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Comparative analysis of Rapid antigen test and RT-PCR in the detection of Covid-19 Infection

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ABSTRACT

Since the emergence of Covid-19 pandemic, the number of cases have been on an increasing trend. The critical demand and need of hour is rapid screening, testing as well as early contact tracing plays an important role in control of this pandemic. The development of rapid, more reliable and cost-efficient diagnostic test is, which, in contrast to antibody tests, can detect the presence of the virus itself in respiratory sample of the high priority. The study compared a rapid antigen test (RAT) and RT-PCR in 154 patients during a period of 7 months. A sensitivity of 65.78%, specificity of 100%, PPV of 100% and NPV of 89.92% was observed along with Ct values (Ct > 25.1) that were significantly higher in false negative RAT cases. The high sensitivity of RAT supports the fact this rapid diagnostic have a significant impact on the identification of COVID-19 in areas where high prevalence is noted.

KEYWORDS: COVID-19, RAT, standard Q COVID-19, RT-PCR.

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INTRODUCTION

December 2019 witnessed the presence of severe acute respiratory syndrome Coronavirus 2 (SARs CoV-2) and the disease it caused, now known as Coronavirus disease 2019 (COVID-19) in Wuhan, China and it spread like wild fire to over 200 nations in the world. Worldwide, as of April 2021, there have been 129,215,179 confirmed COVID-19 cases, including over 2,800,000 deaths, reported to WHO.[1]

Since March 2020, we started real time reverse transcription polymerase chain reaction (RT-PCR) for the detection of positive cases amongst suspects of COVID-19. A challenge faced with RT-PCR is the need for special equipment and skilled laboratory personnel familiar with molecular techniques making the test expensive and time consuming.[2] In July 2020, Rapid Antigen Test(RAT) was done on suspected cases and close contact patients on OPD as well as IPD basis for early detection and intervention.

The RT-PCR targets RNA polymerase dependent RNA and the nucleocapsid gene at 3'-end of the viral genome where as the standard Q COVID -19 Antigen test is a rapid chromatographic immunoassay for qualitative detection of specific antigen to SARS-COV-2 present in human nasopharynx.[3] The rapid antigen test is approved by ICMR in combination with RT-PCR test and results can be obtained in 20-30 mins.

OBJECTIVES

The aims of the study are to determine the sensitivity, specificity, positive predictive value and negative predictive value of Rapid antigen Test and to obtain comparative analysis of Rapid antigen Test and RT-PCR.

MATERIALS AND METHODS

Study Design

This is a retrospective study from September 2020 to March 2021, with a sample size of 154 cases, includes review of clinical records of cases of Covid-19 admitted in a tertiary care teaching hospital of MGM's Medical College in Navi Mumbai region of Maharashtra state of India. The study includes reports of Rapid antigen test and RT-PCR test of suspected cases of COVID-19 in the OPD and admitted patients who underwent both RT-PCR and RAT.

Specimens

National guidelines on laboratory biosafety related to SARS-CoV2 were strictly adhered to, and sample collection was done by trained personnel under all personal protective precautions. A nasopharyngeal (NP) swab and/or an oropharyngeal (OP) swab were employed for testing of COVID-19. Nasophyrangeal swabs were taken for RT-PCR and RAT in highly suspected patients of COVID-19.

Tests

STANDARD Q COVID-19 Antigen Test by SD Biosensor is a ready to use test which allows rapid and qualitative detection of SARS-CoV-2 antigen in nasopharyngeal secretions. The test device consists of two pre-coated lines, "C" Control line and "T" Test line on the nitrocellulose membrane which cannot be seen before applying the specimen. The control line should always appear to confirm whether the test has been conducted adequately, the test line is seen if SARS-CoV-2 antigens are present in the patient's specimen.[4]

IntaQ96 RT-PCR Machine by HiMedia was used to run RT-PCR specimens. PCR characterized samples (Viral transport medium with swabs) were kept at 4°C and tested within 48 hours. A single NP swab is found most tolerable for the patient and is also considered safer in terms of exposure to the sample collector.[5] Results of the RAT were compared to those of RT-PCR as the reference method. Tests were repeated for samples showing inclusive results.

Exclusion Criteria

All proven negative cases on RT-PCR, patients aged less than 5 years and those patients who did not undergo both RT-PCR and RAT on the same day were excluded from the study.

Statistics

The Statistical analysis was done using SPSS 21 software. Sensitivity, Specificity, positive predictive value and negative predictive value of Rapid Antigen test was calculated.

