Original Article

Sensitivity of SARS-CoV-2 Rapid Antigen Test as Compared to RT-PCR Test

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It is well known that for detection of COVID-19, Rapid Antigen Test (RAT) is less sensitive than RT-PCR test. Here, we have compared both the tests when done in same patients, at same time, in a series of 352 patients from our lab. Symptoms of patients were also taken into consideration. This data is quite relevant as Government statistics include results of RAT and RT-PCR combined to determine daily and weekly positivity rates. The decision of Government restrictions to be imposed by civic authorities also depends on the positivity rate. RAT testing numbers are increasing for their low cost, less time to report and ease of doing the test. However, considering its low sensitivity, it portrays a false picture of low prevalence.

[*J Indian Med Assoc* 2024; **122(7):** 32-6]

Key words **: SARS-CoV-2, COVID-19, Real-time PCR, Rapid Antigen Test (RAT).**

A sequence of numerous mysterious viral pneumonia cases of strange cause emerged in Wuhan, Hubei, China, in December, 2019. It was later on recognized as Corona Virus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)[1]. All countries world over have been battling various waves of COVID-19 since it was declared as virus with pandemic potential by WHO in January, 2020[2].

Corona viruses are enveloped viruses with a positive-sense single stranded RNA and a nucleocapsid of helical symmetry with characteristic club shaped spikes on the surface[3]. They are highly diverse due to constant mutations and recombinations. Corona virus belongs to Coronaviridae family and Orthocoronavirinae subfamily. There are about 40 different varieties of corona viruses distributed mainly into 4 genera namely alpha, beta, gamma and delta. SARS-CoV-2 belongs to β (beta) corona virus, subgenus Sarbeco virus, 150-200 nm in diameter with a genome size of about 30 kb. Corona viruses cause mild to moderate illness and majority of the infected patients recover without any hospitalization[4,5]. The possible modes of transmission for SARS-CoV-2 include droplet, airborne, contact, fomites, faecal-oral, blood borne, mother-to child and animal-to-human transmission[6,7]. Frequently observed symptoms are dry cough, fever, headache, body ache, and less frequent symptoms are conjunctivitis, diarrhoea, loss of smell, skin rash, or discolouration of fingers or toes. The severe symptoms are breathlessness, chest discomfort and loss of speech or movement[8].

Editor's Comment :

- Rapid Antigen Tests (RAT) for COVID-19 detection are not very sensitive. However, positive tests help in early detection of COVID-19 till the confirmation from RT-PCR is awaited.
- RAT positive tests also help in segregation and isolation of the patients from epidemiological point of view. However, they should not be considered for determination of positivity rates in a geographical area.

The novel Corona Virus Disease (COVID-19) pandemic has affected all the countries without any discrimination. Till August 5, 2021, 202,573,486 COVID-19 cases were detected. This includes 4,294,265 deaths. The total cases in India were 31,895,385[10]. In India, first case of COVID-19 infection was reported in Kerala on January 27, 2020. A 20 year old, female patient, presented to the Emergency Department in General Hospital, Thrissur, Kerala, with a one-day history of dry cough and sore throat. In Maharashtra state, first case was confirmed on March 9, 2020. Now as of August 5, 2021, the total cases in Maharashtra are 63,41,759. The first positive case in Nagpur was detected on March 12, 2020 and now as of August 5, 2021 the total positive cases of COVID-19 in Nagpur are 4,93,045[11].

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Received on : 09/08/2021

Accepted on : 07/02/2024

All the countries are fighting against COVID-19 with all the available resources. The important pillars in any country's strategy to tackle COVID-19 are trace, test, isolate and treat[9]. As the symptoms are very much similar to other endemic viral illnesses, it is quite prudent to confirm diagnosis of COVID-19 by various modalities available. The diagnostic modalities most commonly employed for detection of COVID-19 are molecular and serological methods for direct confirmation; and radiological and other laboratory blood parameters for indirect evidences.

Most commonly used Nucleic Acid Amplification Test (NAAT) for detection of COVID-19 is Reverse Transcription Polymerase Chain Reaction (RT-PCR). RT-PCR has very high sensitivity and specificity and becomes positive within few days of exposure. It is considered as a gold standard during this pandemic. All other diagnostic modalities are compared with the results of RT-PCR only. In a multiplex PCR, at least 1 screening gene (E) and 2 specific genes (N/RdRp/ORF) are included. S gene though specific, is not included in many kits as this gene is likely to undergo many mutations owing to lack of good proof reading mechanism in the RNA viruses[12].

