

Diagnostic Accuracy of Rapid Antigen Test for COVID-19 Infection: A Retrospective Analysis

DHWANI N CHAUHAN¹, SHAMBHAVI VERMA², KISHOR V JADHAV³, M KIRAN KUMAR⁴, NARESH T CHAUHAN⁵, ABHAY B KAVISHVAR⁶, JAYESH K KOSAMBIYA⁷



ABSTRACT

Introduction: For the diagnosis of Coronavirus Disease 2019 (COVID-19), Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) is a laboratory-based technique and is considered a gold standard test, but is time consuming. A Rapid Antigen Test (RAT) is used for screening which is an immunoassay that identifies the presence of a viral antigen causing infection at the point of care. The RAT is quick, inexpensive, easily accessible and doesn't need lab handling or sample preprocessing.

Aim: To measure the sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV) of RAT in comparison to RT-PCR.

Materials and Methods: This retrospective study was conducted in Department of Community Medicine at Government Medical College (tertiary care centre), Surat, Gujarat, India, using secondary data from 1st July 2020 to 5th Dec 2020. The samples were collected from all the patients of Acute Respiratory Illness (ARI), Severe Acute Respiratory Illness (SARI), Influenza Like Illness (ILI), the suspected COVID-19 cases and all walk-in patients for testing

or treatment purposes. A total of 264 participants enrolled in the study underwent both the RAT and RT-PCR tests. The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated using MS Excel Statistical Package for the Social Sciences (SPSS) version 17.0.

Results: Total 264 cases were analysed, amongst which 161 (60.9%) were males and 103 (39.1%) were females and the mean age of the patient was 41.6 years and 36.8 years for males and females, respectively. The overall sensitivity was 52.47%, specificity was 87.11%, PPV was 71.62% and the NPV was 74.73%. While among symptomatic patients, sensitivity was 55.55%, specificity was 88.54%, PPV was 76.97% and NPV was 74.35%.

Conclusion: Because of the low sensitivity of the RAT, if used alone, a high number of false negative cases will be resulted. Hence, it is employed in community and clinical settings as sequential screening in conjunction with RT-PCR, which results in improved net gain and aids in disease transmission control.

Keywords: Coronavirus disease 2019, Positive predictive value, Sensitivity, Specificity

INTRODUCTION

The Coronavirus Disease 2019 (COVID-19) now known as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) is a global pandemic disease that emerged in 2019, is a major public health challenge and continued to cause devastation worldwide [1]. Until now, as per the World Health Organisation (WHO), COVID-19 situation update, there were more than 100 million confirmed cases and more than 2 million deaths reported worldwide. In India, it has affected more than 11 million cases out of which more than 11 million cases were recovered and more than 1 lac died while more than 50 million have been vaccinated according to WHO coronavirus global data [2]. The clinical features of this disease vary from strain to strain it ranges from asymptomatic cases to mild and severe respiratory illness. The symptoms may be fever, cough, cold, breathlessness and diarrhoea. People aged above 65 years and people of all ages with severe chronic medical conditions like hypertension, diabetes mellitus, lung diseases, heart diseases are at higher risk of succumbing to severe COVID-19 [3].

It is very important in clinical fields to confirm the clinical diagnosis with laboratory tests which is an integral part of diagnosis because medical decisions are 70% dependent on laboratory tests [4]. One important aspect of limiting SARS-CoV-2 spread is through laboratory tests which detects the presence of causative virus to ensure the detection of the cases earlier and accurate diagnosis of the infection through breaking the chain of transmission by isolation and contact tracing, help in deciding the appropriate treatment decreasing patient's expenditure and other harms of misdiagnosis [1,5]. But, it is rare to find a test that is perfect in terms of 100%

sensitivity and specificity [4]. Therefore, it has to be evaluated for its accuracy, technique, site and quality of sampling [4]. Validity can be measured by sensitivity and specificity which can be done by comparing it with the gold standard test [4].