RESULT

The nasopharyngeal samples were collected from subjects with the following presentation: (1) Asymptomatic with upper respiratory tract infection, individuals who had history of contacted with confirmed cases or were from COVID-19 risk areas, (2) patients with acute respiratory infections, (3) unknown causative agents of pneumonia, (4) travellers screened at port of entry or in quarantine places.

In our study, a total of 154 symptomatic patients for detection of SARS CoV-2, COVID-19 by both RT-PCR and RAT were studied. Of these, 38 (24.68%) were RT-PCR positive for SARS-CoV-2 RNA and 116 (75.32%) tested negative. Out of 154 total samples,25 (16.23%) were positive and 129 (83.77%) were negative as shown in table and Fig.1.

Among them, 87 were males and 67 were females. Patients were from age of from 6 years to 87 years. The median age was 39 years. Most samples were taken during the initial phase of the disease, with median duration of symptoms of 3 days (interquartile range (IQR) 1–5 days). The mean Ct value of RT-PCR-positive samples was 20.2(IQR 14.6–25.8). (Table 2) Of the total COVID-19 cases, 73.6% (n=28) of patients had direct contact with a variety of confirmed cases, such as family members and friends. Most of the patients showed signs and symptoms of upper respiratory tract infections (60.5%; n=23). Around 15.7% (n=6) of COVID-19 cases were presented with pneumonia and were admitted to an intensive care unit (ICU).

Table 1: Overall RT-PCR test results and Rapid antigen test results

	RT-PCR (+)	**RT-PCR (-)**	**Total**
RAT (+)	25	0	25
RAT (-)	13	116	129
Total	38	116	154

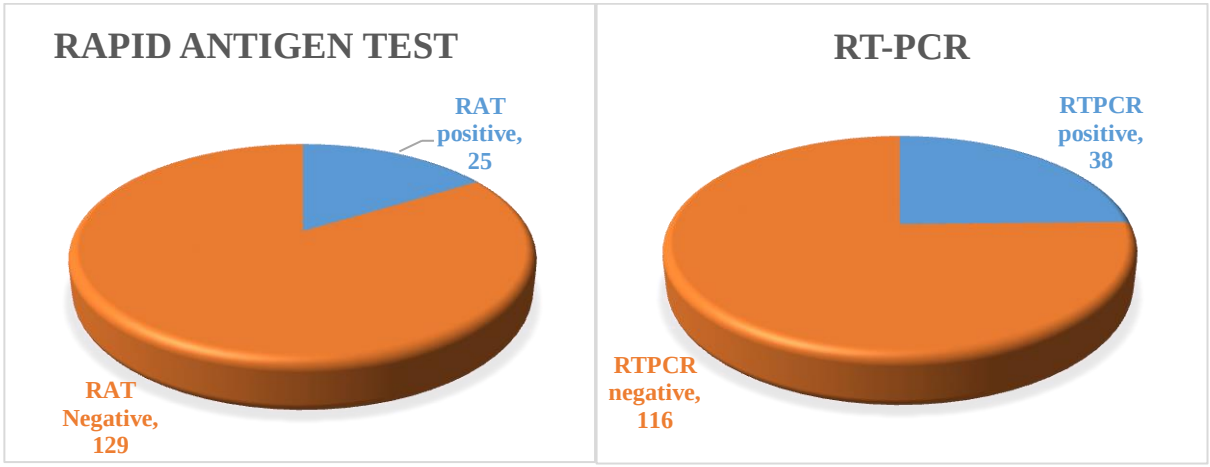

Figure- 1 - Overall RT-PCR test results and Rapid antigen test results

Table 2: Data representing absolute numbers (%).

		Total	RTPCR (+)	RTPCR (-)
Total		**154**	**38**	**116**
Sex	Male	87	21	66
	Female	67	17	50
	Median	39	39	39
Age (years)	**Range**	**5yrs-87yrs**	**14yrs-84yrs**	**5yrs-87yrs**
	5yrs-17yrs	11	3	8
	18yrs-59yrs	108	26	82
	≥60 yrs	35	9	26
Days of symptoms	**Range**	**0-10**	**0-10**	**0-10**
	Day 0-3	115	28	87
	day 4-7	34	9	25
	Day≥8	5	1	4
Ct value	IQR		14.6-25.8	
	Mean		20.2	

It was found that the sensitivity of Rapid antigen test was 65.78% and specificity of 100%. Sensitivity was significantly reduced in the subgroup of samples with Ct values >25.1, indicating lower viral loads. No significant difference within other subgroups was identified. All false-negative results (n =13) corresponded to samples with RT-PCR Ct values >26.