Rapid Antigen Tests (RAT) are rapid immunoassays to sense the presence of a particular viral antigen, which implies present viral infection. These point-of-care Rapid Antigen Tests have specific viral antigens such as the Nucleocapsid (N) protein and Spike (S) protein[13,14].

Materials and Methods

Study Design and Participants :

This is a retrospective study to compare the results of RT-PCR and RAT. The study compared the two tests that were conducted at Dhruv Labs between November 1, 2020 and May 31, 2021. Dhruv Molecular Lab is a lab of repute in Central India. It is not attached to any single hospital and gets referral from various hospitals in Central India. It is NABL accredited. It is approved by Government of India and ICMR for COVID testing. The lab participates in proficiency testing/ external quality assurance programs for COVID-19 conducted by ICMR and AIIMS, Nagpur. As of August 5, 2021 the lab has conducted 159,356 RT-PCR tests and 2,608 RAT tests.

Sample Collection :

Sample collection and analysis were done as per standard guidelines recommended by ICMR. Nasopharyngeal and Oropharyngeal swabs were collected at our sample collection facility with due care. Samples were also collected from hospitals or homes by personnel wearing all protective gear. Cotton swabs were not used for sample collection. Dacron or nylon swabs were used. The swabs were immersed in Viral Transport Medium (VTM)/ Viral Lysis Medium (VLM) and the tube was capped.

For Rapid Antigen Test — After taking the sample, nasopharyngeal swab was immediately transferred to the pre-labelled extraction buffer and transferred to the lab in cold chain for testing.

For RT-PCR — After taking sample, both nasopharyngeal and oropharyngeal swabs were immersed in pre-labelled Viral Transport Medium (VTM) or Viral Lysis Medium (VLM). The tubes were then put in zipper polythene bags and transported immediately in cold chain to the testing centre. Samples were processed by qualified personnel. Patient data was gathered as per ICMR guidelines in a printed requisition form. This compulsorily included address and phone number. Aadhar card was not mandatory. Total 150,209 RT-PCR samples were processed from May, 2020 to June, 2021, out of which 90,769 were negative and 58,200 were positive.

COVID-19 Antigen Lateral Flow Test :

Throughout the study period, Antigen testing was done by using PathoCatch Ag lateral flow test kit from Mylab Discovery Solutions. It is based on Immuno-chromatoghaphy principle. Nitrocellulose membrane used in this device is coated with control specific antibodies on Control line (C) and SARS-CoV-2 specific monoclonal antibodies on Test line (T). Colloidal gold conjugate pad consist of control solution specific antibodies and SARS-CoV-2 specific monoclonal antibodies conjugate with colloidal gold nano particles. When sample (specimen and lysis buffer mixture) is added on the sample port of test device, the sample migrates along with the colloidal gold nanoparticles. If sample contains detectable level of COVID-19 antigen, it reacts with the conjugated monoclonal antibodies in colloidal gold particles to form Ag-Ab complex. This complex then migrates on the membrane and reacts with coated SARS-CoV-2 monoclonal antibodies on the test line to form a test band (coloured line on test side)[15].

Antigen test was performed within 30 min of collection and results noted at 20 min after loading the swab samples along with buffer. Results were interpreted based on internal control line C and Test line T. If only internal control line C was seen, it was interpreted as negative for COVID-19 antigen. If both the internal control Line C and the Test line appeared, it was interpreted as positive for COVID-19 antigen. If the internal control Line C is not observed, the test

was invalid regardless of whether there was Test line. Then the test was repeated again with fresh card[16]. A total of 2593 sample were received from November 1, 2020 to June 2, 2021 for Antigen testing for COVID-19. Out of which 197 were found positive and 2396 were found negative.

Extraction of Ribonucleic Acid (RNA) from SARS CoV-2 virus :

The SARS-CoV-2 Ribonucleic Acid (RNA) was extracted by various methods, including manual spin column extraction and automated extraction. Automated extraction reduces the extraction time, optimizes the yield of RNA, increases the quantity and quality of isolated RNA and reduces probability of cross contamination. The extracted RNAs were stored at 2 to 8°C until amplification[17].

