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Comparative evaluation of SARS-CoV-2 antigen rapid immunoassay with real-time Polymerase Chain Reaction for diagnosis of COVID-19 in symptomatic patients

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ABSTRACT:

Background:SARS-CoV-2 is a new Betacoronavirus, subgenus Sarbecovirus, family Coronaviridae causing pandemic of coronavirus disease 2019(COVID 19).The study aims to evaluate the efficiency and accuracy of Rapid antigen tests compared to the RTPCR assay in order to improve early detection and triaging of SARS CoV2 infected patients presenting to the COVID outpatient department, The objectives of the study were as follows.1.To Determine the sensitivity and specificity of rapid antigen detection assay among symptomatic patients in comparison with the RTPCR test for COVID 19.2. To analyse the result of RAT and RTPCR based on the time since onset of symptoms.3.To analyse the RAT result with the cyclic threshold values of RTPCR.

Materials and Methods

This cross-sectional study was conducted in the Department of Microbiology, Govt Stanley medical college for a period of three months from July to September 2021 after obtaining an approval from Institutional ethics committee.

Results: Among 200 samples, 27(13.5%) samples were positive and 173(86.5%) samples were negative by RT-PCR. By RAT assay 10(5%)samples were positive and 190(95%) samples were negative.Antigen testing sensitivity was 37%, specificity was 100%, Positive Predictive Value (PPV) was 100% and Negative Predictive Value (NPV) was 91.1%. Accuracy between the two test assays was 91.5% and Kappa coefficient value is 0.504

Conclusion: The use of RAT among symptomatic patients is useful for the early identification of COVID-19, but symptomatic patients who test negative by Rapid antigen test require confirmation by real time RT-PCR.

Keywords:SARS-CoV-2, COVID-19, Rapid Antigen Test, RT-PCR

I. BACKGROUND

SARS-CoV-2 is a new Betacoronavirus, subgenus Sarbecovirus,family Coronaviridae causing pandemic of coronavirus disease 2019(COVID 19). The first case was recorded in the city of Wuhan, China in December 2019 and has been recognized as a public health emergency on January 30,2020.Then it was subsequently declared as a pandemic on March 2020(1,2). As the SARS COV 2 pandemic ravages the world,an unprecedented incidence of COVID 19 cases was witnessed in India recently in the second wave. Thus far,the most commonly used and the most reliable gold standard test for COVID-19 diagnosis has been the reverse transcription-quantitative PCR (RT-qPCR)(3).

Though RTPCR is a gold standard test, there is need for trained personnel, special equipment, expensivereagents and Biosafety level II laboratory. In addition, turnaround time of3- 4 hours causes undue delay in triaging patients.

Rapid Antigen Tests (RAT) used as a Point of Care (POC) were developed for the rapid and inexpensive detection of SARS-CoV-2. RAT uses a principle of Lateral flow Immunochromatographic assay using monoclonal anti-SARS-CoV-2 antibodies, which target SARS-CoV-2 Nucleocapsid (N) antigen has a short turnaround time of 15-30 minutes and thus offers a huge advantage of quick detection of cases to isolate and treat them early for curbing transmission.Nucleocapsid phosphoprotein,a structural protein located in 3' region forms a helical capsid structure with genomic RNA and plays an important role in structure, replication and transcription of virus(4,5). The incubation period ranges from 1 to 14 days. Infectivity period is typically 1 to 3 days prior to onset of symptoms and during the first 5 to 7 days after the course of

illness and thereafter clearance of virus occurs.The test is highly sensitive if RAT is performed within 5 to 7 days of symptom onset(6).

The sensitivity and specificity of the rapid antigen test tests depends on several factors, including the time from onset of illness, viral load in the specimen, the quality of the specimen collected from a person and processing of specimen as per kit recommendation.The performance of RAT is best when performed in symptomatic people. Nasopharyngeal samples are recommended for most of the SARS-CoV-2 Ag-RATs.Positive Rapid AntigenTests (RATs)are attributed to high viral loads in patients' sample. False negative results occur when viral loads fall below the test's limit of detection. Data from various studies shows thatthe sensitivity of RATs varies from 0% to 94% and specificity is more than 97%(7).