For diagnosing COVID-19, there were major challenges faced [6]. Diagnostic strategies used in COVID-19 to identify current infection, rule out other infections, identify the appropriate people in need of hospitalisation, to test for past infections and immune response [6]. The Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) is considered as a gold standard diagnostic test [7-9], while Rapid Antigen Test (RAT) as a screening test [9]. Such tests identify previous COVID-19 infections and may help to confirm the presence of current infection [6]. The RT-PCR requires proper centralised laboratory infrastructure and laboratory facilities. It is time consuming as it takes around 5-6 hours. Moreover, transportation can cause a delay in the result and requires more precautions and preprocessing of the samples which becomes difficult and is not available every time. But, as the turn-around time for the diagnostic result is more, it limits the potential for diagnosis to lead to reductions in transmission [5,9]. Moreover, the demand for RT-PCR has outrun its precarious availability [7]. So, it is not feasible to carry out RT-PCR tests under every circumstance. So, the need for rapid diagnostic tests arises that can be performed at a time.

A RAT is quick, inexpensive, easily accessible, easily transportable and doesn't need lab handling or sample preprocessing. Therefore, it can decrease the pressure on overburdened centralised testing and the shortage of RT-PCR reagents that have occurred worldwide [4]. It does not require laboratory infrastructure, precautions during

transportation and preprocessing of samples, though tests active infection [4,5,10,11]. But, its lower sensitivity has limited adoption in clinical settings [5]. At the same time, the requirements of RT-PCR have outrun its stock. Thus, RATs used for mass screening or community surveillance could control the pandemic by quickly isolating individuals during their incubation period to prevent disease transmission [10].

In few studies [4,12], overall sensitivity of the RAT compared with laboratory-based testing was reported 94% (95% CI: 86-98) and an overall specificity of 100% (99-100) but its accuracy has not been studied in Gujarat. So, the present study may help to evaluate the rapid test and will give valuable insight into COVID-19 testing strategy.

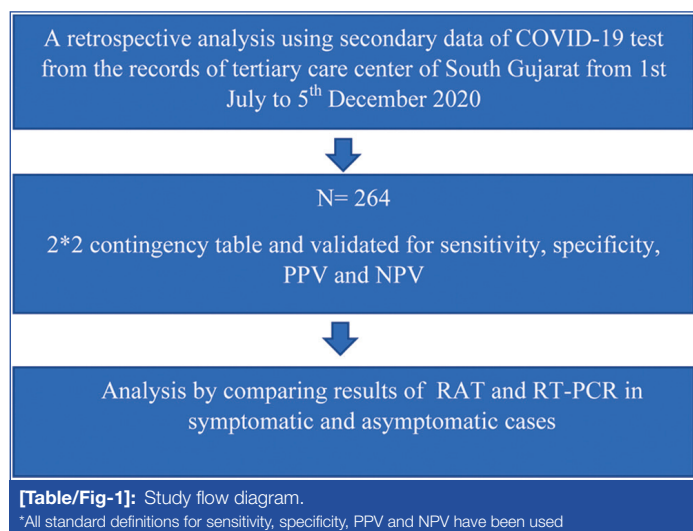
The objective of the present study was to evaluate the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the RAT which has remained a mainstay of identifying cases in the field, in comparison to RT-PCR.

MATERIALS AND METHODS

This retrospective study was conducted in Department of Community Medicine at Government Medical College (tertiary care centre), Surat, Gujarat, India, using secondary data from 1st July 2020 to 5th Dec 2020 and were analysed in August 2021. The study was conducted in accordance with the ethical standards and the approval has been obtained from the Institutional Human Research and Ethical Committee (No. GMCS/STU/ETHICS/Approval/8673/20).

Inclusion and Exclusion criteria: All the cases having details of both the tests i.e, RAT and RT-PCR tests were included in the study and cases with incomplete records were excluded from the study.

Total 264 cases admitted within the study period were enrolled in the study. The COVID-19 test data were obtained from the records from 1st July to 5th December 2020. This secondary data has the records of the samples collected from all the Acute Respiratory Illness (ARI) patients, Severe Acute Respiratory Illness (SARI), Influenza Like Illness (ILI), suspected COVID-19 cases or asymptomatic and presented for screening for testing or treatment purposes [Table/Fig-1].



Study Procedure

A nasopharyngeal swab was taken through sterile technique from each patient and was examined for RAT (SD Biosensor) immediately whereas another sample was collected in viral transport media and was sent for RT-PCR test to microbiology laboratory at tertiary care hospital keeping it in cold boxes maintaining 2-8°C temperature and taking all the proper precautions for transportation.

The following data were collected which included age, sex, address, date of OPD, the onset of symptoms, presence of symptoms like fever, cough, cold, breathlessness, diarrhoea, RAT results and RT-PCR results.

