromatographic immunoassay that may be performed quickly. The test was carried out in accordance with the manufacturer's instructions.[7] At the influenza clinic, testing and sample collection were done concurrently, and results were analysed within thirty minutes of the test.

RT-qPCR Protocol for SARS-CoV-2 Detection

The automated extraction of nucleic acids kit (Qiagen) was used to extract RNA from viruses from samples in accordance with the manufacturer's instructions. We used a Biorad CFX96® heat cycler for RT-qPCR. 5 µL of RNA, which is 12.5 µL of 2× reaction solution containing Platinum Taq Polymerase, which 0.5 µL of reversing transcriptase/ Taq combine, 1.5 µL of primers and probe mix of NIV, and 5.5 µL of water without nuclease were all included in each 25 µL reaction mix. Thermal cycling consisted of 45 repetitions of 95 °C for 15 seconds and 58 °C for 30 seconds, after which reverse transcription was carried out for 10 minutes at 55 °C and initial denaturation for 3 minutes at 95 °C. Firstly, the SARS-CoV-1 E gene was checked for in the samples. After

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receiving favourable results, the N gene was checked again for verification. According to the NIV protocol, samples having a growing exponential curve and a Ct level ≤ 35 were deemed positive. Only samples that tested positive for both the N and E genes were considered RT-PCR positive, and the N gene Ct values—rather than the E gene result—were used to assess the RAT kit's performance.[9,10]

Statistical Analysis

Statistics from SPSS version 25 and Microsoft Excel were used for the data analysis. Percentages (%) were used to display the results. Sensitivity, particularity, positive as well as negative predictive numbers, and overall precision were used to assess the antigen test's predictive performance; the gold standard for this analysis was the RT-qPCR results. To evaluate agreement with RT-qPCR, Cohen's Kappa coefficient was employed, and the significance level was set at $p < 0.05$.

Results

Clinico-Demographic Characteristics of Patients

During the study, a total of 180 samples were collected from the patients for RT-qPCR and fast antigen testing. Out of them, 45 samples (25%) were from female, and 135 samples (75%) were from male. The average age of participants was 35.15 ± 13.17 decades, and the oldest representation was in the 20–40 years age group (51.1%), followed by the 41–65 years of age group (48.9). Most of the patients were asymptomatic 75%, while 25% were presented with symptoms. Among symptomatic patients, fever was the most common symptom in 65% of patients, followed by cough (25%) and body ache (10%).

Diagnostic Performance of Rapid Antigen Test for SARS-CoV-2 Diagnosis Compared to RT-qPCR

With an accuracy rate of 76.19%, the fast antigen test was able to accurately identify roughly 76.19% of people who tested positive for SARS-CoV-2 confirmed by RT-qPCR. Its specificity was perfect at 100%, signifying that it correctly identified all individuals who did not have the virus, as determined by RT-qPCR. The positive predictive value of 100% shows that all individuals who tested positive with the rapid antigen test were indeed positive for the virus according to RT-qPCR, highlighting the test's reliability in confirming active infections. However, the negative predictive value of 75% indicates that there is a 25% chance of false negatives among those who tested negative with the rapid antigen test. Overall, the accuracy of the test was 86.11%, suggesting that fast antigen testing is a trustworthy method for SARS-CoV-2 diagnosis, particularly in settings where immediate results are needed (Table 1).

Table 1.Comparison of Diagnostic Performance of RT-qPCR with the Rapid Antigen Test for SARS-CoV-2 Diagnosis

Test Result	Antigen Positive	Antigen Negative	Total
RT-qPCR Positive	80	25	105
RT-qPCR Negative	0	75	75
Total	80	100	180

Rapid Antigen Test Diagnostic Performance for SARS-CoV-2 Diagnosis in Symptomatic and Asymptomatic Patients

The SARS-CoV-2 fast antigen test demonstrated varying diagnostic performance between symptomatic and asymptomatic patients. Sensitivity was higher among symptomatic individuals at 80.95%, while it dropped to 57.3% for asymptomatic patients, indicating reduced effectiveness in detecting infections without symptoms. Both groups showed 100% specificity, accurately identifying all non-infected patients. The positive predictive value (PPV) was also 100% for both groups, confirming that all positive results were true infections. However, the negative predictive value (NPV) was lower in asymptomatic patients at 70.09% compared to 78.95% for symptomatic patients. Overall accuracy was 88.89% for symptomatic and 78.6% for asymptomatic patients, emphasising the test's reliability in symptomatic cases but highlighting its limitations in asymptomatic detection (Table 2).

Table 2.Diagnostic Performance in Symptomatic and Asymptomatic Patients

Characteristics	Symptomatic Patients (%)	Asymptomatic Patients
Sensitivity	80.95	57.30
Specificity	100.00	100.00
Positive predictive value	100.00	100.00
Negative predictive value	78.95	70.09
Accuracy	88.89	78.60

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Cohen Kappa Coefficient for Concordance between RT-PCR and Rapid Antigen Test

The Kappa coefficient for the quick antigen test in contrast to RT-qPCR is approximately 0.73, indicating a moderate agreement between the two testing methods. The Kappa coefficient for symptomatic patients was 0.84 (indicating strong agreement) and that for asymptomatic patients was 0.70 (indicating moderate agreement). It suggests that the rapid antigen test performs well in both symptomatic and asymptomatic populations, with strong agreement observed in symptomatic cases and moderate agreement in asymptomatic cases.

