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Research Article

RAPID DETECTION OF SARS COV-2 INFECTION IN COMPARISION WITH RT-PCR IN TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: The goal of the present study was to assess the SARS-CoV-2 antigen detection test's performance features and compare them to the real-time reverse transcription polymerase chain reaction (RT-PCR) test, the gold standard test for the diagnosis of COVID-19 cases.

Methods: From October 2020 to May 2021, patients attending the OPD, including those undergoing surgery, at a Tertiary Care Teaching Hospital in Telangana provided 1000 respiratory samples, primarily nasopharyngeal swabs. A skilled technician had collected two nasopharyngeal swabs from each person in a COVID sample collection room while wearing personal protective equipment and following strict infection control procedures. One swab was used for the rapid antigen test given by the standard Q COVID-19 Ag test kit and placed into the extraction buffer tube. Second swab was kept in the viral transport medium and used for Allplex™ 2019-nCoV Assay (Seegene, Korea), which targets envelope gene (E), and RNA dependent RNA polymerase (RdRp) and nucleocapsid (N) genes of SARS CoV-2, was used for SARS-CoV-2 RNA detection according to the manufacturer's instructions.

Results: Out of 1000 samples tested for COVID-19, 623 (63.7%) were males and 377 (36.3%) were females. Out of 1000 samples, 347 samples were RT-PCR positive and 653 were RT-PCR negative. Out of 347 RT-PCR samples positive, 341 were Rapid antigen test positive samples and six were negative. Overall sensitivity and specificity are 98.27% and 99.85%, respectively.

Conclusion: The real-time RT-PCR assay's sensitivity and specificity were comparable to those of the rapid assay for SARS-CoV-2 antigen detection. It can be utilized for contact tracing measures to control the COVID-19 pandemic in places such as border crossings, airports, interregional bus and train stations, and mass testing campaigns needing quick findings. This is especially true in areas with a high prevalence of the disease.

Keywords: Severe acute respiratory syndrome coronavirus 2, Rapid antigen test, Reverse transcription-polymerase chain reaction, Sensitivity, Specificity.

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INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-caused coronavirus illness 2019 (COVID-19) has spread around the globe since the first case was found in the Chinese city of Wuhan in December 2019 [1]. The virus spreads swiftly and is hard to control; in March 2020, it was declared a pandemic [2]. Due to the rapid spread of the increasing number of COVID-19 cases and the significant morbidity brought on by the coronavirus, the Indian Council of Medical Research (ICMR) published testing methodologies for viral detection, management of the sources of infection, and prevention of illness progression. Although results might often take 3–4 h to complete and specialized lab equipment and skilled staff are required, real-time reverse transcription polymerase chain reaction (RT-PCR) is the gold standard test for detection [2,3]. The significant gap between the large number of patients/contacts and the laboratory capacities to perform RT-PCR in a timely manner is one of the primary drawbacks of current public health containment strategies (WHO, 2020). Therefore, there is an urgent need for alternative assays, such as antigen detection tests, which, in contrast to antibody testing, may detect the presence of the virus itself in respiratory samples (WHO, 2020). Rapid antigen tests (RAT) for SARS-CoV-2 may be performed in batch on-site, cost less than real-time RT-PCR, do not require pricy specialized equipment, and yield results in 15 min (CDC, 2021) [2,4]. The Standard "Q" COVID-19 antigen detection kit from SD Biosensor (South Korea) was the first fast antigen kit to achieve ICMR approval (2) This test is easy to administer, easy to interpret, and gives results in 30 min. Accurate and quick detection of acute SARS-CoV-2 infection is essential. To speed up disease prevention and control, rapid and accurate tests for SARS-CoV-2 screening are required, as well as pre-operative screening for invasive operations. If the accuracy of lateral flow immunoassays, which use monoclonal anti-SARSCoV-2 antibodies to target SARS-CoV-2 antigens, was equivalent to that of real-time RT-PCR assays, they could serve as complementary screening tools. The goal of the present study was to assess the quick SARS-CoV-2 antigen detection test's performance features and compare it to the industry-recognized real-time reverse transcription-polymerase chain reaction (RT-PCR) assay for the diagnosis of COVID-19 cases [4].

