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Original Research Article

Diagnostic utility and performance of rapid antigen test in SARS CoV- 2 in symptomatic and asymptomatic patients during the second pandemic wave in Kashmir, North India

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ABSTRACT

Purpose: Real time reverse transcriptase PCR (rRT PCR) although gold standard test for the diagnosis of SARS CoV-2, carries disadvantages of a sophisticated set up, long time to results and centralized services. The rapid antigen tests (RAT) can be used as a primary screening tool with the advantages of rapid turnaround time and ease of use. The study was conducted to determine the performance of rapid antigen test (standard Q COVID 19 Ag) in comparison to rRT PCR in symptomatic patients and asymptomatic contacts and asymptomatic patients with no apparent contact history.

Methods: Nasopharyngeal swabs taken in duplicate from 1034 patients were collected over a 5 months period. These included 248 (23.98%) symptomatic, 386 (37.34%) asymptomatic contacts and 400 (38.68%) asymptomatic subjects who were routinely screened in pre-operative period, as a prerequisite for travel, or pregnant females. Both rRT PCR and RAT were performed as per manufacturers' instructions. Performance of test in different subgroups of patients was evaluated. Performance of RAT test on basis of duration of illness and Ct values was also analyzed.

Results: In this study, 445 (43.04%) were rRT PCR positive, out of which 374 samples were RAT positive as well. 31 samples were RAT positive but PCR negative. The sensitivity, specificity, PPV and NPV of the rapid antigen test was 84.04%, 94.74%, 92.35% and 88.71% respectively. The negative predictive value of the test in asymptomatic patients without any significant contact history was 97.07%.

Conclusions: This study recommends the use of the antigen test as a method of diagnosis for SARS CoV-2. However a negative result with RAT in suspected patients and their contacts should be viewed with caution. This study also finds the utility of using RAT test in the community settings as a screening test in schools, colleges and mass gatherings.

1. Introduction

Ever since the pandemic of COVID 19 started, a lot of focus had been on the development of diagnostic modalities to accurately identify the causative agent SARS CoV-2. Timely detection of the infection is important in identifying cases and their contacts in order to interrupt the transmission of disease [1,2]. Although real time reverse transcriptase polymerase chain reaction (rRT PCR) is the gold standard for diagnosis of COVID -19, it requires sophisticated laboratory set up, highly trained personnel, is labor intensive and time consuming with a reportable time of 24–48 h. Despite strengthening of molecular laboratories across the country, there are considerable challenges faced by such laboratories due to centralized services and escalated demand in testing. There is need of a point of care test that is sensitive and specific, requires minimal technical skill, can be used to test large number of samples in a short time and can be performed at primary health care level as a screening tool [3,4]. Antigen tests are used to detect the presence of specific viral antigens in the patients' sample. They are the indicators of current infection while rRT PCR tests can remain positive for longer periods due to shedding of genomic and subgenomic RNA. Use of such rapid tests can improve access to testing in the community and can be used for surveillance [5–7].

The Standard™ Q Covid-19 antigen kit test was the first rapid antigen test (RAT) approved by Indian Council of Medical Research (ICMR) for identification of SARS CoV-2. This test is a lateral flow based

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immunoassay that can qualitatively detect the presence of nucleocapsid antigen in the specimen. The manufacturers recommend its use in symptomatic patients and their contacts [8].

Testing for asymptomatic patients is equally important as they are contagious and act as silent spreaders of infection in the community. Therefore, besides symptomatic suspects and their contacts, it is equally important that testing be expanded to include the asymptomatic population. To our knowledge, there are very few studies that have evaluated the role of RAT in different study groups; symptomatic patients and their asymptomatic contacts and the persons who were tested as a part of screening policy. This study was done with the aim to evaluate the diagnostic performance of rapid antigen test (Standard Q COVID 19 Ag) in different study groups; symptomatic patients and their asymptomatic contacts and asymptomatic population with no apparent contact history.

2. Materials and methods

2.1. Study design and setting

This cross sectional, single blinded study was conducted in the Department of Microbiology, Government Medical College, Srinagar. The laboratory has been actively involved in testing of suspected COVID-19 patient since March 2020 after getting approval from ICMR. This study was carried out over a period of 5 months from December 2020 to April 2021. At the time of this study, the valley witnessed an unprecedented increase in the number of cases and this subsequently marked the onset of second pandemic wave. Patients who visited the collection center at SMHS hospital, an associated hospital of GMC, Srinagar in order to get tested and consented to participate were included in the study. These patients were divided into three categories:

1. Symptomatic group that included patients suspected of having COVID- 19 infection.
2. Asymptomatic contact group that included persons with known exposure to a laboratory confirmed COVID- 19 case.
3. Asymptomatic screening group: patients screened for surgical procedure, pregnant women and travel purposes.