Table 3: Analysis of Rapid antigen test

Analytical parameters	**Value**
Sensitivity	65.78%
Specificity	100%
Positive predictive value	100%
Negative predictive value	89.92%

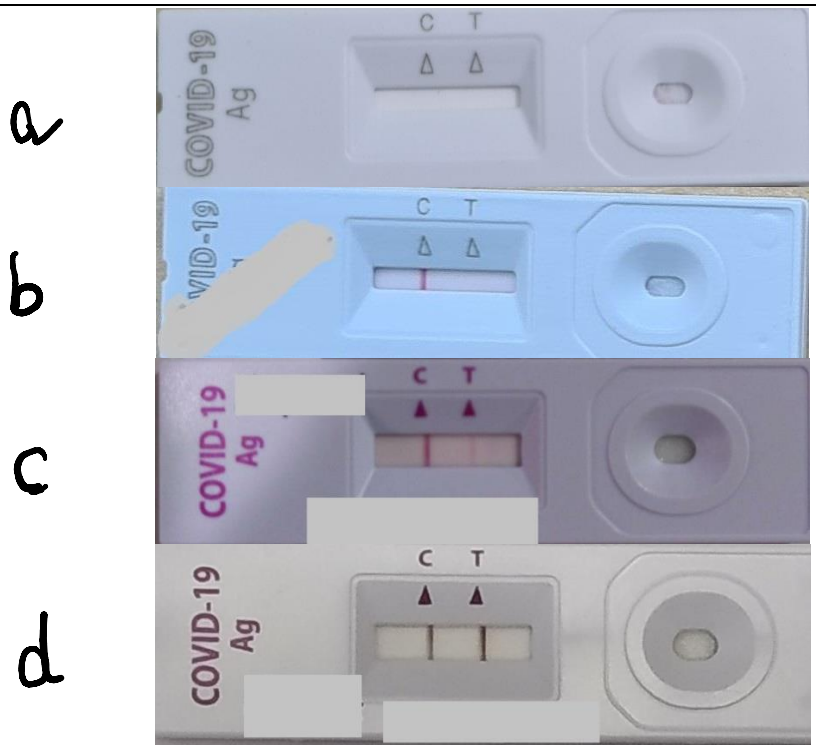

Figure 2: Interpretation of rapid SARS-CoV-2 antigen detection assay (Standard Q COVID-19 Ag Test). Demonstration of (a)- test strip for viral transport media control, (b)- test strip, which was interpreted as negative SARS-CoV-2 antigen, (c)- test strip, which was interpreted as (weakly) positive SARS-CoV-2 antigen, and (d)- test strip, which was interpreted as positive SARS-CoV-2 antigen.

DISCUSSION

COVID-19 outbreak was declared as public health emergency of international concern by WHO in January 2020. Since then the disease has spread worldwide, which resulted in increasing needs for the availability of a diagnostic testing in order to limit the spread of virus.[6] A Nasopharyngeal swab rather than oropharyngeal swab is recommended for early diagnosis or screening because it provides higher diagnostic yield since the nucleo-capsid protein of the virus is present in abundance in the nasopharyngeal samples is better tolerated by the patient and is safer for the operator.[7] An NP swab can be combined with an OP swab to increase sensitivity but requires twice the number of swabs.[5] A rapid, accurate, and affordable diagnostic test for COVID-19 can be highly beneficial by limiting the spread of the infection in the initial stages. RAT can also help in early detection of the virus in asymptomatic contacts of symptomatic patients.[8] In a country like India, where SARS-CoV-2 testing by RT-PCR is a challenging task, due to the large population and longer reporting time, RAT could prove as a boon in the diagnosis of COVID-19. Rapid Antigen test is a point-of-care test, although different diagnostic test manufacturers have developed several commercially available rapid tests based on SARS-CoV-2 proteins detection in respiratory samples.

The analytical performances of these tests depend on factors like viral load, quality of the specimen, proper testing techniques and reading of results. The performances also depend on the setting of patients tested.[9] The COVID-19 Rapid antigen test has several advantages over RT-PCR in terms of ease of performance, quicker results, lower cost and the non-requirement of any special equipment, lab or skills as compared to molecular techniques.[10] However the data presented here in this study suggests that the rapid test is falling short in sensitivity.