Real-Time PCR for Detection of SARS CoV-2 :

Multiplex RT-PCR (3/4 channel) were done to detect SARS-CoV-2 by targeting E, N and RdRp genes. RT-PCR was done on Quant Studio 5, from Thermo Fisher Scientific company by following manufacturer's instructions regarding choice of the dyes and PCR conditions. The kits used were either from Mylab, HiMedia or Genetix Biotech. For HiMedia, the dye selection was as follows: N gene - FAM; E gene - Rox; RdRp gene - Cy5; RPPH1 gene -VIC. Temperature conditions for HiMedia were as follows: 50°C for 15 min; 95°C for 180 sec; 95°C for 15 sec, 58°C for 30 sec (40 cycles). Acquisition was done at last cycle.

For Genetix Biotech, the dye selection was as follows: RNaseP gene - VIC; E gene - FAM; RdRp - Cy5. Temperature conditions for Genetix Biotech kits were as follows: 50°C for 15 min; 95°C for 3 min; 95°C for 10 sec; 60°C for 30 sec (45 cycles). Acquisition was done at last cycle.

The cycle threshold (Ct) of 35 was kept to determine positive and negative samples as per ICMR guidelines.

The lab reports of RT-PCR included Ct values. The lab believes that it is not of much relevance in depicting the viral load. Ct values are dependent on how the sample has been taken, transported and stored. Thus, it depends more on pre-analytical variables[18-20]. All the RT-PCR and RAT test results were submitted to the health department of Nagpur Municipal Corporation and Indian council of Medical Research (ICMR) health portal as per the guidelines proposed by Indian Government for national surveillance.

Results

We first identified 352 patients where both COVID-19 RAT and RT-PCR tests were done. One hundred and twenty two (34.6%) patients who voluntarily discussed their COVID-19 symptoms were further identified. Out of those who reported their symptoms, around 96 (78.7%) patients had reported common symptoms ie, respiratory infections, such as fever, cough, body ache, chest tightness and dyspnoea. Whereas, 24 (19.7%) patients had reported less common symptoms like loss of smell or taste, chills, chest pain, gastrointestinal symptoms, arthritis, anorexia, allergy-like symptoms, insomnia, ear and eye related symptoms, skin problems, dry skin, rash, and itching. Moreover, 2 (1.6%) patients have reported rare symptom such as, loss of appetite and hiccups.

While interpreting data, we noticed that 83 patients were RAT Positive; PCR Positive (ie, True Positives); while 58 patients were RAT Negative; PCR Positive (ie, False Negatives) (Table 2).

The demographics (gender distribution and prevalence) amongst positive patients are mentioned in Table 2. We have also noticed that the average Ct value of RAT+PCR+ was 19 (CI 16-21); whereas in patients that were RAT-PCR+ was 24 (CI 22-29) (Table 3). The statistical assessment and diagnostic accuracy of RAT is elaborated in Table 4.

Discussion

RAT is an immunoassay that detects the presence of specific proteins on the outer portion of the virus, such as the spike protein which implies recent viral infection. Rapid Antigen Tests (RAT) are comparatively

Table 1 — *Interpretation of RT-PCR and RAT for COVID-19*

RT-PCR	RAT	Interpretation
Negative	Negative	Negative
Negative	Positive	Positive
Positive	Negative	Positive
Positive	Positive	Positive
Positive	Inconclusive	Positive
Negative	Inconclusive	Negative
Inconclusive	Positive	Positive
Inconclusive	Negative	Repeat RT-PCR after 3 days

Table 2 — *Table showing RAT and RT-PCR test results*

	RT-PCR (Positive)	RT-PCR (Negative)	Total
RAT (Positive)	83 (True Positive)	00 (False Positive)	83
RAT (Negative)	58 (False Negative)	211 (True Negative)	269
Total	141	211	352

Table 3 — *Demographics and Ct values of Positive samples*

Variables	N	Value
Age in years - Mean ±SD	352	41±17.7
Male - No. (%)	352	209 (59.37%)
Female - No. (%)	352	143 (40.62%)
Prevalence - No. (%)	352	141 (40.05%)
Ct Value of RAT + and RT-PCR + Cases - Median (IQR)	58	19 (16-21)
Ct value of RT-PCR Positive cases	83	24 (22-29)