People who had COVID- 19 infection in the past or had come for follow up sampling were excluded from the study.

2.2.1. Ethical clearance

The study protocol was approved by the institutes' ethical clearance committee (IEC-GMC-Sgr/41)). An informed consent was obtained from all the study subjects.

2.2. Specimen collection

For RT-PCR, nasopharyngeal swabs were collected from patients and put in viral transport medium and transported to the laboratory at 4 °C. The samples were processed in BSL-3 facility on the same day. For rapid antigen testing, another nasopharyngeal swab was taken by the same lab personnel and the test was performed immediately at site of collection itself. The technical staff was trained to interpret the results accurately.

2.3. Real time RT PCR testing

RNA extraction: Once the sample was received in the laboratory, RNA was extracted by automated RNA extraction system (nextractor NX-48S) using genolution extraction kit or manual method using PureLink™ Viral RNA/DNA Mini Kit (Invitrogen) depending upon the availability as per the manufacturer's recommendations. Viral RNA was eluted and used for rRT PCR assay. An aliquot of residual sample was stored at −70 °C.

Real time RT- PCR assay: Detection of SARS-CoV-2 in specimens was done by commercial multiplex PCR kit (LabGun™ COVID-19 RT-PCR Kit) which targets E gene and RdRp gene. Amplification was done using

CFX-96 real time thermocycler (Biorad laboratories). Assay performance was monitored using positive template controls, no-template controls, and internal controls in all runs. A run was considered valid when all controls gave the expected results. Specimen was considered positive for SARS-CoV-2 when both E gene and RdRp plots crossed the threshold line within 35 cycles ($C_t < 35$). If only the internal control was present, the test result was interpreted as negative. If all 3 plots for E, RdRp and IC were not seen, RNA extraction and amplification of the same sample was repeated. If retest results were the same as initial test, the samples were rejected and resampling of the patient advised.

2.4. Antigen testing

The antigen testing was done using the Standard™ Q Covid-19 antigen kit (SD Biosensor, Inc). The test was performed according to the manufacturers' instructions. Briefly, after collection, the nasopharyngeal swab was put in the tube containing extraction buffer and squeezed while taking out. After putting the nozzle, 3 drops of buffer was added to the cassette. The test result was read in 15–30 min. A sample was considered positive, if the violet band appeared at both the control line and test line. If the band appeared at only the control line, it was interpreted as negative. If no band was seen, the result was considered invalid and repeat testing was done. The test was interpreted as positive even if the control line was faint or test line was not uniform.

2.5. Demographic data

Patient details were collected in a preformed structured proforma. These included demographic data such as name, age sex, residence, and reason for testing. Patients' symptoms and its duration were also noted in the symptomatic group.

2.6. Statistical analysis

Data was recorded in Microsoft excel. Continuous variables such as age, gender were interpreted as mean or median while categorical variables were interpreted as numbers, percentage and 95% confidence interval. The sensitivity, specificity, positive predictive value and negative predictive value of RAT was calculated with RT PCR as reference standard using open epi software (version 3.01) available online.

3. Results

A total of 1050 patients were included in the study from whom paired nasopharyngeal swabs were taken. Out of these, details were not available for 12 patients and 4 gave invalid results and were excluded from the study. The eligible patients in the study were 1034. The mean age of the patients included in the study was 37.55 years (range 0–95 years) and majority of them were males (n = 674; 65.18%) (Fig. 1). Among the subjects included in the study, 248 (23.98%) were symptomatic, 386 (37.34%) patients were asymptomatic contacts and 400 (38.68%) asymptomatic subjects with no apparent contact history. The most common presenting complaint was fever (79.8%) followed by sore throat (36%), cough (35.5%) and fatigue (32.26%). Patients with more than one symptom were 176 (70.97%) and 169 (68.15%) presented within 5 days of illness.

