

SHORT COMMUNICATION

Evaluation of a Rapid Antigen Test Using a Point of Care Real-Time RT-PCR as a Comparator for the Detection of SARS-CoV-2

J. P. Punchihewa, B. N. Iqbal, S. R. M. Shihab and F. Noordeen*

Evaluation of a rapid antigen test using a point of care real time RT-PCR as a comparator for the detection of SARS-CoV-2			
Prospective diagnostic accuracy study			
50 (25 positives : 25 negatives) Nasopharyngeal swabs	 STANDARD™ Q COVID-19 Ag rapid test kit A rapid antigen test to detect SARS-CoV-2	 Gold standard Point of care real time RT-PCR (Molbio - Trueprep Auto POC RT-PCR)	
Sensitivity	48 % [27.80 – 68.69%]	Specificity	96 % [79.65 – 99.90 %]
Positive predictive value	92.31 % [62.75 – 98.84 %]	Negative predictive value	64.86 % [55.68 – 73.07 %]
• RAT performance aligns with viral load levels in the samples • 100 % sensitivity for samples with high viral load (Ct ≤ 20.3) • Sensitivity dropped to 48 % for samples with low viral load (Ct = 20.9 – 33.0) • 96% sensitivity for the detection of SARS-CoV-2			

Highlights

- Performance of the Rapid Antigen Test (RAT) positively aligns with viral load in nasopharyngeal samples.
- RAT showed 100% sensitivity for high viral loads (Ct ≤ 20.3).
- Sensitivity dropped to 48% for low viral loads (Ct = 20.9–33.0).
- RAT demonstrated 96% specificity for the diagnosis of COVID-19.

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Abstract

A rapid antigen test (RAT) was compared with a point-of-care real-time RT-PCR for the diagnosis of COVID-19 using “50 nasopharyngeal swabs (25 positive and 25 negative by POC-rtRT-PCR)”. The samples were collected from symptomatic patients at the Teaching Hospital, Peradeniya (March–August 2021) and tested using the STANDARD™ Q COVID-19 Ag RAT. The diagnostic performance of the RAT was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) using Microsoft Excel 2010 and MedCalc software. The RAT showed 100% sensitivity for high viral loads ($Ct \leq 20.3$) with 96% specificity. Sensitivity dropped to 48% for samples with low viral loads ($Ct = 20.9–33.0$). The RAT was highly reliable at detecting high viral loads but showed reduced sensitivity at lower viral loads, making it most suitable for detecting highly infectious COVID-19 cases.

Keywords: Point of care real-time RT-PCR; Rapid antigen test; SARS-CoV-2

INTRODUCTION

There are several molecular and immunological diagnostic methods available for the detection of SARS-CoV-2 infection (COVID-19). Of these diagnostic methods, the real-time reverse transcription polymerase chain reaction (rtRT-PCR) is the gold standard method due to its high sensitivity and specificity (Dhama et al., 2020). However, it is arduous, time-consuming and resource-intensive. The rtRT-PCR also requires a sufficient number of samples in a batch for a single run and thus delays the diagnosis in individual cases of concern. Short supply of diagnostic resources for rtRT-PCR and the high demand for prompt diagnosis increases the use of rapid antigen detection tests (RAT) for the laboratory diagnosis of COVID-19 (Grant et al., 2020).

RAT is simple and cost-effective, with a short turnaround time (Gremmels et al., 2021; Jarrom et al., 2022). On the other hand, RAT is suitable for detecting samples with moderate to high viral load (Porte et al., 2020). There are several RATs available for the detection of SARS-CoV-2 infection with high specificity but varying sensitivity (Hirotsu et al., 2020; Scohy et al., 2020). However, their

diagnostic performance may vary (Deeks et al., 2020) and RATs also produce false negative and false positive results (Gremmels et al., 2021). The present study compared the diagnostic performance of a RAT for the detection of SARS-CoV-2 infection in symptomatic patients using a point-of-care (POC) rtRT-PCR by evaluating the test indices for the RAT.

MATERIALS AND METHODS

We collected 50 nasopharyngeal swab samples from an array of samples collected from symptomatic patients who visited the Teaching Hospital, Peradeniya, from March to August 2021 and were referred to POC-rtRT-PCR (Molbio-Trueprep Auto POC RT-PCR, Molbio Diagnostics Private Limited, India) testing for the laboratory diagnosis of COVID-19. The study samples included 25 positive and 25 negative samples tested for COVID-19 by POC-rtRT-PCR. These samples were subjected to RAT (STANDARD™ Q COVID-19 Ag rapid test kit, SD biosensor Inc., Republic of Korea) and the results obtained for these tests were used to calculate and compare the test indices [sensitivity, specificity, Positive Predictive Value (PPV) and Negative

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Predictive Value (NPV)] using MS Excel 2010 and MedCalc statistical package (MedCalc Software Ltd, Ostend, Belgium online).

$$\text{Sensitivity} = TP / (TP + FN)$$

$$\text{Specificity} = TN / (TN + FP)$$

$$\text{Positive Predictive Value (PPV)} = TP / (TP + FP)$$

$$\text{Negative Predictive Value (NPV)} = TN / (FN + TN)$$

TP: True positives; TN: True negatives; FP: False positives; FN: False negatives

The ethical clearance for the study was exempted by the Ethics Review Committee of the Postgraduate Institute of Science, University of Peradeniya.