METHODS

From October 2020 to May 2021, patients visiting the OPD, including those undergoing surgery, at a Tertiary Care Teaching Hospital in Telangana provided 1000 respiratory samples, primarily nasopharyngeal swabs. A skilled technician collected two nasopharyngeal swabs from each person in a COVID sample collection room while wearing personal protective equipment and following strict infection control procedures. One swab was put into the extraction buffer tube of the fast antigen test that included with the standard Q COVID-19 Ag test kit (SD Biosensor). Squeezing the walls of the tube allows the liquid to be extracted from the swab when it is removed. The extraction tube was firmly clamped on by the filter nozzle cap. The second swab was maintained in Himedia Pvt. Ltd, India's viral transport medium (VTM). Samples were transported at 2–8°C to the molecular laboratory, for processing within a few hours. All specimens were processed in Biosafety level-2 TYPE B (BSL -2, TPYE -B) with full Personal Protective Equipment.

Rapid SARS-CoV-2 antigen detection assay

The Standard Q® COVID-19 Ag test (SD Biosensor HEALTH CARE PVT.LTD., HARYANA, INDIA) is a fast chromatographic lateral flow immunoassay with a sensitivity of 84.38% and a specificity of 100% for the detection of SARS-CoV-2 Nucleocapsid (N) antigen in respiratory specimens. On the result window of this fast antigen test device, there are two pre-coated lines: The control (C) and test (T) lines. Mouse monoclonal anti-chicken Ig antibody is coated on the control (C) region, whereas mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test (T) region. The detectors for this device are mouse monoclonal anti-SARS-CoV-2 antibodies that have been coupled with colored particles. The antigen-antibody color complex is created during the test when the SARS-CoV-2 antigen in the specimen interacts with monoclonal anti-SARS-CoV-2 antibody coupled with a color particle. The mouse monoclonal anti-SARS-CoV-2 antibody coated on the test (T) area captures this antigen-antibody color particle complex as it migrates through the capillary force. The amount of SARS-CoV-2 N antigen present in the sample determines how intense the colored test (T) line is. A test gadget received three drops of the extracted material, and within 15–30 min, the test result was read. Two colored lines representing the control (C) and test (T) lines were displayed for positive COVID-19 antigen results (1).

Viral RNA extraction

Viral RNA was extracted using the QIAamp® Viral RNA MINI Kit. SARS-CoV-2 RNA was extracted using the QIACUBE CONNECT automated extraction platform (QIAGEN). 560 liters of lysis buffer are mixed with 5.6 L of c-DNA and 140 L of viral transport medium before being fed into a QIAGEN machine for RNA elution. The elution of RNA takes 45 min. Extracted RNA was tested immediately using RT-PCR, and any leftover samples were kept at −80°C.

RT-PCR test

As directed by the manufacturer, the AllplexTM 2019-nCoV Assay (Seegene, Korea), which specifically targets the nucleocapsid (N) and RNA dependent RNA polymerase (RdRp) genes of SARS-CoV-2, was used to identify SARS-CoV-2 RNA. The 2019-nCoV MuDT Oligo Mix (2019-nCoV-MOM), 5 L of 5X Real-time One-step Buffer, 2 L of Real-time One-step Enzyme, and 5 L of RNase-free water are combined to create the Mastermix. 8 L of viral RNA that has been extracted is added to this master mixture. For amplification, the Light Cycler® 96 Roche (Roche Diagnostics India) is employed under the following cycling conditions: 1 cycle lasting 20 min at 50°C, 1 cycle lasting 15 min at 95°C, then 45 cycles lasting 15 s at 94°C, 30 s at 58°C. A cycle threshold value (ct value) 40 for each of the three target genes was considered a favorable outcome when analyzing the results with seegene viewer (seegene, Korea).