Of the total 1034 patients, 445 (43.04%) were positive by rRT PCR whereas 589 (56.96%) were negative. The positivity rate in the study was around 43.04%. The rRT PCR positive cases included 175 (39.33%) symptomatic persons, 186 (41.8%) contacts of COVID positive patients and 84 (18.81%) routinely screened persons. Of the 445 samples that came positive by rRT PCR, 374 (84.04%) samples were RAT positive as well whereas 71 (15.96%) came RAT negative. Of the 589 rRT PCR negative samples, 31 (5.26%) came positive on RAT testing and 558 (94.74%) came negative (Fig. 2). The sensitivity, specificity, PPV and NPV of the RAT was 84.04%, 94.74%, 92.35% and 88.71% respectively
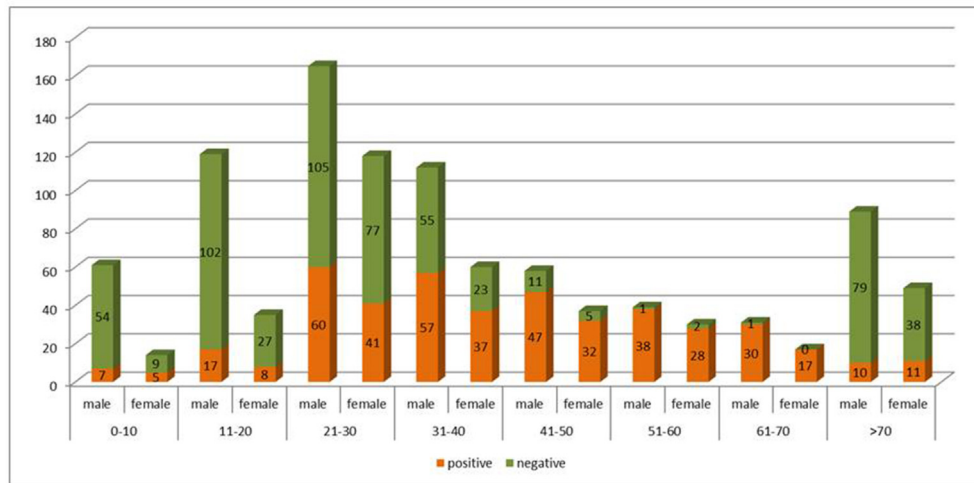
Fig. 1. Age and gender characteristics of total and positive patients. Footnote to Fig. 1: * 3 infant under the age group 0–10 included in the study. No neonate included in the study.
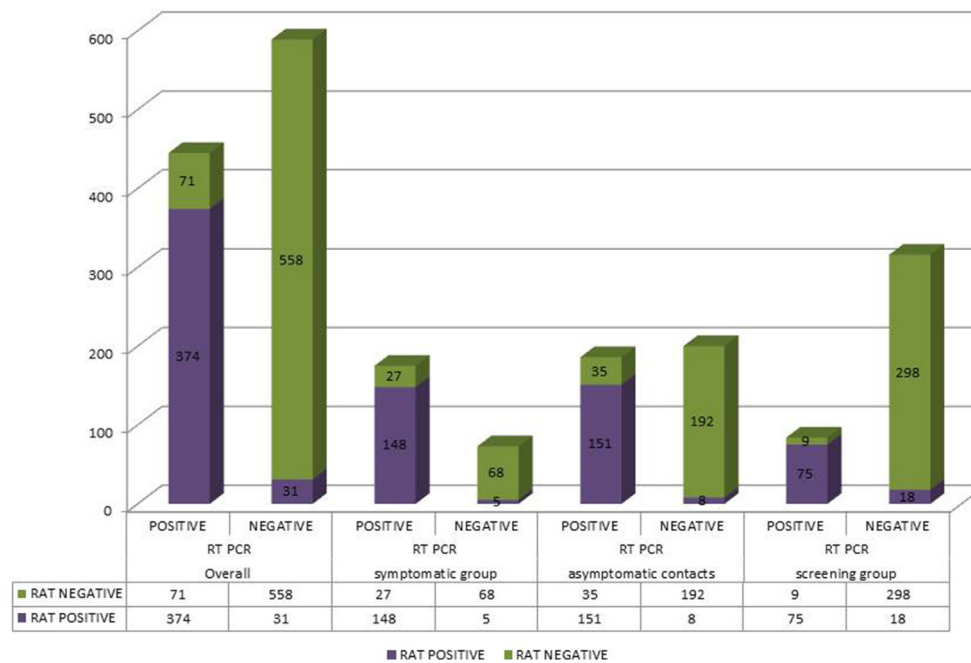

	RT PCR Overall		RT PCR symptomatic group		RT PCR asymptomatic contacts		RT PCR screening group	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
RAT NEGATIVE	71	558	27	68	35	192	9	298
RAT POSITIVE	374	31	148	5	151	8	75	18

Fig. 2. Diagnostic performance of RAT in comparison to rRT PCR in different subgroups.

