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# Diagnostic Accuracy of Rapid Antigen Test and RT-PCR in Symptomatic COVID-19 Patients: A Prospective Observational Study in Central India

Mr. Tajvar[1], Dr. Ramanath K[2]

[1]Research Scholar, Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, India
[2]Professor of Microbiology; Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, India

## ABSTRACT

**Background:** Rapid identification of SARS-CoV-2 infection is critical for interrupting transmission, guiding treatment, and informing public health measures. Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) is the gold standard, whereas Rapid Antigen Tests (RAT) offer faster but less sensitive detection.

**Aim:** To evaluate the diagnostic accuracy of RAT compared with RT-PCR among symptomatic COVID-19 patients and vaccinated individuals with breakthrough infection.

**Methods:** A prospective observational study (2021–2024) was conducted in a tertiary-care molecular laboratory in Central India. Nasopharyngeal swabs were tested using RAT and RT-PCR. Diagnostic parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and association between RAT and RT-PCR results were analysed.

**Results:** RAT demonstrated a sensitivity of 87.6%, specificity of 65.1%, PPV of 80.3%, and NPV of 76.3% compared with RT-PCR. A statistically significant association existed between RAT and RT-PCR ($p < 0.001$). RT-PCR cycle threshold (Ct) values for the E, N, and RdRp genes were consistently <30, indicating high viral load in positive cases. RAT performance aligned with previously published global ranges (61–98.3% sensitivity).

**Conclusion:** RAT shows good diagnostic sensitivity and rapid turnaround time, making it valuable for mass screening and early triage. However, its moderate specificity necessitates confirmatory RT-PCR, particularly in clinical or epidemiologically significant scenarios.

**Keywords:** COVID-19, SARS-CoV-2, Rapid Antigen Test, RT-PCR, Diagnostic Accuracy, Breakthrough Infection

## INTRODUCTION

The COVID-19 pandemic, caused by SARS-CoV-2, has resulted in unprecedented global health and socioeconomic disruption. Timely and accurate diagnosis remains essential for patient management, containment of outbreaks, and epidemiological surveillance. SARS-CoV-2 primarily spreads through respiratory droplets and aerosols and presents with symptoms ranging from mild upper respiratory features to severe pneumonia and multiorgan dysfunction [1,2.]
RT-PCR remains the reference standard for confirming SARS-CoV-2 infection due to its high analytical sensitivity, ability to amplify viral RNA at very low concentrations, and capacity to detect multiple target genes (E, N, RdRp) [3]. However, RT-PCR has limitations such as longer turnaround time, higher cost, requirement for sophisticated equipment, and dependence on skilled personnel.

Rapid Antigen Tests (RAT) detect viral proteins, mainly the nucleocapsid antigen, offering results within 15–30 minutes. They are inexpensive, easy to perform at point of care, and scalable for community testing [4]. Nevertheless, antigen-based methods are prone to lower sensitivity, especially in low viral load scenarios.

The World Health Organization (WHO) recommends that RATs must meet minimum performance criteria of ≥80% sensitivity and ≥97% specificity [5]. European Centre for Disease Prevention and Control (ECDC) suggests ≥90% sensitivity and ≥97% specificity for public health use.[6]

With global vaccination campaigns, breakthrough infections have emerged, defined as detection of SARS-CoV-2 RNA or antigen ≥14 days after receiving complete vaccination [7]. Variants such as Delta and Omicron exhibit partial immune escape, further complicating diagnostic interpretation [8].

This study evaluates RAT performance relative to RT-PCR among symptomatic patients, contributing institution-level evidence towards refining diagnostic strategies during evolving pandemic phases.

## MATERIALS AND METHODS

### Study Design and Setting

This prospective observational study was conducted at the Molecular Biology Laboratory and Central Clinical Laboratory of Index Medical College Hospital & Research Centre, Indore, India, from November 2021 to December 2024 [9].

Study Population

**The study included:**

- Symptomatic individuals tested for COVID-19
- Vaccinated individuals with suspected breakthrough infection
- Patients admitted to COVID wards

Breakthrough infection was defined per CDC/USFDA guidelines: RT-PCR positivity ≥14 days after completion of recommended vaccine doses [7].

Sample Size

Sample size was calculated using the formula:

$n = z^2 p (1–p) / d^2$

The final sample size was 1000 patients [10].