Successful implementation of rapid antigen testing protocols depends on technical, preanalytical, analytical and clinical assay performance and interpretation and verification of test results.Thus, this study aims to evaluate Rapid antigen test for SARS CoV2 among symptomatic patients presenting to the COVID outpatient departmentwith the RTPCR assay.

II. AIMS & OBJECTIVES

The study aims to evaluate the efficiency and accuracy of Rapid antigen tests compared to the RTPCR assay in order to improve early detection and triaging of SARS CoV2 infected patients presenting to the COVID outpatient department,

The objectives of the study were as follows.
1.To Determine the sensitivity and specificity of rapid antigen detection assay among symptomatic patients in comparison with the RTPCR test for COVID 19.
2. To analyse the result of RAT and RTPCR based on the time since onset of symptoms.
3.To analyse the RAT result with the cyclic threshold values of RTPCR.

III. MATERIALS AND METHODS

Thiscross-sectional study was conducted in the Department of Microbiology, Govt Stanley medical college for a period of three months from July to September 2021 after obtaining an approval from Institutional ethics committee.

Clinical specimens

One Nasopharyngeal swab for RAT assay and Nasopharyngeal swab and one nasopharyngeal swab were obtained from 200 symptomatic patients suspected of COVID -19. Standard and droplet precautions were followed. The Swab collected for RT-PCRwas sent to the laboratory in Viral transport medium in cold chain and subjected to SARS-CoV-2 RT-PCR under Biosafety level 2 precautions.The second swab was inserted into the specimen extraction buffer tube provided with the rapid antigen kit.

RNA Extraction

SARS-CoV-2 RNA was extracted using 96 well KingFisher Flex (ThermoFisher Scientific) from 200µL sample. Using manufacturer's manual, extraction was performed and 60 µL of template RNA is eluted and it was used for RT-PCR assay.

SARS-CoV-2 RNA detection using real-time RT-PCR

LabGun COVID-19 ExoFast RT-PCR Kit which targets RdRP(RNA dependent RNA Polymerase) gene and Nucleocapsid (N) gene of SARS-CoV-2 was used for detection of SARS-CoV-2 RNA detection according to manufacturer's instructions. 5µL of template RNA was added to 4µL of ExoFast 1step buffer, 2µL of ExoFast 1 step Enzyme, 4µL of Assay and 5µL of RNase Free water. The CFX96 Real- Time PCR detection system (BIORAD) thermal cycler was used for amplification. The PCR conditions applied were 1 cycle of 5 min at 50℃, 1 cycle of 1 min at 95℃, 10 cycles of 1 sec each at 95℃, 60℃ and 95℃ and followed by 32 cycles of 1 sec at 60℃. The results were analysed, in which a cycle threshold value (Ct value) of less than or equal 30 for two target genes were interpreted as positive result.

Rapid antigen test:

CoviRAT^{TM} is a rapid qualitative lateral flow immunochromatographic assay for detection of nucleocapsid antigens in the nasopharyngeal specimen. This device has two precoated lines on the result window: Control (C) and Test (T) lines. After bringing kit components to room temperature,provided Extraction buffer bottlewas opened and 8 drops of Extraction buffer was dispensed into it. Nasopharyngeal swab with collected specimen was inserted into Extraction Buffer tube and rolled for more than 5 times within the tube. Then swab was safely removed and discarded as per BMWM guidelines. The nozzle cap was tightly fitted into extraction buffer tube

International Journal Dental and Medical Sciences Research
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and device pouch was opened and labelled. Four drops of the extracted specimen were added to the sample window and the result was read within 15 to 30 minutes. For positive test, two pink-purple coloured lines of control (C) and test (T) lines were presented (Figure.1).

