Original Article

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Microbiology Section

Can Rapid Antigen Tests Lessen the Burden on Testing Laboratories? An Evaluation of the Testing Methods during the COVID-19 Pandemic

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ABSTRACT

Introduction: Timely diagnosis and isolation of cases is of paramount importance to contain the spread of a pandemic. The Coronavirus Disease-2019 (COVID-19) has emerged as a major health problem that needs concerted efforts for mitigation and control. Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR), the gold-standard diagnostic modality, has high cost and can be performed in special laboratories. Rapid Antigen Tests (RAT) has been developed to serve as an alternative and is recommended to use at point-of-care testing.

Aim: To compare the case detection rate of RAT and RT-PCR and the possible role they may play in the pandemic mitigation efforts.

Materials and Methods: In this retrospective study, all the samples collected during a nine-months period were analysed. Depending upon the criteria, either a RAT or RT-PCR was done on the samples. Data was analysed using descriptive statistics (frequencies, mean, standard deviation, and percentages).

Results: A total of 8,29,745 samples were tested during the study period among which number of positive samples was 19,414 giving an overall positivity rate of 2.34% (0.20% to 12.58%). RAT positivity was 1.58% while RT-PCR gave a positivity of 4.26. Total number of positive cases identified by RAT and RT-PCR were 9,382 and 10,032, respectively.

Conclusion: RAT is a low-cost alternative to the expensive RT-PCR with the added advantage of giving accurate and timely results. This can be a game changer especially in low-resource settings, which had witnessed a increase in the spread of COVID-19 during the latter part of the pandemic.

Keywords: Case fatality rate, Rapid antigen testing, Severe acute respiratory syndrome-coronavirus-2, Tests per million

INTRODUCTION

By the end of December 2019, China reported an outbreak of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) which soon progressed to engulf the entire world as COVID-19 pandemic [1]. At the time of writing this article, there have been 500 million confirmed cases and more than six million deaths around the world [2]. The emergence of new variants due to mutations in the structure of the virus had kept the pandemic raging around the world [3]. Timely identification and isolation of COVID-19 patients is of paramount importance to contain the spread of the pandemic [4]. As of now, RT-PCR is the gold standard diagnostic modality for the diagnosis of SARS-CoV-2 infection [5]. The major limitation is that it requires specialised and costly equipment and is time-consuming, taking up upto three days depending on the sample load and positivity [6].

During the later stages of the pandemic, RAT kits were developed as an alternative to the time-consuming RT-PCR tests. RAT uses an immunochromatographic technique which involves fixation of specific antigens on a nitrocellulose membrane that has prefixed antibodies. The antigen-antibody interaction is visible as colour change either manually or by using an immunofluorescence machine reader. Similar tests are available for the diagnosis of Human Immunodeficiency Virus (HIV), Malaria, Influenza, and other diseases [7]. RAT is an inexpensive and simple point-of-care test that provides rapid and reliable results. The rapidity of results is vital in control measures during pandemic mitigation [8].

RT-PCR results usually take one to three days during which asymptomatic patients can further spread the infection in the community. Comparatively, RAT results can help in circumventing this issue of time delay. Additionally, the less cost of RAT makes it an ideal candidate for mass screening in the community [9]. In this study, authors had compared the case detection rate of RAT and RT-PCR for COVID-19 infection in a low-resource division during nine months encompassing two waves of the pandemic. This study compares the RAT and RT-PCR test results from the same geographical area and is one of the first of its kind from this region and demonstrates the prevalence of COVID-19 among the population of a resource limited division in Northern India; therefore, the data generated in this study would enable us to better plan the testing strategies in future. In addition, this study emphasises the use of RAT in resource limited settings and advocates reserving RT-PCR testing for asymptomatic but otherwise vulnerable people so that country's economic sources are utilised more efficiently.

