

PERFORMANCE OF RAPID ANTIGEN TEST IN COMPARISON TO REAL-TIME RT-PCR IN DIAGNOSIS OF COVID-19 INFECTION: A SHORT STUDYARITRA BISWAS^{id}, RAJA RAY^{id}, ARPITA KHAMRAI^{id}, DIPANKAR PAUL*^{id}

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ABSTRACT

Objective: The global pandemic of Coronavirus Disease 2019 (COVID-19) is still spreading. Because of this, it is urgently necessary to develop quick, easy, and accurate assays to identify severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. For the purpose of diagnosing COVID-19 cases, the quick SARS-CoV-2 antigen detection test's performance attributes should be assessed and contrasted with the gold standard real-time reverse transcription-polymerase chain reaction (RT-PCR) assay.

Methods: A retrospective analysis was done during January to May, 2022 at IPGME&R and SSKM Hospital, Kolkata, India. 2842 paired samples were taken from all patients reporting to the hospital for Rapid Antigen Test (RAT) and RT-PCR. The sensitivity and specificity were calculated to evaluate the performance of the RAT.

Results: Of 2842 samples, 229 (8%) were positive, and 2613 (92%) were negative for SARS-CoV-2 RNA by real-time RT-PCR assay. The rapid SARS-CoV-2 antigen detection test's sensitivity and specificity were 77.29% (177 out of 229) and 99.54% (2601 out of 2613), respectively. Positive predictive value was 93.65% and negative predictive value was 93% with the Ct value ≤ 35 of real-time RT-PCR result.

Conclusion: RAT-positive samples are also positive by real-time RT-PCR test though RAT-negative patient with symptom, should further test for real-time RT-PCR as sensitivity is bit low particularly in symptomatic patient respect to real-time RT-PCR result. Hence, we can conclude that specificity- and sensitivity-wise real-time RT-PCR method is best, but during pandemic/outbreak situation, RAT is good alternative for fast screening and point-of-care test of COVID-19 detection.

Keywords: COVID-19, RAT, Real-time RT-PCR, Pandemic, Outbreak.

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INTRODUCTION

In Wuhan, Hubei, China, since December 2019, a number of instances of pneumonia with no known origin have been reported [1]. The clinical presentations are strikingly similar to viral pneumonia. Subsequently, pathogenic gene sequencing confirmed that the infected pathogen was a novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Similar to previous outbreaks of two beta coronaviruses, SARS-CoV in 2003 and Middle East respiratory syndrome coronavirus in 2012, were fatal in nature and have caused more than 10,000 deaths in the past two decades. SARS-CoV-2-infected disease named as coronavirus disease 2019 (COVID-19), its outbreak developing into an epidemic that quickly spread all over China and to all over the world [2,3]. Most COVID-19 virus-infected individuals will experience a mild-to-severe respiratory infection and recover without the need for special care. Serious sickness is more likely to strike older persons and those with underlying medical conditions including cancer, diabetes, cardiovascular disease, or chronic respiratory diseases and chronic kidney diseases [4,5]. The COVID-19 virus spreads primarily through droplets of saliva or discharging from the nose when an infected person speaks and coughs.

This study aims with the following objectives: (1) to compare the performance of a rapid antigen test (RAT) (index test) with a gold standard test real-time reverse transcription polymerase chain reaction (RT-PCR) in the diagnosis of COVID-19 and (2) to evaluate the sensitivity and specificity of a particular RAT kit with respect to real-time RT-PCR results.

METHODS**Ethical statement**

To participate in this study, informed sample referral form (SRF) developed by ICMR was obtained from each patient. This study protocol was approved by the Institutional ethical committee of Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata.

Place of work

All tests were performed at Virus Research and Diagnostic Laboratory (VRDL), unit of Microbiology department of the Institute of Post-Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital (IPGME&R and SSKM Hospital).

Sample collection

A total of 2842 nasopharyngeal swab (NPS) samples in duplicate were collected by health-care workers at IPGME ANNEX SNPH hospital fever clinic with a SRF. One swab was immediately tested at the facility using the ICMR approved SARS-CoV-2 RAT KIT, and the result were interpreted according to the manufacturer's guidelines. The second swab was preserved in viral transport medium (VTM) and transported to VRDL Lab, IPGME&R and SSKM Hospital for RNA extraction and real-time RT-PCR testing under cold chain condition.