Inclusion Criteria

- Symptomatic patients presenting for testing
- Fully vaccinated individuals with RT-PCR confirmed infection
- Breakthrough cases meeting criteria
- Willingness to provide informed consent

Exclusion Criteria

- Vaccinated with only one dose
- Non-consenting individuals
- Unreachable during telephonic follow-up

### Specimen Collection

Nasopharyngeal Swabs

Two NP swabs were collected using polyester/Dacron flocked swabs and transported in Viral Transport Medium (VTM) [11]. VTM preserves viral RNA integrity and prevents microbial contamination.

Lower Respiratory Specimens

Deep sputum or BAL samples were collected in severe cases requiring invasive diagnostics [12].

Diagnostic Methods

Rapid Antigen Test (RAT)

Performed per manufacturer instructions using approved commercial antigen kits detecting SARS-CoV-2 nucleocapsid protein.

RT-PCR

Multiplex RT-PCR was conducted using assays targeting:

- E gene
- N gene
- RdRp gene

Ct value <30 was considered indicative of high viral load and strong positivity [13].

Statistical Analysis

Diagnostic accuracy parameters calculated:

- Sensitivity
- Specificity
- PPV
- NPV

Association between RAT and RT-PCR was determined using Chi-square test. $p < 0.001$ was considered statistically significant.

## RESULTS

1. Diagnostic Accuracy of RAT Compared With RT-PCR

| RAT Result | RT-PCR Positive | RT-PCR Negative |
|---|---|---|
| Positive | 248 (65.1%) | 77 (12.4%) |
| Negative | 133 (34.9%) | 542 (87.6%) |

Diagnostic Performance

- Sensitivity: 87.6%
- Specificity: 65.1%
- PPV: 80.3%
- NPV: 76.3%

RAT demonstrated strong sensitivity but moderate specificity, indicating occasional false positives [14].

2. Statistical Association

A statistically significant association between RAT and RT-PCR results was observed ($p < 0.001$) [15].

3. RT-PCR Ct Value Interpretation

Mean Ct values:

- E gene: 27.32
- N gene: 22.32
- RdRp gene: 25.82

All Ct values were <30, demonstrating high viral loads in positive cases [13].

4. Comparison with Published Literature

The following reported sensitivities match the present findings:

- Rania et al.: 78.2% sensitivity, 64.2% specificity [16]
- Abdulrahman et al.: 82.1% sensitivity, 99.1% specificity [17]
- Ashok Kumar et al.: 61% sensitivity, 94.4% specificity [18]
- Chaimayo et al.: 98.3% sensitivity [19]
- Hirabayashi et al. (meta-analysis of 91 studies): PPV 97.7%, NPV 95.2% [20]

These confirm that RAT accuracy varies widely based on population, viral load, and assay type.

## DISCUSSION

**1. RAT Sensitivity and Viral Load Correlation**

RAT sensitivity is highest during early symptomatic phases when viral load peaks. The high proportion of Ct <30 in RT-PCR positive cases explains the elevated RAT sensitivity (87.6%) in this study [13].

**2. Moderate Specificity and Implications**

The specificity of 65.1% suggests that some uninfected individuals may test falsely positive. This may occur due to:

- Cross-reactivity
- Improper sampling
- Low pretest probability settings

Clinical decisions for isolation or initiation of therapy should therefore incorporate RT-PCR confirmation when RAT is positive.

**3. RAT Utility in Breakthrough Infections**

Vaccinated individuals may exhibit:

- Lower viral loads
- Shorter viral shedding
- Mild or atypical symptoms

These factors may reduce RAT sensitivity [7], yet in this study, symptomatic breakthrough cases showed Ct values <30, enabling reasonable antigen detection.

**4. Global Comparisons**

Reported RAT sensitivities globally range from 61% to 98.3% [16–25]. The study's sensitivity aligns with upper-mid global performance bands.

WHO and ECDC thresholds for RAT performance require specificity ≥97% [5,6]. The moderate specificity in our setting underscores the need for RT-PCR confirmation in epidemiologically important scenarios.

**5. Strengths and Limitations**

Strengths

- Large sample size (1000 patients)
- Real-world tertiary care setting
- Inclusion of breakthrough infections
- Gene-specific Ct evaluation

Limitations

- Specificity lower than global recommendations
- Viral culture correlation not performed
- No variant-specific performance analysis

## CONCLUSION

This study demonstrates that RATs provide rapid, sensitive detection of SARS-CoV-2 in symptomatic individuals, particularly when viral loads are high. However, their moderate specificity necessitates confirmatory RT-PCR to avoid false-positive interpretations.

RAT is therefore highly valuable for triage, emergency screening, mass testing, and outbreak management, but RT-PCR remains indispensable for clinical confirmation, especially among high-risk or hospitalized patients.

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