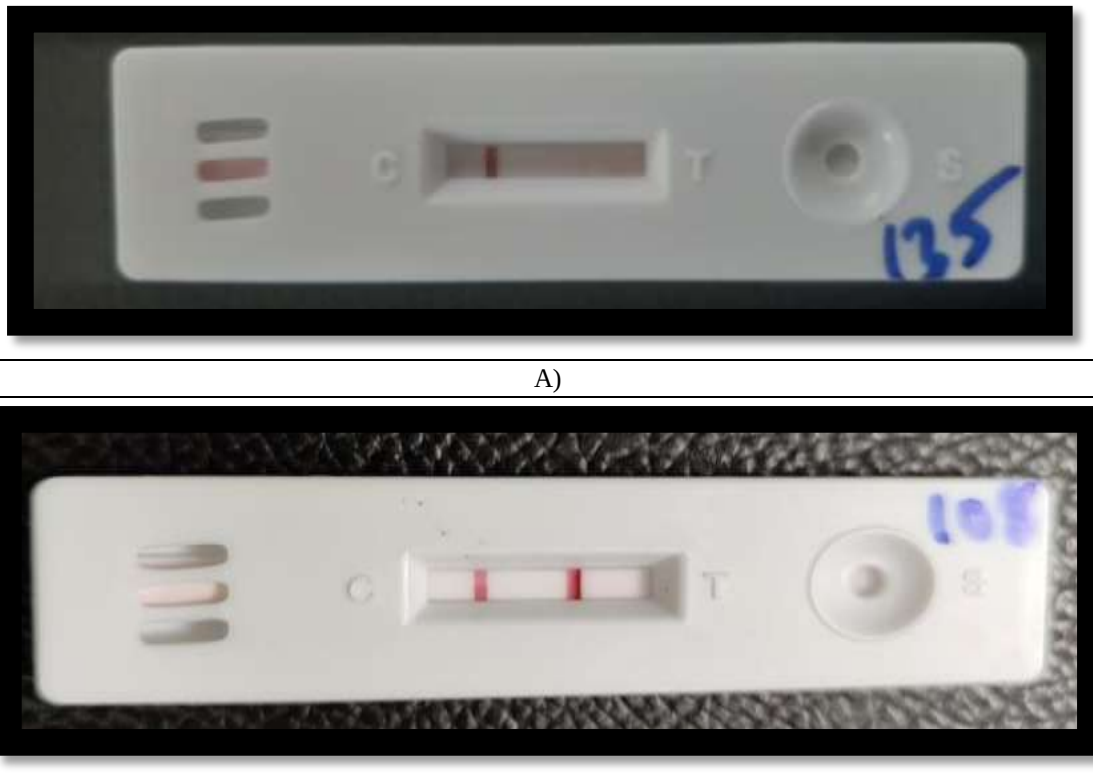

Figure.1 Interpretation ofCoviRAT™ Rapid antigen Test: A) - interpreted as Negative and B) interpreted as Positive

IV. STATISTICAL ANALYSIS

The collected data were analysed with IBM SPSS Statistics for Windows, Version 23.0.(Armonk, NY: IBM Corp).To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean & S.D were used for continuous variables. Sensitivity,Specificity,PPV, NPV, accuracy and Kappa coefficient were calculated.

V. RESULTS

This study evaluated the performance of the SARS-CoV-2 RAT assay (CoviRAT™) on comparison with real time RT-PCR for SARS-CoV-2 detection among symptomatic patients. A total of 200 duplicate nasopharyngeal swabs and 200 oropharyngeal swabs were collected from patients with following clinical symptoms of suspected or probable SARS COV2 infection presenting to the outpatient department(acute onset of Fever, cough, general weakness/fatigue, headache, myalgia, sore throat, running nose, vomiting and diarrhoea). The mean age of study group was 35.06 (± 11.86)(Table 1) with a sex ratio of 0.3 (49 females and 151 males) (Table 2).Among 200 patients tested, it was noted that all 200 patients (100%) had fever, 17.5% had nasal congestion, 8.5% had myalgia and sore throat, 8% had cough, 3.5% had headache, 1.5% had vomiting, 1% had abdominal pain and loss of smell, 0.5% had diarrhea and loss of taste (Figure 2). Table 3 shows that 17 patients had comorbidities like Hypertension, Diabetes mellitus, Bronchial Asthma, COPD and Coronary artery disease (8.5%). Of the samples tested for COVID-19 by RT-PCR, 13.5% (n=27) samples were positive and 86.5% (n=173) samples were negative for SARS-CoV-2-RNA (Table 4). Of the 200 samples, 5% (n=10) samples were found to be RAT positive and 95% (n=190) samples were found to be RAT negative (Table 5).Table 7 shows the Cycle threshold (Ct) value among RT-PCR positive individuals. The lowest Ct value for N gene is 9 and highest Ct value is 27. Similarly, for RdRP gene thelowest Ct value is 9 and the highest Ct value is 28.Figure 3 shows that 50% (n=5) of RAT