In the present study, while the specificity was 100%, the overall sensitivity of the COVID-19 Ag test was only 65.78 %. We have considered RT-PCR as the gold standard test for the detection of COVID-19 with a 100% specificity. Since one test came positive on RAT but negative on RT-PCR, we considered it as a false positive test. Such an error can occur if the test kit is read beyond the time which has been specified by the manufacturer, or if the storage temperature requirements of the kit are not met.[11] Out of the RAT positive cases a majority were tested in the first three days of onset of symptom, hence we conclude that RAT is more sensitive in initial days of COVID-19 disease when the viral load is high.

The advantage of the Standard Q COVID-19 Ag test as a screening for COVID-19 is its simple procedure and quick results with high NPV, but its disadvantage is low PPV in a low prevalence area. Owing to the poor sensitivity of the COVID-19 Ag test the number of false negative results increase, which in these times of pandemic can be of great consequence.[12] Since a negative result cannot rule out SARS-CoV-2 infection, this test can only be used as a screening test and is of little use during a pandemic. Pending more evidence of their performances, our data suggest that COVID-19 Ag test should not be used alone for COVID-19 diagnosis and RT-PCR should be done for confirmation in RAT negative patients.

CONCLUSION

The rapid assay for SARS-CoV-2 antigen detection showed comparable sensitivity and specificity with real-time RT-PCR assay. Taken together, we conclude that sensitivity of the antigen assay is inferior to the RT-PCR assay. However, the antigen assay may be a quick and easy-to-perform approach to allow differentiation of individuals contagious for SARS-CoV-2 from non- or less contagious individuals. We believe that there is a potential use of this rapid and simple SARS-CoV-2 antigen detection test as a screening assay, especially in a high prevalence area.

CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES:

1. World Health Organisation. 2021 April 3. Retrieved from https://covid19.who.int/table.
2. Mak GC, Cheng PK, Lau SS, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. *J Clin Virol*. 2020;129:104500. doi:10.1016/j.jcv.2020.104500
3. World Health Organisation. Laboratory Testing for Coronavirus Disease 2019 (COVID-19) in Suspected Human Cases: Interim Guidance, 2 March, Available from: (2020) https://apps.who. int/iris/handle/10665/331329.
4. World Health Organisation. October 2020. WHO EUL Public Report. WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs PUBLIC REPORT. EUL-0563-117-00
5. Yi-Wei Tand, Jonathan E. Schmitz, David H. Persing, Charles W. Stratton. Laboratory Diagnosis of COVID-19: Current Issues and Challenges. doi: 10.1128/JCM.00512-20
6. World Health Organisation. Coronavirus disease (COVID-2019) Situation Report – 51. 11 March 2020. Available from: https://www.who.int/docs/defaultsource/coronaviruse/situation-reports/20200311-sitrep-51-covid19.pdf?sfvrsn=1ba62e57_10
7. Khandker SS, Nik Hashim NHH, Deris ZZ, Shueb RH, Islam MA. Diagnostic Accuracy of Rapid Antigen Test Kits for Detecting SARS-CoV-2: A Systematic Review and Meta-Analysis of 17,171 Suspected COVID-19 Patients. *J Clin Med.* 2021;10(16):3493. Published 2021 Aug 8. doi:10.3390/jcm10163493.
8. Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol 2020 published online ahead of print, 2020 May 21.
9. Torres I, Poujois S, Albert E, Colomina J, Navarro D. Evaluation of a rapid antigen test (Panbio™ COVID-19 Ag rapid test device) for SARS-CoV-2 detection in asymptomatic close contacts of COVID-19 patients. *Clin Microbiol Infect*. 2021;27(4):636.e1-636.e4. doi:10.1016/j.cmi.2020.12.022
10. Albert E, Torres I, Bueno F, et al. Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres. *Clin Microbiol Infect*. 2021;27(3):472.e7-472.e10. doi:10.1016/j.cmi.2020.11.004
11. Yamayoshi S, Sakai-Tagawa Y, Koga M, et al. Comparison of Rapid Antigen Tests for COVID-19. *Viruses*. 2020;12(12):1420. Published 2020 Dec 10. doi:10.3390/v12121420

12. Chaimayo C, Kaewnaphan B, Tanlieng N, Athipanyasilp N, Sirijatuphat R, Chayakulkeeree M, Angkasekwinai N, Sutthent R, Puangpunngam N, Tharmviboonsri T, Pongraweewan O, Chuthapisith S, Sirivatanauksorn Y, Kantakamalakul W, Horthongkham N. Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand. Virol J. 2020 Nov 13;17(1):177. doi: 10.1186/s12985-020-01452-5. PMID: 33187528; PMCID: PMC7665091.