MATERIALS AND METHODS

This retrospective study was conducted at a tertiary care hospital in the Northern Kashmir which caters to a population of more than 10 lakh people. The evaluation period included samples collected from May 2021 to February 2022 and the details of the patients were available with data management cell of the Department of Microbiology, Government Medical College, Baramulla, Jammu and Kashmir, India. Following the advisory issued by the Indian Council of Medical Research (ICMR) on 14th June 2020, RAT was introduced as a point of care screening test for the rapid diagnosis of COVID-19 throughout India (https://www.icmr.gov.in/pdf/covid/strategy/Advisory_for_rapid_antigen_test14062020.pdf) [10].

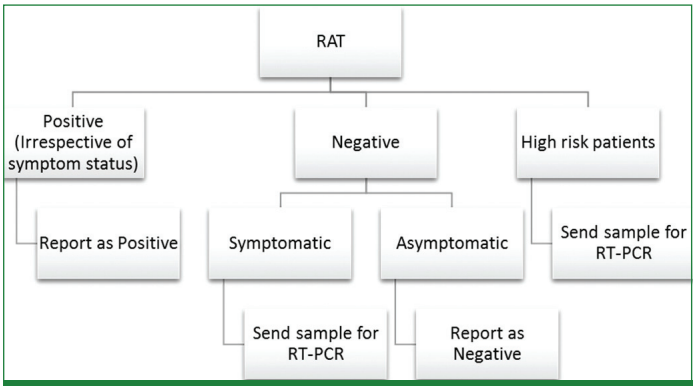
Before sampling, the patients were examined by a doctor, after which a nasopharyngeal swab was taken based on the doctors' advice. The Case Fatality Rate (CFR) was calculated based on the proportion of people confirmed positive for COVID-19, who end up dying of it.

Inclusion criteria: All symptomatic patients who self-reported to the collection centre with symptoms of COVID-19, no longer than five days before testing, were included in the study.

Exclusion criteria: Improperly labelled samples, samples with incomplete details and samples with leakage were excluded from the study.

Setting and Sample

A total of 8,29,745 patients were tested during the study period. All the healthcare workers involved in sample collection and transport were given relevant training regarding the standard operating procedures. Patient identifiers including personal information were removed from the samples to ensure patient confidentiality. The scheme of testing is depicted in [Table/Fig-1].



[Table/Fig-1]: Flowchart showing scheme of testing followed during the study period.

RAT Testing

RAT is an in-vitro diagnostic test based on an immunochromatographic assay designed for the qualitative detection of SARS-CoV-2 antigens. The kit contents are a lateral flow cassette, a sterile swab for sample collection, an extraction tube with viral lysis buffer to inactivate and lyse the virus, and a nozzle. The COVID-19 antigen test cassette has two lines: a control line and a test line. After sample collection, the swab is immersed in the viral lysis buffer and mixed thoroughly. The buffer tube cap is replaced with a nozzle. This sample-buffer mixture is only stable for less than an hour and as such needs to be tested at the sample collection site. A total of two to three drops are put into the well of the cassette. The cassette is observed for up to 15 minutes for the appearance of the control and test lines. According to the protocol, a test showing both test and control lines was considered positive for SARS-CoV-2, while as the ones showing control line alone were designated as negative for SARS-CoV-2.The control line is always displayed when the sample migration is successful. Testing was repeated if there was no appearance of a control line [Table/Fig-2]. The maximum duration for interpretation of a positive or negative result was 30 minutes.