Table 4 — *Assessment of the Diagnostic Accuracy of RAT*

Variables	Formula	Value	95% Confidence Interval (CI)
Prevalence	$\frac{TP+FN}{TP+FN+TN+FP} \times 100$	40.05%	27.1 – 52.9%
Sensitivity	$\frac{TP}{TP+FN} \times 100$	58.86%	50.0 - 67.7%
Specificity	$\frac{TN}{TN+FP} \times 100$	100%	100%
Accuracy	$\frac{TP+TN}{TP+FN+TN+FP} \times 100$	83.5%	78.7 - 88.3%
False Positive Rate (FPR)	$\frac{FP}{TN+FP} \times 100$	0 %	0 %
True Positive Rate (TPR)	$\frac{TP}{TP+FN} \times 100$	58.86%	50.0 – 67.7%
Positive Predicted Value	$\frac{TP}{TP+FP} \times 100$	100%	100%
Negative Predicted Value	$\frac{TN}{FN+TN} \times 100$	78.4%	72.9-83.9%
Positive likelihood Ratio (+LR)	$\frac{Sensitivity}{1-Specificity}$	0 %	0 %
Negative likelihood Ratio	$\frac{1-Sensitivity}{Specificity} \times 100$	0.41%	-
False Discovery Rate (FDR)	$\frac{FP}{FP+TP} \times 100$	0%	0%

low-cost, and mainly can be used at the point of care. Most of the currently authorized tests return results in roughly 15-30 minutes. The real-time reverse Transcription Polymerase Chain Reaction (RT-PCR) and other Nucleic Acid Amplification Tests (NAATs) are more sensitive as compared to the antigen test for detecting the presence of viral nucleic acid. On the other hand, RT-PCR can remain positive for weeks to months after early infection and can identify levels of viral nucleic acid even when virus cannot be cultured. Information about antigen tests interpretation is mentioned in Table 1.

Whole Generation Sequencing (WGS) is done for highly sensitive and specific results. It provides complete information and can identify novel strains and mutations as well.

The most common clinical symptoms of COVID-19 infections are mild respiratory symptoms like sore throat, dry cough, fever sometimes gastrointestinal symptoms like loose motions may be the presenting complaint. Serious clinical symptoms like pneumonia, stroke, cardiac complications are highly atypical. The present study also shows a similar picture.

Though laboratory and radiological parameters aid in diagnosis and management of COVID-19, molecular detection of SARS CoV-2 remains gold standard. There are other supportive and prognostic modalities which help in the management of COVID-19. Imaging modality, CT thorax is the most useful in detecting the extent of lung involvement. The laboratory investigations that help in management include Complete Blood Counts, blood sugar levels, d-dimer, C-reactive Protein (CRP), ferritin, Interleulin-6, LFT and KFT.

The Maharashtra State Government had included reports of both RAT and RT-PCR to determine the daily and weekly positivity rate. In May and June, 2021, the dependence upon RAT had increased drastically. While the ratio used to be 70:30 between RT-PCR and RAT, only 41% tests conducted in June, 2021 were done using RT PCR. After a recent order to exclude RAT for determining positivity rate in Maharashtra state, the daily and weekly positivity rate (period June, 19 to 25) jumped from 5.2% to 8.7%. The positivity rate determines the level of restrictions to be imposed in various cities. Twelve districts, including Kolhapur, Sangli, Palghar, Sindhudurg, Pune and Satara have a positivity rate of over 10% that puts them in stringent level 4 restrictions.

Districts of Buldhana, Gadchiroli, Sangli, Osmanabad and Palghar where RAT number is more, reported a positivity rate of less than 20% during the peak months. The rates of other districts have also been revised. The rate for Mumbai has been revised from 3.7 to 4.7% now. On June, 25, Mumbai city performed 17,200 RAT and 16,600 RT-PCR tests. The positivity rate of RAT was close to 1% and that of RT-PCR was almost 4%. The overall positivity rate was close to 2%. Although RAT delivers quick results, they are not very accurate and many times give false negative results. This is because of low sensitivity of

RAT. Experts do not recommend use of RAT. Random RAT screening of general population gives a false belief of decreased positivity rate and has implications on restrictions to be enforced (TOI, June 27, 2021).

