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Research Article

Transcriptase Polymerase

Comparative Evaluation of Rapid Antigen Detection with Reverse Transcriptase Polymerase Chain Reaction for Detection of Novel SARS-CoV-2

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Introduction: Swift and precise detection of SARS-CoV-2 is essential for managing outbreaks both within communities and hospitals. Real-time reverse transcriptase polymerase chain reaction (rRT–PCR) stands as the benchmark diagnostic test for SARS-CoV-2. However, its reliance on specialized equipment and technical expertise, alongside the necessity for a sophisticated laboratory, limits its widespread use. Rapid antigen tests have emerged as convenient point-of-care diagnostic assays. Evaluating the diagnostic accuracy of these tests compared to RT-PCR is crucial. While numerous studies have been conducted for this purpose globally, many have assessed performance using separate samples, potentially leading to variations in findings.

Aim: In our study, we aimed to comparatively assess rRT-PCR and Rapid Antigen Tests, with rRT-PCR considered the gold standard, by conducting both tests using samples collected in the same Viral Transport Medium (VTM) Tube.

Materials and Methods: We collected a total of 300 nasopharyngeal/oropharyngeal swabs from patients suspected of having COVID-19. Rapid antigen tests were performed directly from the tube using the STANDARD Q COVID-19 Ag test. RT-PCR of the sample was conducted post-RNA extraction. Both tests were performed using the same VTM tube.

Results: The rapid antigen detection test (RADT) demonstrated a sensitivity and specificity of 86% and 90%, respectively. The positive predictive value (PPV) and negative predictive value (NPV) of RADT were 91% and 88%, respectively.

Conclusion: RADT conducted directly from VTM exhibited high sensitivity and specificity, suggesting its potential utility during pandemics.

Keywords: SARS CoV-2, RTPCR, RADT, Pandemic, Point of care

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Introduction

Coronaviruses (CoVs) encompass a diverse array of viruses infecting various animal species, capable of causing respiratory infections ranging from mild to severe in humans [1]. Notably, two highly pathogenic CoVs, namely the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, emerged in humans, causing fatal respiratory illnesses [2,3]. However, SARS-CoV-2 has eclipsed both SARS and MERS in terms of the number of individuals infected and the global spread [4]. Diagnostic methods for SARS-CoV-2 include reverse transcriptase polymerase chain reaction (rRT-PCR), TrueNat, CBNAAT (cartridge-based nucleic acid amplification test), rapid antigen detection (Standard Q COVID-19 Ag detection kit), and rapid antibody detection tests. Currently, rRT-PCR serves as the gold standard molecular diagnostic technique for COVID-19 detection. Its advantages lie in both its detection accuracy and the capacity to process up to 96 samples in a single run, with a turnaround time of 4-5 hours. However, all these platforms necessitate specialized laboratory infrastructure in terms of equipment, biosafety, and biosecurity. Rapid antigen tests have emerged as point-of-care diagnostic assays, complementing the gold standard RT-PCR test. These tests operate as rapid chromatographic immunoassays, qualitatively detecting specific antigens of SARS-CoV-2. Nonetheless, many studies worldwide have assessed performance using two separate samples, potentially introducing variation in findings. To mitigate this sample bias and evaluate the performance of antigen detection assays for diagnosing SARS-CoV-2, our study was conducted using swabs collected from the same VTM tube.

Methodology

Study design and settings: This observational study was carried out in the Department of Microbiology of a tertiary care hospital in North India for a period of 18 months from November 2020 to May 2022.

Inclusion criteria: All symptomatic patients suspected of having COVID-19 infection, all asymptomatic direct and high-risk contacts of a confirmed case and all asymptomatic patients who had to undergo any surgical or invasive procedure, All pregnant females or people who had to travel were included in the study [5].

Exclusion criteria: Patients previously positive for SARS-CoV-2 were excluded from the study.

Sample collection and processing:

Nasopharyngeal/oropharyngeal swabs were collected from patients suspected of having COVID-19 in VTM tubes. The standard protocol for the collection of nasal and oral swab samples as given by the WHO was followed [6].

Swabs were placed immediately into a sterile transport tube containing 2-3ml of the viral transport medium (VTM). The tubes were labelled with the patient's name, ID number and date of sample collection and all the required information regarding the patient was recorded in the proforma given by ICMR.

The collected samples were transported to the laboratory and RADT was done immediately and in case of any delay, the tubes were stored at 4-8^{o}C till further processing for RT-PCR.

Rapid antigen test:

RADT was performed as per the manufacturer's instructions with few modifications. Using a micro-pipette, 350µl of specimen was collected from the VTM tube containing the swab.

The specimen was mixed with an extraction buffer provided with the kit. The nozzle cap was pressed tightly onto the tube. Three drops of extracted specimen were then applied to the specimen well of the test device and results were read in 15-30 minutes.

The presence of a coloured band in the top section of the result window i.e. the control line 'C' indicated that the test has been performed correctly. A coloured band (of any intensity) that appears in the lower section of the result window was taken as positive for SARS-CoV-2.