Result	Control line	Test line
RAT positive	Present*	Present*
RAT negative	Present	Absent
Invalid (Repeat test)	Absent	Present/Absent

[Table/Fig-2]: Interpretation criteria for Rapid Antigen kits.
*Presence of any line, even if faint, was considered positive

RT-PCR Testing

Combined oropharyngeal and nasopharyngeal swab specimens in a single Viral-Transport Medium (VTM) tube were obtained. Samples were immediately immersed in the VTM and transported in triple-layered packing. On receipt, the samples collected were processed in the Biosafety Level II lab (BSL II) in a Biological Safety Cabinet (BSC-class II type A2). Ribonucleic Acid (RNA) extraction and purification were done for all the specimens using various extraction kits such as QIAamp Viral RNA Kit (Qiagen), Imperial life sciences RNA extraction kit, Genetix RNA extraction kits, as per the availability. Extracted and purified RNA was reverse transcribed to complementary Deoxyribonucleic Acid (cDNA) and subsequently amplified by following manufacturer's instructions using the thermocycler Applied Biosystems 7500/7500 Fast Real-Time PCR Instrument System (ThermoFisher Scientific). Two or more SARS-CoV-2 specific genes were targeted for the detection of RNA. A combination of Nucleoprotein (N gene) and Open reading frame 1b (ORF-1b) [Meril Diagnostics, Meril COVID-19 One-step RT-PCR Kit] or N gene), Spike (S gene) and ORF-1b (Taqpath, COVID-19 combo kit, ThermoFisher scientific, USA) were used. Samples were processed and results were dispatched within six to eight hours, if a delay was expected then samples were stored at 2-8°C for upto 72 hours after collection. The extracted nucleic acid was stored at -70°C in a freezer for long-term storage. To ensure the integrity and verification of RT-PCR assay results, an Internal Control (IC) was analysed for each patient sample, also testing one replicate of the positive control and one replicate of the negative control in each batch. A Cycle threshold value (Ct value) <35 was declared as a positive test result, and a Ct value of ≥38 was declared as a negative test result. A Ct value of 35 to less than 38 was reported as inconclusive and was asked to go for repeat sampling. Assessment of the test results was performed after the positive and negative controls had been examined and determined to be valid and acceptable.

STATISTICAL ANALYSIS

Data was compiled and entered into a Microsoft excel sheet and analysed using software Statistical Package for the Social Sciences (SPSS) version 22.0. Data was analysed using descriptive statistics (frequencies, mean, standard deviation, and percentages).

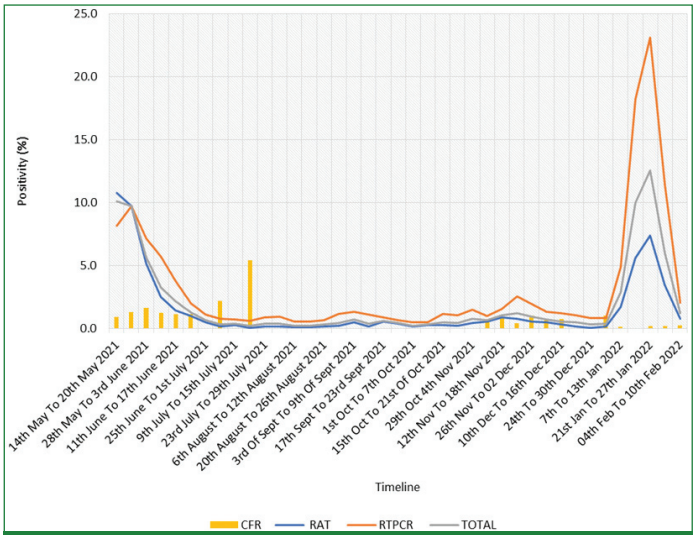
RESULTS

A total of 8,29,745 patients were tested during the study period out of which RAT was performed on 5,94,520 patients while the remaining 2,35,225 were tested by RT-PCR [Table/Fig-3]. Amongst the patients tested by RAT, 9,382 tested positive for COVID-19 giving a positivity of 1.58%. The positivity ranged from 0.05% to 10.76% during different intervals. For the RT-PCR tests, 10,032 patients tested positive giving a positivity rate of 4.26% (0.5% to 23.12%) [Table/Fig-4]. The total no of positive samples was 19,414 giving an overall positivity rate of 2.34% (0.20% to 12.58%). The total number of deaths recorded was 89 with a CFR of 0.46% [Table/Fig-5]. The tests conducted per million populations remained fairly constant during the study period with an average of 19,690 (12,214-36,675) [Table/Fig-6].