Conclusion

The results of RAT tests should be interpreted considering the symptoms of the patients. It should be done only in acute cases. Whenever done, RT-PCR tests should be done in parallel. We also believe that RAT should be excluded while deciding the daily and weekly positivity rates as these rates determine the level of restrictions to be imposed in a particular district, city or town.

References

1 She J, Jiang J, Ye L, Hu L, Bai C, Song Y — Novel coronavirus of pneumonia in Wuhan, China: emerging attack and management strategies. *Clin Transl Med* 2020; **9:** e19.

2 Ghosh A, Nundy S, Mallick T — How India is dealing with COVID-19 pandemic, Sensors International, 2020; **1:** 100021.

3 Malik YA — Properties of Coronavirus and SARS-CoV-2. *Malays J Pathol* 2020; **42(1):** 3-11.

4 Li H, Liu SM, Yu XH, Tang SL, Tang CK — Coronavirus disease 2019 (COVID-19): current status and future perspectives. *Int J Antimicrob Agents* 2020; **55(5):** 105951.

5 Singh A, Mehra M, Gulyani S — A modified variable-order fractional SIR model to predict the spread of COVID-19 in India. *Math Meth Appl Sci* 2021; 1-15.

6 Girolamo P — Assessment of the potential role of atmospheric particulate pollution and airborne transmission in intensifying the first wave pandemic impact of SARS-CoV-2/COVID-19 in Northern Italy. *Bull of Atmos Sci & Technol* 2020; **1:** 515-50.

7 Karia R, Gupta I, Khandait H, Yadav A, Yadav A — COVID-19 and its Modes of Transmission. *SN Compr Clin Med* 2020; **1:** 1-4.

8 Robson F, Khan K, Le T, Paris C, Demirbag S, Barfuss P, *et al* — Coronavirus RNA Proofreading: Molecular Basis and Therapeutic Targeting. *Mol Cell* 2020; **79(5):** 710-27.

9 La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM — Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. *Reprod Biomed Online* 2020; **41(3):** 483-499.

10 Available from: https://covid19.who.int/ [Last access on August 5, 2021]

11 Available from: https://www.covid19india.org [Last access on August 5, 2021]

12 Mustafa Hellou M, Górska A, Mazzaferri F, Cremonini E, Gentilotti E, De Nardo P, *et al* — Nucleic acid amplification tests on respiratory samples for the diagnosis of coronavirus infections: a systematic review and meta-analysis. *Clin Microbiol Infect* 2021; **27(3):** 341-351.

13 Osterman A, Baldauf HM, Eletreby M, Wettengel JM, Afridi SQ, Fuchs T, *et al* — Evaluation of two rapid antigen tests to detect SARS-CoV-2 in a hospital setting. *Med Microbiol Immunol* 2021; **210(1):** 65-72.

14 Enjuanes L, Gorbalenya AE, de Groot RJ, Cowley JA, Ziebuhr J, Snijder EJ — *Nidovirales. Encyclopedia of Virology* 2008; 419-30.

15 Brian D, Baric R — Coronavirus genome structure and replication. *Curr Top Microbiol Immunol* 2005; **287:** 1-30.

16 Tanna J, Singha B, Nayak A, Husain A, Raje D, Desai S, *et al* — Incidence and Epidemiological study of COVID-19 in Nagpur urban region (India) using Molecular testing. https://doi.org/10.1101/2021.05.11.21256719

17 Mukherjee TK, Malik P, Maitra R, Hoidal JR — Ravaging SARS-CoV-2: rudimentary diagnosis and puzzling immunological responses. *Curr Med Res Opin* 2021; **37(2):** 207-217.

18 Carter L, Garner L, Smoot J, Li Y, Zhou Q, Saveson C, *et al* — Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Central Science* 2020; **6(5):** 91-605.

19 Ambrosi C, Prezioso C, Checconi P, Scribano D, Sarshar M, Capannari M, *et al* — SARS-CoV-2: Comparative analysis of different RNA extraction methods. *J Virol Methods* 2021; **287:** 114008.

20 Hou H, Wang T, Zhang B, Luo Y, Mao L, Wang F, *et al* — Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. *Clin Transl Immunology* 2020; **9(5):** e01136.