(Table 1). When observed individually in the symptomatic, asymptomatic contacts and screening groups of patients, the variables did not change much except that the NPV and PPV was slightly lower in the symptomatic group and screening group of patients respectively. In the symptomatic group, out of 175 RT PCR positive subjects (125 and 50 presenting before and after 5 days of illness respectively), 148 were both RT PCR and RAT positive. Of these 148, 118 (94.4%) presented within 5 days of illness while 30 (60%) presented after 5 days of illness. The diagnostic accuracy of the test was 90.14%.

Of the 71 samples that gave false negative RAT, 27 of these patients were symptomatic, 35 were contact of COVID-19 positive patients while 9 were routinely screened. Of the 31 samples that gave false positive RAT, 5 were symptomatic, 8 were contact of COVID-19 positive patients while 18 were routinely screened. The study team tried to contact all the patients that gave RT PCR negative and RAT positive results for retesting. Only 9 of them agreed for retesting on the following day. These included 2 symptomatic, 2 asymptomatic contacts and 5 from the screening group.

All these patients came negative on retesting. Other 10 patients gave a repeat sample for RT PCR after 7 days of the first report which was not included in the study. However, the 11 patients were lost to follow up.

Of the cases that tested positive by rRT PCR, the average cycle of threshold (Ct) value for E gene was 26.17 ± 5.27 (min 13.15; max 35.2) and RdRp was 26.19 ± 5.66 (min 12.86; max 35.05). On comparison of viral load of the samples detected by the Ct value and the positivity of RAT, it was found that the samples with low Ct on rRT PCR testing were also positive on rapid antigen testing. The diagnostic sensitivity was 100%, 93.65%, 82.71% and 62.62% respectively for Ct < 20, 21–25, 26–30 and > 30. It was observed that as the Ct value of the rRT PCR increased, the positivity rate of RAT proportionately decreased (Fig. 3).

4. Discussion

In the event of COVID pandemic where testing of large population is necessary and rRT PCR testing facilities are overstretched and working to

Table 1
Diagnostic performance of the rapid antigen test with comparison to PCR as reference standard.

	Overall performance	Symptomatic group	Asymptomatic contact group	Asymptomatic screening group
Sensitivity	84.04% (95% CI 80.35–87.15)	84.57% (95% CI 78.48–89.17)	81.18 (95% CI 74.96–86.15)	89.29 (95% CI 80.88–94.26)
Specificity	94.74% (95% CI 92.63–96.27)	93.15 (95% CI 84.95–97.04)	96 (95% CI 92.31–97.96)	94.3 (91.18–96.37)
PPV	92.35% (95% CI 89.34–94.56)	96.73 (95% CI 92.58–98.6)	94.97 (95% CI 90.39–97.43)	80.65 (95% CI 71.47–87.39)
NPV	88.71% (95% CI 86–90.95)	71.58 (95% CI 61.81–79.67)	84.58 (95% CI 79.31–88.7)	97.07 (95% CI 94.52–98.45)
Diagnostic accuracy	90.14% (95% CI 88.17–91.81)	87.1 (95% CI 82.35–90.71)	88.86 (95% CI 85.33–91.62)	93.25 (95% CI 90.36–95.32)
Cohen value	0.7966	0.7145	0.7758	0.8043

their maximum capacity, it is necessary to have a point of care test with good sensitivity and specificity. In this study, a rapid antigen test was evaluated against a commercially available real time rRT PCR kit for detection of SARS CoV-2 [9–11].

The positivity rate in our study was 43.04%. The reason for this was that the study was conducted at a time when the state witnessed the second wave of SARS CoV-2 pandemic. The sensitivity and specificity of the RAT in our study was found to be 84.04% and 94.74% respectively. The average sensitivity and specificity of SD Biosensor in several studies has been 79.3% and 98.5% respectively while that reported by the manufacturer is 84.38% and 100%. While many studies have reported low sensitivity of the RAT, our studies demonstrates a fairly acceptable one [12–15]. Although the manufacturers have recommended testing in symptomatic patients and their contacts only, this study was also performed in asymptomatic patients with no apparent positive contact history. The results obtained were comparable and did not alter the overall sensitivity and specificity of the test in our study. This is in contrast to the observation by Dinnes et al. that showed marked differences in sensitivities between symptomatic (72.0%, 95% CI 63.7%–79.0%) and asymptomatic patients (58.1%, 95% CI 40.2%–74.1%). The low sensitivity of RAT in the community setting has also been reported by Jeewandra et al. and A. Fernandez-Montero et al. [16–18].