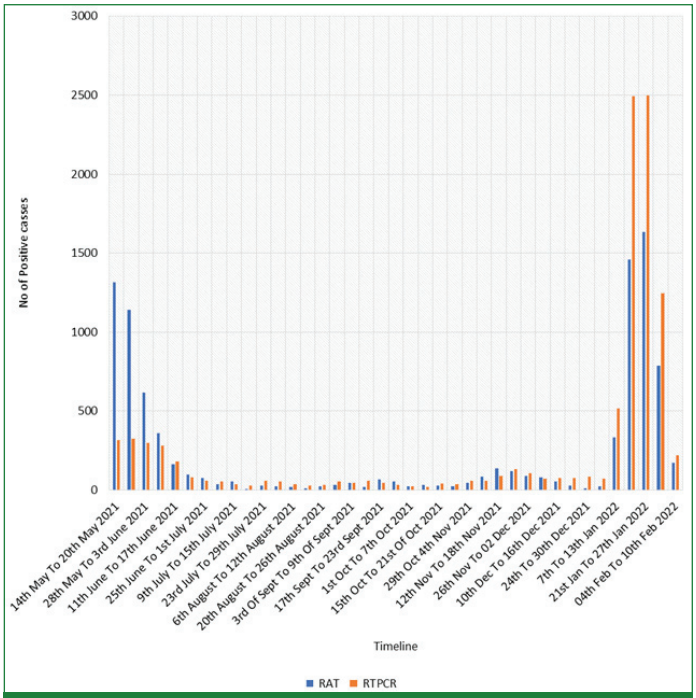
Time period	Samples tested by RAT	Samples tested by RT-PCR	Total samples tested
14th May to 20th May, 2021	12217	3879	16096
21st May to 27th May, 2021	11690	3323	15013
28th May to 3rd June, 2021	12036	4143	16179
4th June to 10th June, 2021	14441	4941	19382
11th June to 17th June, 2021	11228	4862	16090
18th June to 24th June, 2021	10019	3996	14015
25th June to 1st July, 2021	15162	5131	20293
2nd July to 8th July, 2021	19429	6751	26180
9th July to 15th July, 2021	18493	5327	23820
16th July to 22nd July, 2021	13661	5022	18683
23rd July to 29th July, 2021	17467	6476	23943

30th July to 5th Aug, 2021	15422	5613	21035
6th Aug to 12th Aug, 2021	17986	6572	24558
13th Aug to 19th Aug, 2021	14402	5175	19577
20th Aug to 26th Aug, 2021	14490	5113	19603
27th Aug to 2nd Sept, 2021	14550	4762	19312
3rd Sept to 9th Sept, 2021	9816	3381	13197
10th Sept to 16th Sept , 2021	14092	5253	19345
17th Sept to 23rd Sept, 2021	12806	5339	18145
24th Sept to 30th Sept, 2021	13467	4864	18331
1st Oct to 7th Oct, 2021	14764	4439	19203
8th Oct to 14th Oct, 2021	11364	3807	15171
15th Oct to 21st Oct, 2021	10740	3518	14258
22nd Oct to 28th Oct, 2021	10107	3637	13744
29th Oct to 4th Nov , 2021	9559	3931	13490
5th Nov to 11th Nov, 2021	15285	5575	20860
12th Nov to 18th Nov, 2021	15307	5710	21017
19th Nov to 25th Nov, 2021	15817	5163	20980
26th Nov to 2nd Dec, 2021	15953	5667	21620
3rd Dec to 9th Dec , 2021	16418	5354	21772
10th Dec to 16th Dec, 2021	17450	6199	23649
17th Dec to 23rd Dec , 2021	15229	7466	22695
24th Dec to 30th Dec, 2021	16934	9844	26778
31st Dec, 2021 to 6th Jan, 2022	15031	8451	23482
7th Jan to 13th Jan, 2022	19098	10551	29649
14th Jan to 20th Jan, 2022	25961	13666	39627
21st Jan to 27th Jan, 2022	22069	10813	32882
28th Jan to 3rd Feb, 2022	22889	10849	33738
4th Feb to 10th Feb, 2022	21671	10662	32333
Total	594520	235225	829745