The results of RAT were compared with the gold standard RT-PCR tests to obtain sensitivity, specificity, PPV and NPV. The sensitivity of the RAT was calculated as the proportion of all those who resulted positive through the confirmatory RT-PCR method, while, the specificity of the RAT was calculated from the proportion of all those who resulted negative through the RT-PCR method.

STATISTICAL ANALYSIS

Analysis was done using Microsoft Excel and Statistical Package for the Social Sciences (SPSS) version 17.0. The information obtained from the results of the test was summarised in the form of a 2x2 contingency table.

RESULTS

Total 264 cases were analysed for diagnostic accuracy. Amongst them 161 (60.9%) were male and 103 (39.1%) were female. The mean age of the patient was 41.6 years and 36.8 years for males and females respectively. The median duration for the onset of symptoms and visit to health facility was two days (IQR:1-3). The data in [Table/Fig-2] showing distribution according to the results of RAT and RT-PCR.

Rapid antigen test result	RT-PCR result		Total
	Positive	Negative	
Positive	53	21	74
Negative	48	142	190
Total	101	163	264

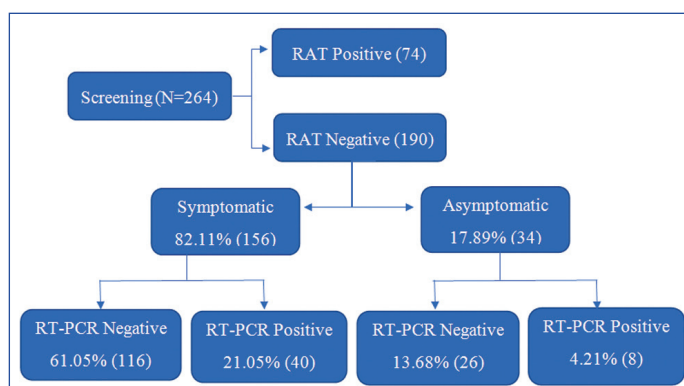
[Table/Fig-2]: Distribution according to the results of Rapid Antigen Test (RAT) and Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

The RAT had higher overall specificity than sensitivity compared to RT-PCR [Table/Fig-3]. The sensitivity, specificity and PPV of RAT increased in symptomatic cases than overall cases [Table/Fig-3].

Variables	Total	Symptomatic patients	Asymptomatic patients
Sensitivity (%)	52.47%	55.55%	27.27%
Specificity (%)	87.11%	88.54%	81.25%
PPV (%)	71.62%	76.97%	33.33%
NPV (%)	74.73%	74.35%	76.47%

[Table/Fig-3]: Validation of results of Rapid Antigen Tests (RAT) with RT-PCR.

Out of 190 Rapid Antigen COVID-19 negative results, symptomatic cases were higher which on further confirmation through RT-PCR test found 40 (21.05%) positive results [Table/Fig-4].



[Table/Fig-4]: Distribution of the cases as per Indian Council of Medical Research (ICMR) flow chart for testing.

All those negative results of the RAT irrespective of their symptomatic status resulted in 48 (25.26%) positive through RT-PCR that could fail to diagnose, if, RAT is done alone [Table/Fig-4].

Out of the total 40 false negative by RAT but RT-PCR positive symptomatic patients, the majority were having cough 28 (70%)

followed by fever 26 (65%) and both fever and cough 18 (45%) [Table/Fig-5].

Symptoms	Number (%)
Fever	26 (65%)
Cough	28 (70%)
Fever and cough	18 (45%)
Cold	10 (25%)
Breathlessness	10 (25%)
Diarrhoea	1 (2.5%)

[Table/Fig-5]: Distribution of the symptoms among RAT negative but RT-PCR positive (N=40).

DISCUSSION

The SARS-CoV-2 is a major public health challenge globally. During this study, the incidence of the cases in the city of South Gujarat was at its peak. Considering, the RT-PCR test as the standard, the sensitivity of the RAT was found to be 52.47% (55.55% and 27.27% in symptomatic and asymptomatic individuals, respectively) and the specificity was high overall (87.11%). A study by Kanji JN et al., shows that 44.83% was the sensitivity [13]. A study by Lanser L et al., showed Panbio™ antigen test 60.8% sensitivity which seems to be poor [9]. A study in Canada shows that 42% resulted in positive results by polymerase chain reaction method [14]. The similar results of PPV (70%) were seen by Panbio™ test compared to the results of PPV in the present study (71.62%) [13].