The NPV of the test in symptomatic group was lower compared to the overall NPV of the test. This has been explained by Peeling et al. who suggested that in a community with prevalence of infection exceeding >36% such as ours, the NPV of the test will decrease leading to unacceptable number of false positive results. Similarly, the PPV of a test will decrease as the likelihood of infection decreases which explains the low PPV of the test in screening group of patients in our study. The test has a

fairly high NPV and can be employed to test in schools, colleges, mass gatherings and for travel purposes [6].

False negative RAT test was seen in 71 patients. A negative test can occur if the antigen concentration in the specimen is below the limit of detection. This is dependent on variables such as adequacy of sample collection, extraction of antigen in the buffer solution, timing of the testing and the viral load in the specimen. It has been observed that the sensitivity of rapid antigen test has an indirect correlation with the Ct value of the rRT PCR test (indirect determinant of viral load). Our study demonstrated the Ct values ranging from 22 to 35. In our study, false negative results were observed in 20 out of 50 symptomatic patients who reported after 5 days of illness. This occurs because in symptomatic person, the viral load is usually low before symptom onset and in the late course of infection when the viral loads and thereby the viral antigen have decreased to levels that cannot be detected by RAT. It is recommended that in suspected patients, the negative rapid antigen test should be confirmed by molecular assays to maximize the detection of cases [19–21]. Taking RT PCR as the gold standard, false positive rapid antigen test was found in 31 patients. Various reasons have been suggested for false positive antigen test including cross reactivity with other pathogens [12,15].

5. Conclusion

This study recommends the use of RAT in the diagnostic algorithms in view of acceptable sensitivity and specificity. The study confirms the use of RAT in rapid identification of patients so that public health measures such as self -isolation and contact tracing can be employed to break the chain of transmission. A negative result with RAT in suspected patients
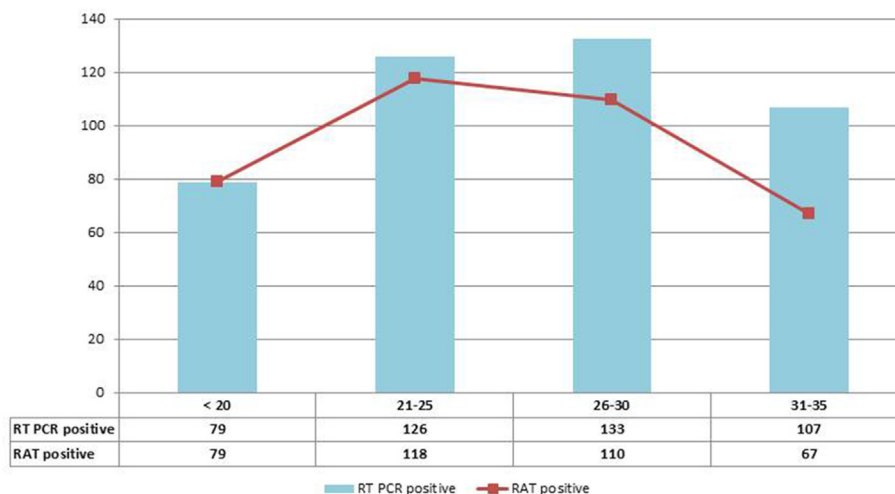

	< 20	21-25	26-30	31-35
RT PCR positive	79	126	133	107
RAT positive	79	118	110	67

■ RT PCR positive ■— RAT positive

Fig. 3. Plot showing correlation of Ct values from RT PCR with the rapid antigen test positivity.

and their contacts should however be viewed with caution and should be followed with RT-PCR to increase probability of detection. This study also finds the utility of using RAT test in community settings as a screening test in schools, colleges and mass gatherings in view of high NPV and high prevalence of the virus.

6. Limitation of the study

First, 31 samples that gave false positive RAT results were not tested for other respiratory viruses to exclude cross reactivity. Second, it was a single centre study wherein participants were recruited from the collection centre. Third, as the prevalence of the SARS CoV-2 is high in our study, this test could be utilized in community settings. However the results need to be further evaluated for the role of RAT in community settings.

Credit author statement

Dr. Anjum Farhana, Conceptualization, Methodology: Dr. Danish Zahoor, Data curation, Methodology , Formal analysis, Resources and software, Writing; Dr. Sanam Wani, Data curation, Formal analysis, Resources and software, Writing; Dr. Reyaz Ahmed Khan, Data curation, Resources and software; Dr. Farhat Kanth, Data curation, Resources and software; Dr. Reyaz Nasir, Data curation, Resources and software.

Declaration of competing interest

The authors' declare no competing interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijmmb.2022.06.007.

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