Statistical analysis

The general information of the patients was described using descriptive statistics. Mean and standard deviation were used to depict continuous data (SD). Numbers, percentages, and the 95% confidence interval (95% CI) were used to present categorical data. An online statistical tool was used to calculate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS

Out of 1000 samples tested for Covid-19, 623 (63.7%) were males and 377 (36.3%) were females (Table 1). Most common age group affected was in the range of 21–40 years (48%) (Table 2). Out of 1000 patients attended the hospital, 168 (48.41%) had contact history, 79 (22.77%) had COVID symptoms, and 36 (10.38%) came for routine checkup (Table 1). Table 3 depicted the results of RT-PCR assay and rapid antigen testing. Out of 1000 samples, 347 samples were RT-PCR positive and 653 were RT-PCR negative. Out of 347 RT-PCR samples positive, 341 were Rapid antigen test positive samples and six were negative. Overall sensitivity and specificity are 98.27% and 99.85%, respectively (Table 4). Positive and negative predictive values were 99.71% and 99.09% (Table 4). Accuracy between the two tests is 99.30%. Cohen Kappa is 0.98, 95% CI: 0.97-0.99 (Table 4). Table 5 showed the statistical analysis results of RT-PCR results.

DISCUSSION

Early diagnosis is crucial for managing and stopping the spread of the COVID-19 epidemic, which has now reached tragic proportions [5]. Point-of-care diagnostic assays that are easy to use, rapid, and economical are therefore urgently needed. In our study, a fast SARS-CoV-2 antigen detection kit (Standard Q® COVID-19 Ag test) and an RT-PCR assay (Allplex TM 2019-nCoV Assay) for the detection of SARS-CoV-2 infection were compared [1]. Thirty-two nasopharyngeal

Table 1: Characteristics of COVID-19 cases

Characteristics	Results
Total number of samples	1000
Males	623 (62.3%)
Females	377 (37.7%)
Contact history with positive person	168 (48.41%)
Travel history	64 (18.44%)
COVID symptoms	79 (22.77%)
Routine checkup	36 (10.38%)

Table 2: Age-wise distribution

Age (years)	Number of patients	Percentage
0–10	59	5.9
11–20	41	4.1
21–30	246	24.6
31–40	234	23.4
41–50	210	21
51–60	68	6.8
>60	142	14.2
Total	1000	100

Table 3: Test results of RT-PCR and rapid antigen detection assay

Test results	RT-PCR positive	RT-PCR negative	Total
Rapid antigen test Positive	341	1	342
Rapid antigen test Negative	6	652	658
Total	347	653	1000

Table 4: Analytical parameters

Statistics	Value	95% CI
Sensitivity	98.27%	96.27-99.36%
Specificity	99.85%	99.15-100%
Positive predictive value	99.71%	97.96-99.96%
Negative predictive value	99.09%	98.01-99.59%
Accuracy	99.30%	98.56-99.72%
Cohen's kappa	0.98450	
Standard error	0.00584	
95% CI	0.97306-0.99594	

Table 5: Statistical results of RT-PCR results

Results of RT-PCR assay	Mean±SD
CT value of E gene	18.97±6.112 (min=12.53; Max=36.06)
CT value of RdRp (RNA-dependent RNA polymerase)	24.48±6.049 (Min=12.96; Max=36.54)
CT value of N gene	24.77±6.715 (Min=10.09; Max=36.41)