RAT kit

Sample was tested by Oscar Corona Antigen Test kit manufactured by Oscar Medicare Pvt. Ltd. (ICMR approved). According to manufacturer's protocol, 10 drops of buffer added in the extraction tube and then inserted a sterile swab into the nostril of the patient reaching the surface of the posterior

Table 1: RNA Extraction processing plate pre-partition

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash Plate 1	2	KingFisher™ 96 Deep-Well	Wash Solution	500 µL
Wash Plate 2	3	Plate	80% Ethanol solution	500 µL
Elution Plate	4		Elution Buffer	500 µL
Tip Comb Plate	5	Place a KingFisher™ Deep Well 96 Tip Comb in a KingFisher™ 96 KF microplate or equivalent plate		

nasopharynx. Then inserted the swab into an extraction buffer tube and stirred the swab more than 10 times swiftly. Then applied 4 drops of extracted specimen to the specimen well of the test device using provided disposable sample dropper. Within 20-min test, result was pointed out.

RNA extraction

Under biosefty level-II B2 cabinet, all the VTM tubes containing samples were sorted out serially. RNA extraction process was performed by MagMAX Viral/Pathogen II (MVP II) nucleic acid isolation kit using KingFisher automated extraction system (Thermo Fisher Scientific) according to manufacturer's protocol. Briefly, Kingfisher instruments automate extraction of RNA, using magnetic beads that capture targeted nucleic acid. Beads bind the nucleic acid more efficiently than glass-fiber filters, resulting in higher and more consistent yields. Using a simple workflow including binding, washing, and elution, KingFisher instruments can automate the extraction of any analyte of interest. Once captured, these nucleic acids can then be eluted in elution buffer for use in downstream applications. The following steps are followed to extract the RNA using KingFisher-automated extraction system.

Set up the instrument (200-µL specimen volume)

KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.

Prepare the processing plates (200-µL specimen volume)

According to manufacturer's instruction, processing plates are prepared as per the following table 1.

Preparation of Binding Bead Mix (200-µL specimen volume)

Binding Bead Mix was prepared on each day as per requirement.

1. Vortex the Binding Beads to ensure that the bead mixture is homogeneous.
2. For the number of required extractions, 265 µL of binding solution and 10 µL of binding beads per well (total 275 µL) was prepared.
3. Mix well by slow inversion, then store at room temperature. The Binding Bead Mix has been shown to be stable for ≤8 h at room temperature.

Preparation of the sample plate (200-µL specimen volume)

1. Invert the binding bead mix 5 times gently to mix, then add 275 µL to each specimen well and the negative control well in the sample plate (KingFisher™ 96 Deep-Well Plate)
2. Add 5 µL of Proteinase K to each specimen well
3. Add 200 µL of specimen to each specimen well, respectively
4. Add 200 µL of nuclease-free water to the Negative Control well
5. Add the following components to each specimen and Negative Control well.

Process the specimens (200-µL specimen volume)

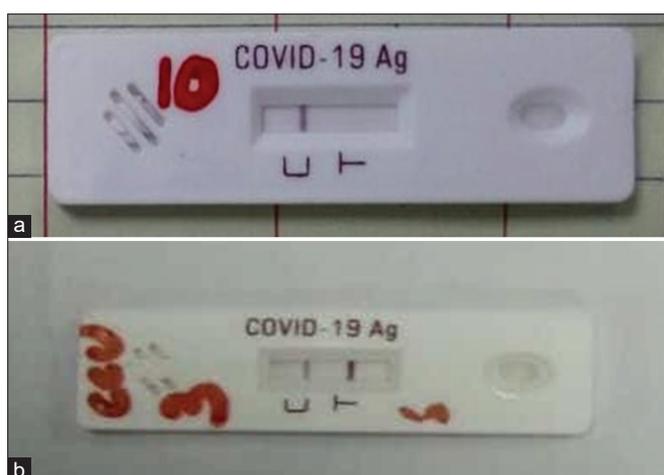
1. Select the MVP 2 Wash 200 Flex on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run and then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~28 min after start), immediately remove the Elution Plate from the instrument, and then cover the plate with MicroAmp™ clear adhesive film.

Real-time RT-PCR

CoviPath COVID-19 real-time RT-PCR Kit (Thermo Fisher) contains the reagents and controls for a real-time reverse transcriptase-polymerase

Table 2: Master mix preparation

Component	Volume per reaction
CoviPath 1-Step Multiplex Master Mix	6.25 µL
CoviPath™ COVID-19 Assay Multiplex	1.25 µL
Nuclease-free water	7.50 µL
Total reaction mix volume	15.0 µL

**Fig. 1: (a) Negative result of RAT (b) Positive result of RAT**

chain reaction. To run real-time reverse transcriptase polymerase chain reaction, Biorad CFX 96 (C1000) touch thermal cycler is used.