RESULTS

Test results of the POC-rtRT-PCR and the RAT for testing for the SARS-CoV-2 infection and the test indices for the RAT are summarized in Tables 1 and 2, respectively. Of the 25 POC-rtRT-PCR positive samples, only 12 were positive (sensitivity = 48%) for the RAT. Of the 25 POC-rtRT-PCR negative samples, 24 were negative (specificity = 96%) for the RAT (Tables 1 and 2). The PPV and NPV indicated that RAT has a lower likelihood of false positive but a higher likelihood of false negative test results (Table 2). The Ct values for the POC-rtRT-PCR positive samples ranged from 10.9 to 33. The RAT showed 100% sensitivity for samples with high viral load (Ct value: 10.9–20.3; $n = 12$). The samples with Ct values ≥ 21.2 ($n = 13$) were identified as negative for SARS-CoV-2 by the RAT.

Table 1: Rapid Antigen Test (RAT) and POC-rtRT-PCR results for the detection of SARS-CoV-2 in symptomatic patients' samples ($n = 50$)

	POC-rtRT-PCR		
	Positive	Negative	Total
STANDARD™ RAT Positive	12	01	13
STANDARD™ RAT Negative	13	24	37
Total	25	25	50

Table 2: Test indices of Rapid Antigen Test (RAT) compared to POC-rtRT-PCR for the detection of SARS-CoV-2 ($n = 50$)

Test indices	Values (95% CI)
Sensitivity	48% (27.80–68.69%)
Specificity	96% (79.65–99.90%)
Positive likelihood ratio	12.00 (1.68–85.47)
Negative likelihood ratio	0.54 (0.37–0.80)
Disease prevalence in the population	50% (35.53–64.47%)
Positive Predictive Value (PPV)	92.31% (62.75–98.84%)
Negative Predictive Value (NPV)	64.86% (55.68–73.07%)
Accuracy	72% (57.51–83.77%)

DISCUSSION

Although rtRT-PCR is the gold standard for the detection of SARS-CoV-2 RNA (Dhama et al., 2020), RATs have been approved for the diagnosis of COVID-19 due to their less turn-around time and technical simplicity (Smith-Jeffcoat et al., 2024). However, their sensitivity varies in different populations during different stages of the infection. Hence, testing the RATs in different settings is important, along with rtRT-PCR for the laboratory diagnosis of COVID-19.

According to the present study results, the sensitivity of the RAT tested was 100% for POC-rtRT-PCR positive samples with high viral load (Ct values: 10.9–20.3; $n = 12$), whereas the sensitivity dropped to 48% when all POC-rtRT-PCR positive samples ($n = 25$) were tested. The POC-rtRT-PCR positive samples with low to moderate viral load (Ct values: 21–33; $n = 13$) were tested negative by the RAT. The manufacturer's claim on sensitivity (96.52%) is in agreement with the highly positive samples only. In general, 48% sensitivity by the current study indicates the inferior detection capacity of the RAT for early and late phases of SARS-CoV-2 infection, as also reported by other studies (Gremmels et al., 2021; Porte et al., 2020). The current study showed that the assay would miss more than 50% of the true positives, increasing the incidence of false negatives (NPV = 64.86%). However, Brihn et al (2021) report that the sensitivity increases from asymptomatic to symptomatic individuals, hence, confirming the RAT results with rtRT-PCR during high prevalence of COVID-19 will help reduce the false negatives.

On the other hand, high sensitivity is crucial for the RAT-based algorithms, particularly in resource-limited settings (Porte et al., 2020). The current study shows 100% sensitivity for samples with high viral load, as suggested by Porte et al (2020) and 96% specificity for all the POC-rtRT-PCR positive samples, in agreement with the manufacturer's claim (specificity = 99.68%). Despite the higher specificity and PPV (92.31%), one negative sample was identified as positive by the RAT. Although it was a false positive, for a pandemic, it is still helpful in not missing a chance to contain the spread of the infection. Moreover, rapidity, cost-effectiveness and simplicity of the RAT make it useful for SARS-CoV-2 detection and surveillance (Porte et al., 2020).

CONCLUSION

The outcomes showed that the RAT could be used in the diagnosis of COVID-19 for patients with high viral load without significantly compromising the detection ability of the test. In those with low to moderate viral load, the use of rapid rtRT-PCR will reduce false-negative results and help in timely and accurate diagnosis.

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DECLARATION OF CONFLICT OF INTEREST

The authors have nothing to disclose and declare no conflicts of interest.

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AUTHOR CONTRIBUTION

Conceptualization, Supervision, Writing–review & editing: **F.N.**; Investigation, Data curation, Formal analysis, Writing–original draft: **J.P.P.**; Formal analysis, Writing–original draft: **B.N.I.**; Formal analysis, Writing–original draft: **S.R.M.S.**; All authors have read, approved and agreed to the published version of the manuscript .

DATA AVAILABILITY STATEMENT

All data are included in the manuscript, however, data is available from the authors upon reasonable request.

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