RT-PCR: Reverse transcription polymerase chain reaction

Table 6: Comparison with other studies

Author	Kit	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Present study	Standard Q® Covid-19 AG Kit/SD Biosensor Allplex™ 2019-N Cov assay (Seegene)	98.27	99.85	99.71	99.09	99.30
Chaimayo *et al.* [1]	Standard Q® Covid 19 AG Kit/AD Biosensor Allplex™ 2019-N Cov Assay (Seegene)	98.33	98.73	92.19	99.74	98.68
Cerutti *et al.* [10]	Standard Q® Covid 19 AG Kit/Sd Biosensor Allplex™ 2019-N Cov Assay (Seegene)	70.64	100	100	87.35	90.30
Pena *et al.* [5]	Standard Q® Covid 19 Ag Kit/Sd Biosensor Genome Cov -2	69.86	99.61	94.44	97.21	97.03
Kruttegen *et al.* [4]	Sars Cov 2 Ag (Roche Switzerland). Real Star RT-PCR (Altona , Germany)	70.67	96.0	94.64	76.0	83.33
Porte *et al.* [9]	Fluorescence Antigen Rapid Test Kit (Bioeasy Biotechnology Co., Shenzhen, China). COVID-19 Genesig Real-time PCR Assay (Primer Design Ltd, Chandler's Ford, UK)	93.9	100	100	99.41	96.1

swabs from symptomatic patients with positive RT-PCR results were used at a trial location in Malaysia to evaluate the test's sensitivity. 170 RT-PCR-negative samples were utilized by the SD Biosensor R&D team to evaluate the test's specificity [1]. According to the manufacturer, the Standard Q® COVID-19 Ag test had a sensitivity and specificity of 84.38% (95% CI, 67.21-94.72%) and 100.00% (95% CI, 97.85-100%), respectively, for the rapid detection of SARS-CoV-2 antigen (total n = 202; positive n = 32; negative n = 170) [1]. Our findings were superior to those of the manufacturer in terms of sensitivity (98.27% vs. 84.38%) but worse in terms of specificity (99.85% vs. 100%). the sample's quality, antigen extraction, sample handling, and processing techniques. In our investigation, six of the 347 RT-PCR-positive samples yielded erroneous negative results. These samples' RT-PCR results showed comparatively high Ct-values, which may be connected to lower viral loads and reduced infectiousness and may help to explain why the Standard Q COVID-19 Ag test came back negative [1, 4-8]. One NP swab was found to be positive for SARS-CoV-2 antigen using the Standard Q COVID-19 Ag test out of 653 RT-PCR negative samples. The outcomes were shown in Table 6 along with comparisons to earlier investigations. Although the reason for the unexpected result is unknown, it may have been because the antigen detection kit tends to give falsely positive findings when tested on thick, highly viscous mucus [1]. According to the most recent testing guidance published by the ICMR on September 4, 2020, any positive antigen test will be reported as COVID-19 positive regardless of whether the patient exhibits symptoms or not. In any event, there is no requirement to confirm such results with RT-PCR [2]. The PPV of antigen testing was 99.71%, showing that SARS-CoV-2 infection in asymptomatic individuals with positive antigen results does not necessitate confirmatory real-time RT-PCR [9-11]. Antigen testing also had a 99.09% NPV, meaning that asymptomatic people with negative antigen results are unlikely to have SARS-CoV-2 infection [1,4]. According to the Centers for Disease Control and Prevention's (CDC, 2021) advice regarding the use of antigen testing, RAT should not take the place of real-time RT-PCR in the diagnosis and surveillance of SARS-CoV-2 infection (CDC, 2021) [4, 12,13]. This is supported by the sensitivity of (98.27%). Given that the number is statistically significant and falls between 0.81 and 1.00, Cohen's Kappa is 0.9845.

CONCLUSION

Real-time RT-PCR (SEEGENE ALLPLEX test) and the fast assay for SARS-CoV-2 antigen identification (Standard Q® COVID-19 AG kit) displayed equal sensitivity and specificity. It can be utilized for contact tracing tactics to control the COVID-19 pandemic in places such as border crossings, airports, interregional bus and train stations, and mass testing campaigns needing quick findings. This is especially true in areas with a high prevalence of the disease.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

AUTHORS' CONTRIBUTION

Author R. Venkata Laxmi contributed conceptual design, performed the work, and wrote the first draft of manuscript. Author A. Ramya corrected the manuscript, D. Mamatha Reddy collected the literature, data collection and S. Gouthami had performed the statistical analysis part of the work.