Another study of comparative evaluation of RATs for diagnosis of COVID-19 shows that RATs show $\geq 80\%$ sensitivity and $\geq 97\%$ specificity [14]. A study by Adamson B et al., shows that RATs lag to detect COVID-19 and it must not be suitable for routine screening of asymptomatic cases to prevent the spread of the virus [15]. Given its sensitivity of around 80%, antigen tests like Panbio™ COVID-19 antigen test, might be suitable for quickly identifying infectious subjects in primary care with symptoms consistent with COVID-19 infections [9].

SARS-CoV-2 infected people with moderate to high virus loads were quickly identified with the Panbio™ COVID-19 RAT. Antigen tests have 1.0% specificity in all types of patients and can be a useful method with a high PPV for COVID pandemic control. It might be utilised as an alternative for PCR in these individuals to avoid the delays and high labour costs caused by the widespread usage of PCRs. The test has a sensitivity of 86.5% in the group of symptomatic patients with fewer than seven days of evolution, which is lower than the sensitivity reported in the technical data sheet of the test [16]. A study by Lefever S et al., shows no false positive results. An 100% sensitivity was obtained with a high viral load in both symptomatic and asymptomatic patients. The proportion of asymptomatic participants with a low viral load was substantially higher than that of symptomatic participants, explaining why asymptomatic people had lower overall sensitivity than symptomatic ones. When compared to RT-PCR, Ag-RDT has a lower sensitivity and a higher specificity [17].

According to a recent Cochrane meta-analysis, the average sensitivity was 75.1% (CI, 57.3-87.1%) and 88.1% (CI, 84.2%-91.1%) in Abbott-Panbio and SD Biosensor rapid testing kits respectively while specificity were 99.5% (CI, 98.7%-99.8%) in Abbott-Panbio and 99.1% (CI, 97.8%-99.6%) in SD Biosensor rapid testing kits amongst symptomatic patients [12]. The Liaison antigen test had a sensitivity of 67.7% and a specificity of 100% reported, which is comparable to the present study. The sensitivity of the Liaison antigen test was 65.7% (CI, 58.9% to 71.9%) and the specificity was 100% (CI, 97.8% to 100%) in a study by Lefever S et al., [17]. A study by Fernandez-Montero A et al., shows Roche SARS-CoV-2 RAT has been proven to be highly sensitive and specific on symptomatic patients, meeting WHO recommended criteria of 80% sensitivity and 97% specificity. Compared with the RT-PCR test, the Roche-rapid test had a sensitivity of 71.43% and a specificity of 99.68% with moderate concordance [18]. Comparison of present study with contrast studies is shown in [Table/Fig-6] [9-18].

Author and year of publication	Place of study	Study design	Sample size	Sensitivity	Specificity	Positive predictive values	Negative predictive values
Lanser L et al., [9] (2021)	Austria	Secondary data analysis	51	60.8%	-	-	-
Berger A et al., [10] (2021)	Switzerland	Prospective cohort	Panbio™535 SD Biosensor 529	85.5% 89.0%	100% 99.7%	100% 99.4%	95.8% 94.1%
Mak GC et al., [11] (2020)	Hong Kong	Secondary data analysis	280 High viral load 72 Normal viral load 132 Low viral load 76	77.8%-100% $\geq 75\%$ 0-11.1%	-	-	-
Dinnes J et al., [12] (2021)	-	Systematic Review	15530 1849 Symptomatic Abbott-Panbio 1094 SD Biosensor 1947	72.0% overall 74.1% symptomatic Abbott-Panbio 75.1% SD Biosensor 88.1%	99.5% overall, 99.9% Abbott-Panbio 99.5% SD Biosensor 99.1%	Abbott-Panbio 89% SD Biosensor 84%	98.7% 99.4%
Kanji JN et al., [13] (2021)	Alberta, Canada	Retrospective review	369 39 confirmed by RT-PCR	-	-	70%	-
Perez-Garcia F et al., [14] (2021)	Madrid, Spain	Secondary data analysis	356	$\geq 80\%$ 66.5, 100% in high viral load samples	$\geq 97\%$ 97.3	-	-
Adamson B et al., [15] (2022)	United States of America	Retrospective cohort	30 individuals with 62 matched pairs	13.33%	-	-	-
Linares M et al., [16] (2020)	Alcala de Henares, Madrid, Spain	Secondary data analysis	255	73.3% overall, 86.5% in symptomatic	-	-	-
Lefever S et al., [17] (2021)	Belgium	Cross-sectional	414	65.7% to 67.7%	100%	-	-
Fernandez-Montero A et al., [18] (2021)	Pamplona, Spain	Cross-sectional	2639 eligible/2543 index test	71.43%	99.68%	81.40%	99.44%
Present study	India	Retrospective analysis	264	52.47	87.11	71.62	74.73

[Table/Fig-6]: Comparison of results of various studies [9-18].