International Journal Dental and Medical Sciences Research
Volume 3, Issue 5, Sep-Oct 2021 pp 970-977 www.ijdmsrjournal.com ISSN: 2582-6018

positive patients had illness for 3 days, 20% (n=2) had illness for 2 days, 20% (n=2) had illness for 4 days and 10% (n=1) had illness for 1 day. The lowest Cycle threshold (Ct) value of N gene for RAT positive individuals was 9 and highest Ct value was 17. Similarly, the lowest Cycle threshold (Ct) value for RdRP for RAT positive individuals was 9 and highest Ct value was 18 (Table 8).Antigen testing sensitivity was 37%, specificity was 100%, Positive Predictive Value (PPV) was 100% and Negative Predictive Value (NPV) was 91.1%. Accuracy between the two test assays was 91.5%and Kappa coefficient value is 0.504(Table 6).

Table 1: Age distribution among symptomatic patients (n=200)

Minimum	Maximum	Mean	SD
9.0	72.0	35.06	±11.86

AGE GROUP	NUMBERS
1-9yrs	1
10-19yrs	13
20-29yrs	56
30-39yrs	70
40-49yrs	40
50-59yrs	13
60-69yrs	5
70-79yrs	2

Table 2: Gender distribution among symptomatic patients (n=200)

Gender	Percentage
Female	24.5(n=49)
Male	75.5(n=151)
Total	200

Table 3: Data of comorbid conditions among symptomatic patients (n=200)

Table 4: Positivity of RT-PCR assay (n=200)

COMORBID CONDITIONS	Percentage
Present	8.5(n=17)
Absent	91.5(n=183)
Total	200

RTPCR STATUS	Number	Percent
Positive	27	13.5
Negative	173	86.5
Total	200	100.0

Table 5: Positivity of Rapid Antigen Test (n=200)

International Journal Dental and Medical Sciences Research
Volume 3, Issue 5, Sep-Oct 2021 pp 970-977 www.ijdmsrjournal.com ISSN: 2582-6018

RAT	Results	Percent
Positive	10	5.0
Negative	190	95.0
Total	200	100.0

Table 6: Correlation of Rapid Antigen Test (RAT) with RTPCR Assay (n=200)

Sensitivity=37%, Specificity=100%

		RTPCR STATUS		
		Positive	**Negative**	**Total**
RAT	**Positive**	10	0	10
	Negative	17	173	190
Total		27	173	200

PPV=100%, NPV=91.1%
Accuracy=91.5%

Table 7: Ct Value among RTPCR positive patients

Descriptive Statistics					
	n	**Minimum**	**Maximum**	**Mean**	**SD**
CT VALUE N GENE	27	9.0	27.0	18.89	±5.23
CT VALUE RDRP GENE	27	9.0	28.0	19.30	±5.57