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REFERENCES

1. Chaimayo C, Kaewnaphan B, Tanlieng N, Athipanyasilp N, Sirijatuphat R, Chayakulkeeree M, *et al*. 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;17:177. doi: 10.1186/s12985-020-01452-5, PMID 33187528
2. Mohanty A, Kabi A, Kumar S, Hada V. Role of rapid antigen test in the diagnosis of COVID-19 in India. J Adv Med Med Res 2020;32:77-80. doi: 10.9734/jammr/2020/v32i1830657
3. Hirotsu Y, Maejima M, Shibusawa M, Nagakubo Y, Hosaka K, Amemiya K, *et al*. Comparison of automated SARS-CoV-2 antigen test for COVID-19 infection with quantitative RT-PCR using 313 nasopharyngeal swabs, including from seven serially followed patients. Int J Infect Dis 2020;99:397-402. doi: 10.1016/j.ijid.2020.08.029, PMID 32800855
4. Krüttgen A, Cornelissen CG, Dreher M, Hornef MW, Imöhl M, Kleines M. Comparison of the SARS-CoV-2 Rapid antigen test to the real star Sars-CoV-2 RT PCR kit. J Virol Methods 2021;288:114024. doi: 10.1016/j.jviromet.2020.114024, PMID 33227341
5. Peña M, Ampuero M, Garcés C, Gaggero A, García P, Velasquez MS, *et al*. Performance of SARS-CoV-2 rapid antigen test compared with real-time RT-PCR in asymptomatic individuals. Int J Infect Dis 2021;107:201-4. doi: 10.1016/j.ijid.2021.04.087, PMID 33945868
6. Schoonjans F. MedCalc's Diagnostic Test Evaluation Calculator. MedCalc Software. 2020. Available from: https://www.medca: Available from: https://www.lc.org/calc/diagnostictest.php [Last accessed on 2020 Jun 01].
7. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, *et al*. Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. Clin Infect Dis 2020;71:2663-6. doi: 10.1093/cid/ciaa638, PMID 32442256
8. Singanayagam A, Patel M, Charlett A, Bernal JL, Saliba V, Ellis J, *et al*. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill 2020;25:2001483. doi: 10.2807/1560-7917.ES.2020.25.32.2001483, PMID 32794447
9. Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R, *et al*. Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. Int J Infect Dis

2020;99:328-33. doi: 10.1016/j.ijid.2020.05.098, PMID 32497809

10. Cerutti F, Burdino E, Milia MG, Allice T, Gregori G, Bruzzone B, *et al*. Urgent need of rapid tests for SARS CoV-2 antigen detection: Evaluation of the SD-Biosensor antigen test for SARS-CoV-2. J Clin Virol 2020;132:104654. doi: 10.1016/j.jcv.2020.104654, PMID 33053494
11. Buder F, Bauswein M, Magnus CL, Audebert F, Lang H, Kundel C, *et al*. Contribution of high viral loads, detection of viral antigen and seroconversion to severe acute respiratory syndrome coronavirus 2 infectivity. J Infect Dis 2022;225:190-8. doi: 10.1093/infdis/jiab415, PMID 34427652
12. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, *et al*. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane database Syst Rev 2021;24:CD013705. doi: 10.1002/14651858.CD013705.pub2, PMID 33760236
13. Jakobsen KK, Jensen JS, Todsen T, Lippert F, Martel CJ, Klokker M, *et al*. Detection of SARS-CoV-2 infection by rapid antigen test in comparison with RT-PCR in a public setting. medRxiv 2021. doi: 10.1101/2021.01.22.21250042