Real-time reverse transcriptase polymerase chain reaction for detection of SARS-CoV-2:

RNA was extracted either manually or using the automated method as per manufacturer instructions. This step was followed by performing rRT-PCR as per the instruction manual using different kits like Biosome, Meril, and SEE gene as per the availability.

All samples exhibiting curves that crossed the cycle threshold (Ct) line at or below 35 cycles were taken as positive for the presence of SARS CoV-2 virus and Ct value > 35 was considered as negative for SARS CoV-2.

Ethical Clearance: Ethical clearance to conduct the study was given by the institutes' ethical committee bearing no: IEC/SKIMS Protocol # 79/2021.

Statistical Analysis: Statistical analysis was done by using SPSS software v24. All categorical data are in the form of frequency and percentage. Also, continuous data are analyzed by knowing the normality of the distribution and using proper parametric and non-parametric tests i.e., chi-square test, and ANOVA. All values are discussed at a 5% level of significance ($P< 0.05$).

Results

Table 1 shows the various demographic and clinical characteristics of the patients included in the study. A total of 300 nasopharyngeal/oropharyngeal swabs were collected from patients suspected of having COVID-19 infection during the study period (Nov 2020 to May 2022). Most of the samples, belonged to male patients, whereas 105 (35%) samples were collected from female patients.

The majority of the samples were received from patients in the age group of 20-39 years (n=107, 35.7%) followed by 40-59 years (n=98, 32.7%), ≥60 years (n=73, 24.3%) and 0-19 years (n=22, 7.3%).

The majority of the samples belonged to patients residing in urban areas, 168 (56%) whereas 132 (44%) samples were received from patients residing in rural areas.

Amongst the 300 patients, 73 (24.3%) were fully vaccinated, 107 (35.7%) had received the first dose of the vaccine and 120 (40%) were unvaccinated against COVID-19.

Among the 300 cases, 220 (73.3%) samples were from symptomatic patients whereas 80 (26.7%) were from asymptomatic patients. The most common presentation in the symptomatic patients was fever, 62 (28.2%) followed by cough, 47 (21.4%); sore throat, 32 (14.5%); myalgias, 27 (12.3%); breathlessness, 24 (10.9%); diarrhoea 16 (7.3%) and loss of taste or smell, 12 (5.5%).

Duration of symptoms was less than one week for 181 (82.3%) patients whereas it was more than one week in 39 (17.7%) patients.

Table 1: Baseline demographic and clinical characteristics of patients

Variable		No. of cases/Percentage	Positive samples	
			RTPCR	RADT
Gender	Male	195(65%)	126 (66.7)	94 (67.1)
	Female	105(35%)	63 (33.3)	46 (32.9)
Age group	0 to 19	22(7.3%)	15 (7.9)	6 (4.3)
	20 to 39	107(35.7%)	53 (28.0)	43 (30.7)
	40 to 59	98(32.7%)	62 (32.8)	49 (35)
	≥60	73(24.3%)	59 (31.2)	42 (30)
Duration of symptoms	< 1 week	181 (82.3%)	114 (85.7)	103 (95.4)
	> 1 week	39 (17.7%)	19 (14.3)	5 (4.6)
Comorbidities	Hypertension	63 (60%)	46 (43.8)	38 (36.2)
	Type 2 diabetes mellitus	28 (26.7%)	22 (20.9)	22 (20.9)
	Chronic kidney disease	11 (10.5%)	11 (10.5)	8 (7.6)
	Malignancy	2 (1.9%)	-	-
	Chronic liver disease	1 (0.9%)	-	-
Vaccination Status	Vaccinated (two doses)	73(24.3%)	27 (14.3)	13 (9.3)
	Non-vaccinated	120(40%)	104 (55)	88 (62.8)
	1st dose of vaccine	107(35.7%)	58 (30.7)	39 (27.9)

In our study out of 300 cases, 105 (35%) had an associated co-morbid condition, the most common being hypertension, 63 (60%) followed by type II diabetes mellitus, 28 (26.7%) and chronic kidney disease (CKD), 11 (10.5%). Two patients had malignancy (1.9%) and one patient had chronic liver disease (CLD) (0.9%) A total of 189 (63%) samples were positive and 111 (37%) negative for SARS CoV-2 by rRT-PCR. On the other hand, 140 (46.7 %) samples were positive and 160 (55.3%) negative for SARS CoV-2 by RADT (Table 2). Out of the 189 samples positive by rRT-PCR, 112 were positive by both rRT-PCR and RADT, whereas 77 samples positive on rRT-PCR were RADT negative. Furthermore, 28 samples negative by rRT-PCR were positive on RADT and 83 samples were negati both the tests under study (Table 2). The sensitivity and specificity of RADT were 86% and 90%

respectively. The PPV and NPV of RADT were 91% and 88% respectively.