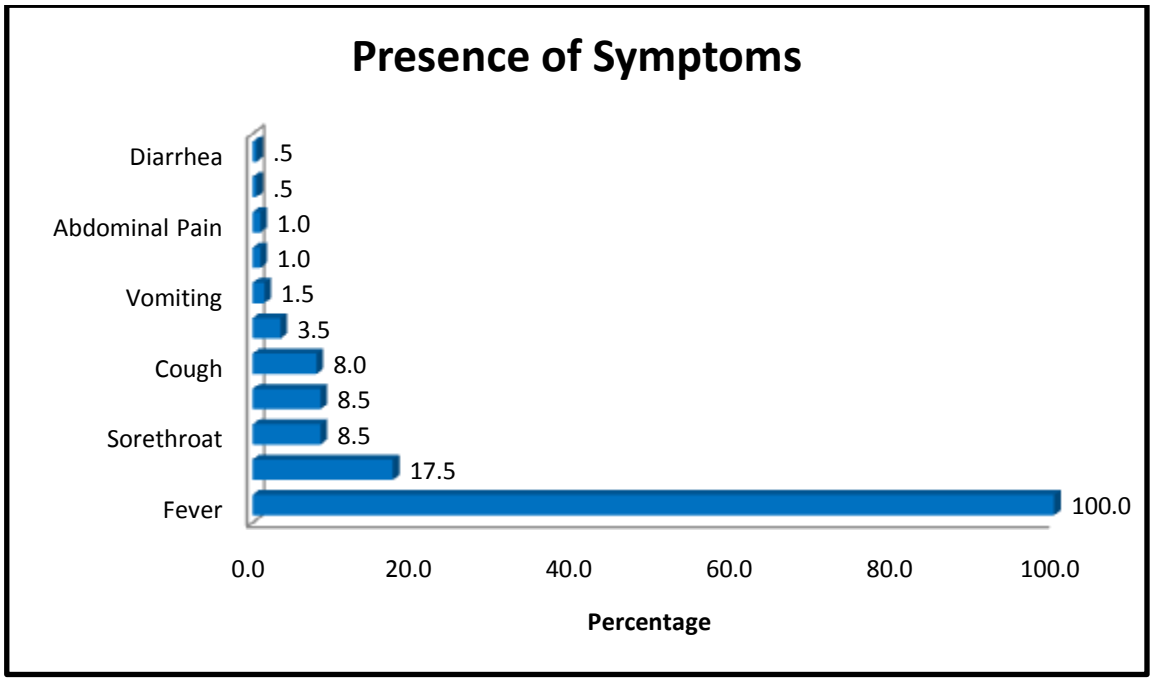

Figure 2:Distribution of symptoms (n=200)

Table 8: Sensitivity of SARS-CoV-2 RAT in respect to Ct value in PCR

International Journal Dental and Medical Sciences Research
Volume 3, Issue 5, Sep-Oct 2021 pp 970-977 www.ijdmsrjournal.com ISSN: 2582-6018

S.NO	SAMPLE ID	CT VALUE OF N GENE	CT VALUE OF RdRP GENE
1	4	17	18
2	5	15	16
3	6	16	18
4	9	9	9
5	15	17	17
6	70	12	13
7	72	9	9
8	107	12	13
9	108	10	9
10	109	14	12