Discussion

This study evaluated the clinico-demographic features and test efficacy amongst sick and asymptomatic patients, comparing the diagnostic results of a fast antigen test of SARS-CoV-2 with RT-qPCR. The results showed that the fast antigen test had a 100% specificity and 76.19% sensitivity, indicating its usefulness in precisely identifying current infections.

Clinico-Demographic Profile

Seventy-five percent of the patients in the trial were male, with an average age of 35.15 years, indicating that younger adults were more represented, particularly in the 20–40 age group. This demographic trend aligns with previous studies, such as one by Zhang et al. (2020), which noted a higher prevalence of COVID-19 cases in younger populations, potentially reflecting social behaviours that increase exposure risk. In this study, most patients were asymptomatic, with fever identified as the most common symptom, followed by cough and body ache. These results correspond with those that Kanaujia et al. found that fever was the leading symptom in their cohort, occurring in 67.4% of cases, with cough following at 39.9%.[3]

Diagnostic Performance

The rapid antigen test demonstrated excellent specificity (100%) and positive predictive value (PPV) across both symptomatic and asymptomatic patients. Such high specificity ensures that false positive results are minimised, thereby supporting the use of this test in clinical settings for immediate diagnosis. Previous studies have reported similar high specificity rates for antigen tests, reinforcing their role as effective screening tools, particularly in outbreak settings.[11–13] The findings of this study were consistent with those of a study conducted by Homza et al. which showed a sensitivity of 61.9% and a specificity of 99.0%,[11–13] whereas Faculty Hospital Motol in Prague, Czech Republic, reported susceptibility of 62.6% and specificity of 99.5%.[11–13] The degree of sensitivity in the present investigation may have been lower than the median obtained in Ristic et al.'s meta-analysis, which found an average accuracy of 72.1% with specificity of 98.6%.[10] Furthermore, this study's sensitivity values were higher than those observed in Pandey et al.'s study (53.6% sensitivity, 97.35% specificity).[14] Several other Indian studies indicated lower sensitivity rates for the test kit compared to the current findings, with Rana et al. reporting 37.5%[9] and Prakash et al. showing 44.5%[15]. However, Kanaujia et al. showed a greater sensitivity of 71.9%, which was greater than what was observed in this study.[3] Overall, the specificity of the test kit in various studies conducted in India was largely comparable.[9,14,15]

However, the fast antigen test's sensitivity varied significantly between symptomatic (80.95%) and asymptomatic patients (57.3%). This finding emphasises a critical limitation in detecting infections in asymptomatic individuals, echoing the concerns raised by Lescure et al., who found that antigen tests often yield lower sensitivity in patients without symptoms.[16] The lower negative predictive value (NPV) in asymptomatic patients further highlights the risk of missed diagnoses in this group, suggesting that confirmatory testing with RT-qPCR may still be necessary, especially in asymptomatic individuals with a known exposure history. The results of this study aligned with those of Dinnes et al., whose meta-analysis revealed particulars to 98.1% and 99.6%, respectively, along with a sensitivity of 80.1% for sick people and 61.1% for asymptomatic individuals.[17] On the other hand, the results were better than those of Pandey et al., which found that patients with symptoms had sensitivities of 61% and those without symptoms of 33.3%.[14] In comparison, the precision rate of 88.6% recorded by Kanaujia et al. was higher than the accuracy rate of this study overall.[3] Furthermore, symptomatic patients had a higher negative predictive value (79.4%) than asymptomatic patients (59.1%). Interestingly, a study by Munne et al. revealed a negative likelihood ratio of 59% in symptoms individuals and 72.3% in patients with no symptoms, conflicting with what was obtained in the present investigation, even though the values for positive prediction were the same at 100% for both categories of patients.[18]

Kappa Coefficient Analysis

The Kappa coefficient of 0.73 indicates moderate agreement between the rapid antigen test and RT-qPCR, with strong agreement in symptomatic patients (0.84) and moderate agreement in asymptomatic patients (0.70). This aligns with findings from a systematic review by Dinnes et al., which reported varying levels of agreement for rapid antigen tests depending on the population tested.[17] These results suggest that while rapid antigen tests are effective, their performance may be influenced by patient symptoms, warranting caution in interpretation and clinical use.

Limitations

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This research has several limitations. The sample size may not be sufficient for generalisation across diverse populations, and the single-institution focus might limit the applicability of the results in different healthcare settings. Additionally, the emphasis on symptomatic and asymptomatic patients could restrict insights into the test's effectiveness for mild or typical cases.

Conclusion

Overall, this study demonstrates that the SARS-CoV-2 fast antigen test is a valuable diagnostic tool, particularly for symptomatic patients, given its high specificity and PPV. However, the reduced sensitivity in asymptomatic individuals underscores the need for careful application and possibly the integration of RT-qPCR testing in this group to ensure accurate diagnosis and management. Future studies should explore strategies to enhance the sensitivity of rapid antigen tests, especially among groups where rates of asymptomatic infections are high.

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