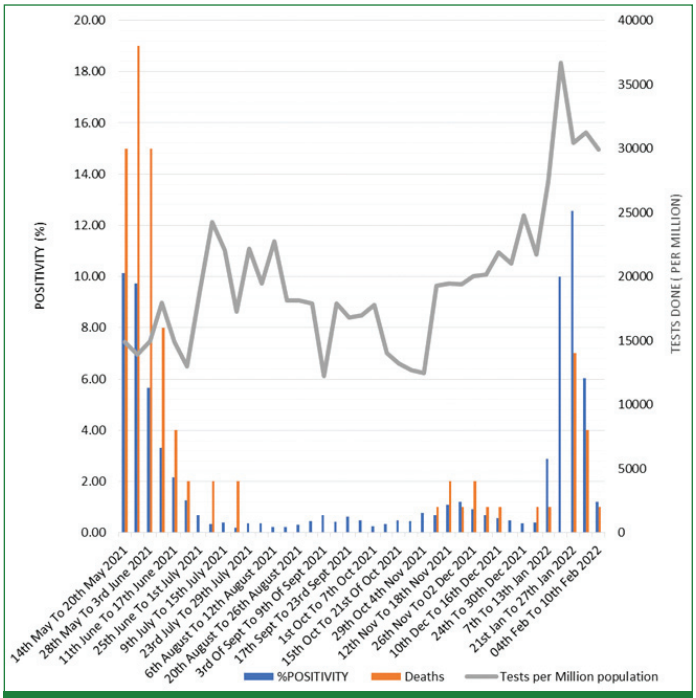
[Table/Fig-3]: Total samples tested during the study period by various modalities.



[Table/Fig-4]: Percentage of positive RAT and RT-PCR results by week along with CFR.



[Table/Fig-5]: Weekly Positive RAT and RT-PCR results.



[Table/Fig-6]: Total positivity along with number of deaths as compared with tests done per million by week.

DISCUSSION

As per research conducted around the world, high morbidity has been attributed to COVID-19, and as such early diagnosis is the need of the hour. ICMR issued testing strategies that were updated as and when needed depending on the stage of the pandemic. A direct impact of this was felt in the clinical microbiology laboratories across the country. Initially, for SARS-CoV-2 detection, different PCRs were used for diagnostics. Real-Time RT-PCR although being the gold standard test for detection is time-consuming, particularly so in resource limited settings. On an average, the time required for confirmation is more than 24 hours and varies depending on the samples received and the positivity. Additionally, the machine is expensive and needs technical expertise. As such the facility cannot be offered by most laboratories. To circumvent this, an antibody test was introduced. Serological tests had a major drawback as the antibodies started to appear only after two weeks of onset of the symptoms. This limited their use for surveillance purposes only [11]. Considering the limitations in testing, there was an urgent need to introduce a reliable point-of-care test to lessen the burden on testing laboratories. In-vivo, viral antigens precede the appearance of antibodies among the infected people. Justifiably, viral antigen detection can act as an alternative to the existing methods while simultaneously being a rapid and cost-effective technique, lessening the economic burden and helping in the detection of the disease at an early stage and achieving the purpose of early diagnosis [12].

In the context of developing countries, the accessibility, and affordability of screening tests are of paramount importance.