Table 2: Comparison of the positive and negative cases by rRT-PCR and RADT

		rRT-PCR		Total
		Positive	Negative	
RADT	Positive	112	28	140
	Negative	77	83	160
	Total	189	111	300

Table 3: Comparison of duration of symptoms and CT values of the rRT-PCR/RADT positive and negative samples

	No.	Mean ± S.D of duration of symptoms	T-value	P-value	Mean ± S.D of CT values	T-value	P-value
rRT-PCR +ve/RADT +ve	112	3.24 ± 1.96	7.84	0.001	22.79 ± 4.54	6.91	0.001
rRT-PCR +ve/RADT -ve	77	5.70 ± 2.27			27.16 ± 3.79		

In 181 symptomatic patients in whom duration of symptoms was <1 week, 114 (85.7%) were positive for SARS CoV-2 by rRT-PCR and 103 (95.4%) patients were positive by RADT. On other hand among 39 symptomatic patients in whom duration of symptoms was >1 week, 19 (14.3%) were positive by rRT-PCR and 5 (4.6%) were positive by RADT (Table 3). The mean duration of symptoms of the patients who tested positive by both rRT-PCR and RADT was 3.24±1.96 in comparison to patients who tested positive by rRT-PCR but negative for RADT it was 5.70±2.27. The difference between these two groups was statistically significant (*P*-value = 0.001) (Table 3). The mean CT value of 112 samples that were positive by rRT-PCR and RADT was 22.79±4.54, whereas 77 samples that were rRT-PCR positive but RADT negative had a mean CT value of 27.16±3.79. The difference between these two groups was statistically significant (*P*-value=0.001) (Table 3).

Discussion

The utilization of Rapid Antigen Detection Tests (RADTs) for COVID-19 diagnosis stands as a pivotal component in disease prevention beyond healthcare settings. These tests, accessible to public, boast simplicity in interpretation and cost-effectiveness. In several studies conducted nationwide, a sample bias was introduced as two separate samples were collected—one for RADT and other for rRT-PCR.

In our investigation, both tests were conducted using swabs from a single Viral Transport Medium (VTM) vial. Out of the 300 samples, 63% tested positive and 37% tested negative for SARS-CoV-2 via rRT-PCR, while 46.7% tested positive and 55.3% tested negative via RADT. RADT demonstrated a sensitivity of 86% and specificity of 90%, with a positive predictive value (PPV) of 91% and a negative predictive value (NPV) of 88%. Our findings align with those of Porte L et al., showcasing comparable sensitivity and specificity of RADT [7]. However, Halfon P et al. reported a lower sensitivity of 72% compared to RT-qPCR, with specificity, PPV, and NPV at 99%, 99%, and 78%, respectively [8]. Amador PM et al. also noted a lower sensitivity but high specificity of RADT in their study [9]. Additionally, Pena M et al. found lower sensitivity but high specificity, PPV, and NPV in asymptomatic patients [10]. Farhana A et al. reported sensitivity, specificity, PPV, and NPV of RADT at 84.0%, 94.7%, 92.3%, and 88.7%, respectively, using the STANDARD Q COVID-19 test kit [11]. Similarly, Chaimayo C et al. reported comparable sensitivity and specificity using the STANDARD Q COVID-19 test kit. Most of the samples in our study were from urban areas, consistent with the observations of Samantaray S et al. [12]. rRT-PCR and RADT yielded more positive results among male patients, with the majority of positive cases in the 40-59 age group, as observed in other studies. The presenting symptoms predominantly included fever and cough, consistent with findings from other investigations.

In our study, 82.3% of patients exhibited symptoms for less than one week, with RADT demonstrating slightly higher positivity among patients with symptoms lasting less than one week compared to rRT-PCR. However, rRT-PCR detected more cases among patients with symptoms lasting over one week. This pattern is consistent with findings from Kritikos et al. [18]. The mean duration of symptoms in patients positive for SARS-CoV-2 by both rRT-PCR and RADT was 3.24 ± 1.96 days, while patients positive by rRT-PCR but negative by RADT had a mean duration of 5.70 ± 2.27 days. Regarding Ct values, RADT performed better when the value was low, consistent with findings from Selvabai AP et al. [20]. Pickering S et al. also observed high specificity at a Ct value of 23.7, with sensitivities increasing to over 90% for samples with values below 25 [21]. Systematic reviews and meta-analyses by Lee J et al. and Brihn A et al. echoed these results [19,22]

Conclusion

Hence, from this study we conclude that rRT-PCR was the most sensitive and effective method to diagnose SARS CoV-2 infection, however, RADT showed a high sensitivity and specificity and can be of potential help in such pandemic situations when the resources are limited. Related studies have been conducted but with this study we have eliminated the sample bias by conducting both tests using samples collected in the same Viral Transport Medium.

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Conflict of Interest:

The authors declare no conflict of interest.

Author contribution statement:

Uksim Qadri: Sample collection, Performed RADT and RT-PCR, Investigation, Writing- original draft, review and editing. **Bashir Ahmad Fomda**: Conceptualization, Methodology, Funding acquisition, Supervision. **Nargis Bali**: Writing- review and editing, Formal analysis, Supervision. **Saleem Javaid Wani:** Resources, Data curation, Software, Writing- review and editing.

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