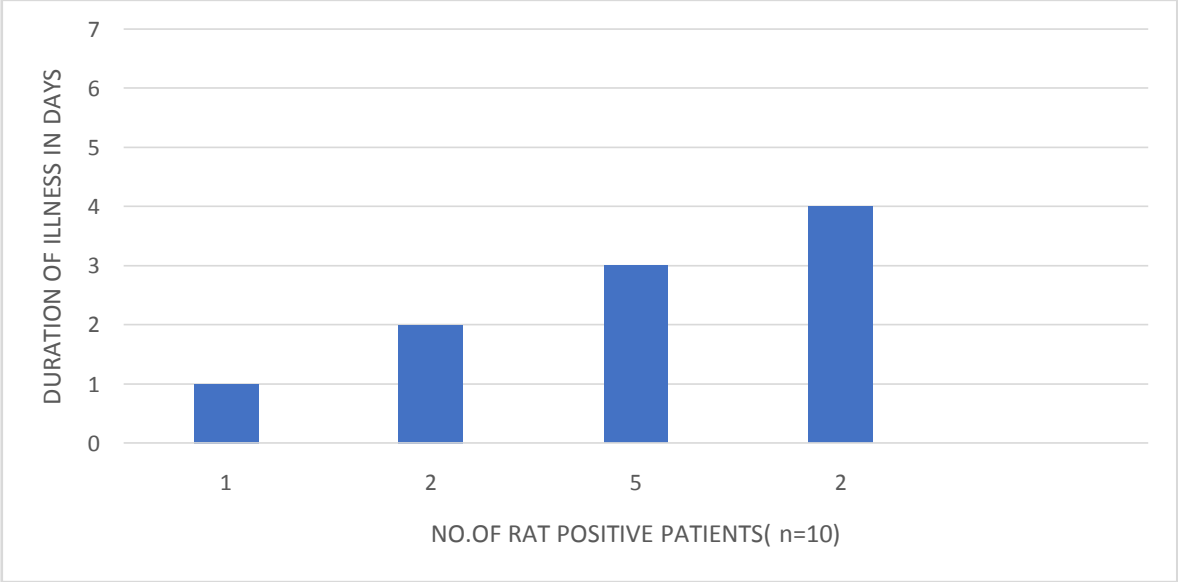

Figure 3: RAT positive patients in relation to duration of illness

VI. DISCUSSION:

In this study we aimed to compare the diagnostic performance of CoviRAT^{TM} COVID 19 Rapid antigentest with real time RT-PCR test which is a gold standard method. The diagnostic testing for SARS-CoV-2 is important for containment as well as management of patients. Many rapid antigen tests have been developed by various manufacturers across India. An ideal RAT should have a sensitivity of >95% and specificity of 100%. The observed sensitivity by CoviRAT^{TM} Rapid antigen test in our study is 37% and specificity is 100%. The sensitivity of the Rapid antigen test mainly depends on the various factors like patient's signs and symptoms,duration of illness, onset of the disease, viral load, quality of specimen and processing of specimen(8). Of 27 positive samples, CoviRAT^{TM} Rapid antigen test kit detects positivity in 10 patients. This is attributed mainly to lower Cycle threshold (Ct) value which indicates higher viral load of SARS-CoV-2 RNA in the patient's upper respiratory

specimens. The remaining 17 samples have a Cycle threshold (Ct) value on a little higher side which indicates lower viral load in a specimen turning RATassay negative. From this data it is obvious that CoviRAT^{TM} Rapid antigen test was more sensitive and more accurate in patients with a high viral load than those with low viral load. This CoviRAT^{TM}Rapid antigen kit isrecommended for severely affected patients with high viral load and at early stages of COVID-19 infection. The positive results shown by our Rapid antigen assay can be taken as positive since those 10 patients also showed positivity in RT-PCR test. The State's positivity rate is <1% since July 2021(9). The low positivity rate may also have an implication on sensitivity of Rapid antigen test.

However, many studies conducted previously in various countries showed differential sensitivity and specificity ranging from 20% to 100%(10). A study by Anais Scohy et al.,2020 showed a sensitivity of 30.2% and specificity of 100% by Rapid antigen test which were in consistent with the present study(1). The overall sensitivity of Rapid antigen antigen test was 27.5% with specificity of 99.6% by another study conducted by Kruttgen et al.,(11).A study conducted by Mboma O et al.,showed a sensitivity of 75% and specificity of 100% by Rapid Antigen Test(12). The main advantage of Rapid antigen test is that it can used as a screening assay for COVID-19 because of its simple procedure and quick results but the disadvantage is its low sensitivity. Rapid antigen test will be having higher sensitivity in symptomatic patients if performed at acute phase while RT-PCR will remain positive for weeks to months after initial infection and is more sensitive such that it can detect even very small amount viral load in a sample(13).

VII. CONCLUSIONS

We conclude that the use ofRAT among symptomatic patients is useful for the early identification of COVID-19, but symptomatic patients who test negative by Rapid antigen test require confirmation by real time RT-PCR and must stay isolated until his result becomes available to avoid transmission.Even with its limitations, still Rapid antigen test can be beneficial to all healthcare workers in managing patients, especially in remote areas where no access to RTPCR facility during current
pandemic times.

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