ICMR approved the antigen-based diagnostic tests COVID-19 in June 2020. In addition to being less expensive than other diagnostic modalities, these tests can be performed on-site and provide results within 15 minutes. Although the sensitivity of rapid tests is less than the RT-PCR tests, the time-consuming nature of the latter is an impediment to its widespread use and usefulness in providing an early diagnosis. The delay in providing reports is a major drawback for RT-PCR tests as the period of infectivity overlaps with the time taken for the results to be dispatched and as such the person-to-person transmission during this period can lead to the potential spread of the infection in the community [9].

At the start of April 2021, RAT accounted for 49% of the total tests conducted in India for COVID-19 [13]. This was a consequence of the explosive nature of the second wave which had emerged due to the Delta variant. The second wave, as opposed to the first wave, had spread from the towns to the villages [14]. During the first wave, about 30% of the infections were reported from rural areas which increased to 50% during the second wave [15]. As the pandemic spread deep into the mainland, the limited availability of RT-PCR tests in the rural set-up led to increased use of RAT. Although numerous laboratories were set-up during the initial phases of the pandemic, the maintenance and running costs of molecular laboratories proved to be a limiting factor for the widespread use of RT-PCR as an initial diagnostic modality [13].

In this study, the positivity rate of RT-PCR was higher than RAT across all time intervals. Although, the sensitivity of RT-PCR has been reported to be greater than RAT in numerous studies [9], the number of patients tested by RAT was far more than those tested by RT-PCR. Consequently, the number of positive patients tested by RAT outnumber those by RT-PCR consistently. This emphasises the protocols in place wherein the widespread use of RAT can lead to favourable epidemiological outcomes. Pekosz A et al., reported that for patients tested within seven days of onset of symptoms, RAT results gave a better correlation of the presence of culturable virus as opposed to that by RT-PCR [16].

In this study, the comparison of RAT and RT-PCR across different intervals was done. The positivity for both testing modalities was highest during increased periods of communicability in the community. Worth mentioning that the difference between RT-PCR and RAT was highest during the onset of the third wave in the country i.e. at the beginning of the year 2022. This wave had a higher proportion of asymptomatic patients and as such RT-PCR showed a higher positivity than RAT [17]. Overall, the difference in positivity between RT-PCR and RAT was found to be 2.68%. Despite the significant difference, testing by RAT is better suited as it allows the reports to be dispatched within 30 minutes as opposed to longer wait time in the case of RT-PCR.

The most important step in controlling the pandemic is prompt testing and isolation of cases to prevent further spread of infection [18]. This goal can more realistically be achieved by swift RAT rather than the more sensitive RT-PCR tests. Also, increased utilisation of RAT as a screening test will lessen the burden on laboratories which will further boost the testing capabilities of such laboratories and reduce the delay between sample collection and generation of reports. Therefore, in resource-limited settings, RAT serves as a viable alternative to RT-PCR testing both in terms of being a cost-effective measure as well as in terms of the rapidity of availability of results. This may lead to flattening the peaks of future waves and provide the means to contain the pandemic.

Limitation(s)

A detailed follow-up of the patients was not undertaken. Future studies need to be conducted to address these issues.

CONCLUSION(S)

India has just emerged from another phase of the pandemic due to the emergence of the Omicron variant. The widespread use of RAT has been vital in reducing the load on the already overburdened laboratories. The substantial cost reduction when shifting from RT-PCR to RAT can be a welcome step in reducing the economic burden on the health sector where most of the RT-PCR tests are still done free of cost. RT-PCR may be reserved for that sub-set of patients having a negative RAT with a strong suspicion of infection and being at risk for severe infection. This exhaustive study gives us an insight into the testing protocols and paves way for future development of strategies to control the pandemic.

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Authors contribution: SAL performed the assays and critically revised the manuscript. TA analysed and interpreted the data and wrote the manuscript. UA acquired the data and reviewed the draft. AHA acquired the data and wrote the manuscript. KJ acquired and analysed the data. APK acquired and analysed the data. JA conceptualised and designed the study. All authors read and approved the final submitted version.

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