Preparation the Master Mix

For each run in a 96-well plate, combine the following components sufficient for the number of RNA samples to be tested plus one negative control from each extraction run and one positive Control (Table 2).

Set up the reaction plate

- a. According to manufacturer's protocol, pipet 15.0 µL of the prepared reaction mix into each well of a MicroAmp™ Optical 96-Well Reaction Plate.
- b. Unseal the plate containing the purified sample RNA and add 10.0 µL extracted RNA to respective well. Negative control from the RNA extraction procedure. Add 10.0 µL nuclease-free water to negative control well and positive cControl to respective well of the reaction plate.

The following PCR cycles/steps were used to perform the run-UNG incubation at 25°C for 2 min, reverse transcription at 53°C for 10 mins, activation at 95°C for 2 mins, and denaturation and extension step (40 cycles) at 95°C for 3 s and 60°C for 30 s, respectively. Here reporter dye FAM and VIC were used to target ORF 1ab and N gene, respectively. ROX reporter dye was used to target RNaseP, as internal control.

RESULTS

As per kit literature, positive and negative RAT test result was evaluated (Fig. 1a and b). Control line (C) should be present in both positive and

Table 3: Month-wise comparison of RAT and real-time RT-PCR of COVID-19

Month	Total sample	Rapid antigen test	% of positive in RAT	Real-time RT-PCR		% of positive in real-time RT-PCR
				Positive	Negative	
January	610	Positive Negative	170 440	27.87	160 40	32.786
February	738	Positive Negative	11 727	1.49	9 7	2.17
March	786	Positive Negative	3 783	0.38	3 2	0.64
April	418	Positive Negative	3 415	0.72	3 2	1.19
May	290	Positive Negative	2 288	0.69	2 1	1.034

Table 4: Overall test result January to May 2022 (RAT vs. real-time RT-PCR)

Total sample	Rapid antigen test	Real-time RT-PCR	
		Positive	Negative
2842	Positive Negative	189 2653	177 (a) 52 (c) 12 (b) 2601 (d)

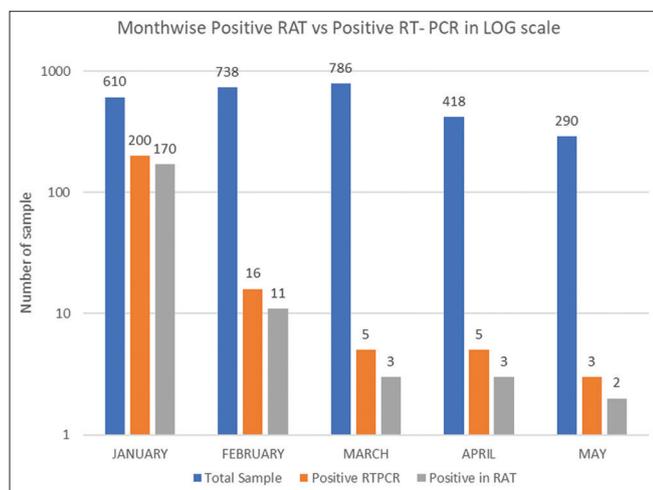


Fig. 2: Month-wise data of total sample with positive RAT and positive real time PCR

negative cases otherwise test is invalid. Based on test line (T), the test result is decided. If the test line (T) is strong or faint, then test is positive and if no line occur in test line, then its negative (negative test result in Fig. 1a and positive test result in Fig. 1b).

A total of 2842 outdoor patients were tested for both RAT and real-time RT-PCR for the period from January to May, 2022. Among 2842 paired NPS samples, 189 were RAT positive (7%) and 229 were real-time RT-PCR positive (8%). Month-wise distribution of RAT and real-time RT-PCR test result is shown in Table 3.

Interpretation of real-time RT-PCR result

Cycle of threshold (Ct) values ≤35 considered positive for the target gene “N” and “ORF 1ab.” The lower the Ct value, the higher the viral load. For the positive control to pass, the N gene and ORF1ab must be detected. For the negative control to pass, the N gene, ORF1ab, and RNase P must not be detected.

Among these 6 months (January to May), highest positivity was seen in January 2022. It corresponds with the 3rd wave of COVID-19 pandemic in India (Fig. 2).