For effective implementation of the test, track and isolation, an accurate diagnostic test is of paramount importance to control the pandemic. SARS-CoV-2 is a serious infectious disease in which efficient measures and timely management is required and its false negative results might lead to free contact of the patient which could lead to transmission of the disease. To overcome this, conduction of the screening RAT along with the confirmatory RT-PCR test simultaneously is necessary [11,19,20].

Rapid diagnostic tests have a crucial role in the foundation of national testing strategy. Because of its low sensitivity, vigilant interpretation of its results is essential for evaluation. Its use has been limited and not completely adopted in the clinical setting but, its increase in the net gain has resulted in controlling disease transmission by providing decentralised and mobile testing rapidly diagnosing results in public places or at community which is well suited to low-resource environments [1].

A study by Mak GC et al., shows that Biocredit COVID-19 Ag was 105 times less sensitive than RT-PCR in terms of LOD (Biocredit COVID-19 Ag: 10⁻²; RT-PCR: 10⁻⁷) in the reference RAD kit. SARS-CoV-2 infection can be detected with the help of RAD kits. The findings were in line with the WHO recommendation to test symptomatic cases within the first 5-7 days of illness. It's important to strike a balance between speed and sensitivity. A COVID-19 filter can be failed, even if, the diagnostic test has a high analytical sensitivity [11].

All those 190 RAT negative results have been confirmed through RT-PCR which led to the detection of 25% (n=48; including symptomatic n=40 and asymptomatic n=8) more cases that could be missed out if RAT is done exclusively. The compatible symptoms of the disease turned out to be more positive on the RT-PCR test. Therefore, the strategy of using a more sensitive test i.e., RT-PCR is strengthened, if there is high clinical suspicion for COVID-19 in cases of having cough (30.11%), fever (27.96%) and both fever and cough (19.35%) which were failed to get diagnosed. Optimum interpretation of the RAT results should take into account the patient's clinical features, history of exposure, the prevalence of COVID-19 in the community and the test's performance characteristics.

Limitation(s)

The outcome of the study was subjected to limitations concerning any COVID-19 compatible symptoms that might not be collected beyond the chief complaint during the time of pandemic and the symptoms might be underestimated. Exposure history was also not assessed. RT-PCR detects the presence of viral Ribonucleic Acid (RNA) detecting past infection too and might not transmit the disease.

CONCLUSION(S)

Indian Council of Medical Research (ICMR) recommended use of RAT use, as point of care screening of COVID-19, so, that we can do isolation, proper treatment and thereby, break the chain of transmission of SARS-CoV-2 disease. As it may happen that the rapid test fails to detect diseases in many cases, thus, it emphasises that it should not heavily relied on rapid test and it should be supplemented by RT-PCR in case of negative results. Finding of the present study document, the sensitivity and specificity of RAT, it also concludes the proportion of symptomatic negative cases found positive on RT-PCR and the proportion of asymptomatic negative cases found positive on RT-PCR. So, the rational use of RAT in combination with RT-PCR is recommended, as per the guidelines.

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PARTICULARS OF CONTRIBUTORS:

1. Resident, Department of Community Medicine, Government Medical College, Surat, Gujarat, India.
2. Resident, Department of Community Medicine, Government Medical College, Surat, Gujarat, India.
3. Tutor, Department of Community Medicine, Government Medical College, Surat, Gujarat, India.
4. Resident, Department of Community Medicine, Government Medical College, Surat, Gujarat, India.
5. Assistant Professor, Department of Community Medicine, Government Medical College, Surat, Gujarat, India.
6. Associate Professor, Department of Community Medicine, Government Medical College, Surat, Gujarat, India.
7. Professor and Head, Department of Community Medicine, Government Medical College, Surat, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Naresh T Chauhan,
B-1103, Shreepad Seasons, Palanpore, Surat, Gujarat, India.
E-mail: dnareshchauhan@rediffmail.com

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