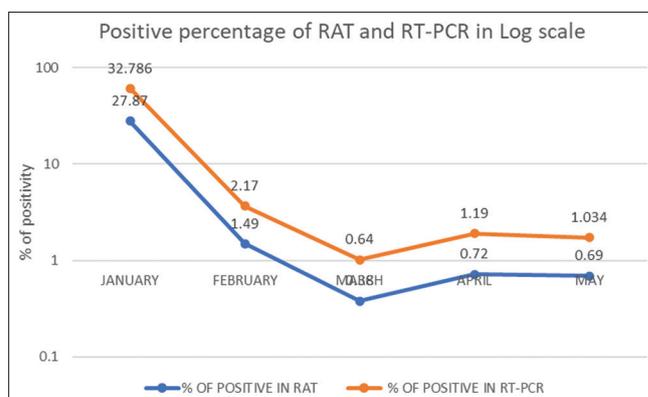


Fig. 3: Month-wise percentage of positivity rate in RAT and real-time RT-PCR

From the month January to May 2022, the percentage of positive sample in both RAT and real-time RT-PCR is shown in Table 3 and it is depicted in Fig. 2. From this figure 3, it was seen the percentage of positivity rate in real-time RT-PCR in every month was always higher than percentage of positivity rate in RAT.

Among 189 RAT-positive samples, 177 samples were also positive in real-time RT-PCR test. However, 12 samples which were RAT positive tested negative by real-time RT-PCR. Among 2653 RAT-negative samples, 52 samples were tested positive by real-time RT-PCR method.

To find out the specificity and sensitivity of both RAT and real-time RT-PCR results, the data were represented at Table 4 and the following equation was applied-

Sensitivity= $[a/(a+c)] \times 100\%$

Specificity= $[d/(b+d)] \times 100\%$

Positive Predictive Value = $[a/(a+b)] \times 100\%$

Negative Predictive Value = $[d/(c+d)] \times 100\%$

From the Table 4, with respect to real-time RT-PCR test result, the overall antigen testing sensitivity was 77.29% (177 out of 229), specificity was 99.54% (2601 out of 2613), and positive predictive value (PPV) was 93.65% and negative predictive value (NPV) was 93%.

DISCUSSION

Our study was a hospital-based study of the analysis of test characteristics of the RAT kit when compared with the real-time RT-PCR (gold standard). The RAT test sensitivity and specificity was found 77.29% and 99.54%, respectively with the PPV of 93.65% and NPV of 93%. The sensitivity and specificity may vary depending on different

RAT and real-time RT-PCR kit. Other studies conducted in India and abroad, reported sensitivity varied from as high as 98.33% to as low as 53.6% and the specificity varied from as high as 99.61% to as low as 97.35% [6-8]. A retrospective analysis at a tertiary care hospital in Eastern Uttar Pradesh, India have shown that the RAT's overall sensitivity and specificity were 53.6% (39.7–67.0) and 97.35% (94.6–98.9), respectively [6], whereas sensitivity was higher in symptomatic individuals (61.0%) (44.5–75.8). It is also noticed that RAT test positivity rates to be higher at a cycle threshold value of 20. It is also reported that sensitivity was 69.86%, specificity was 99.61%, PPV was 94.44%, and NPP was 97.22% with Ct values (Ct >27) that were significantly higher among individuals with false-negative RAT [6]. A study at Siriraj Hospital in Bangkok, Thailand [8] pointed out that the rapid SARS-CoV-2 antigen detection test's sensitivity and specificity were 98.33% (95% CI: 91.06–99.96%) and 98.73% (95% CI: 97.06–99.59%), respectively. Five false positive test results were from samples of pre-operative patients, whereas one false negative test result came from a sample with a high real-time RT-PCR cycle threshold [8]. Based on all studies, it is described that RAT-positive samples are also positive by real-time RT-PCR test though RAT-negative patient with symptoms should further test for real-time RT-PCR as sensitivity is bit low particularly in symptomatic patient respect to real-time RT-PCR result. Hence, specificity- and sensitivity-wise real-time RT-PCR method is best, but during pandemic/outbreak situation, RAT is good alternative for fast screening and point-of-care test of COVID-19 detection.

CONCLUSION

Real-time RT-PCR test is gold standard for COVID-19 testing as it has highest specificity and sensitivity, but it is laborious, time consuming, and expensive. It requires maintenance of cold chain, well-equipped laboratory, and skilled laboratory staff. In a resource-poor country like India, alternative test to diagnose COVID-19 is necessary to serve a huge population. RAT for COVID-19 meets most of the requirements as it is easy to perform, cheap, and does not require well-equipped laboratory and skilled manpower. It is a "point of care" test, can be done in outdoor facility or at the bed side of the patient without any cold chain and result obtained within few minutes. Although RAT test has some limitations, it can be extensively done during outbreak/epidemic period.

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AUTHORS CONTRIBUTION

Biswas, A. Khamrai performed the experiments and wrote the manuscript; R. Ray and D. Paul looked after the overall study design, manuscript preparation, and trouble shoot problems. D. Paul assisted in the sample collection, clinical database generation, and first-line interaction with patients.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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