Original Article

Evaluation of Rapid Antigen Test for Detection of SARS-COV2 Virus in Comparison with Real-Time Reverse Transcription-Polymerase Chain Reaction Assay

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Abstract

Introduction: The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to spread worldwide. Antigen point-of-care tests are needed to help speed up the testing of SARS-CoV-2. In this study, we evaluated the performance of a rapid SARS-CoV-2 antigen detection assay to the SARS-CoV-2 real-time reverse transcription-polymerase chain reaction (RT-PCR) test. **Materials and Methods:** COVID-19 infection suspected patients and contact individuals provided 185 respiratory samples (mostly nasopharyngeal and throat swabs) during the study from April to June 2021. The rapid SARS-CoV-2 antigen detection test was compared to the real-time RT-PCR test for SARS-CoV-2 detection in respiratory specimens. **Results:** By real-time RT-PCR testing, 80 (43.2%) of 185 respiratory samples were positive for SARS-CoV-2 RNA, while 105 (56.8%) were negative. The sensitivity and specificity of the fast SARS-CoV-2 antigen detection test were 63.1% and 90.1%, respectively. **Conclusion:** The sensitivity and specificity of the fast assay for SARS-CoV-2 antigen detection were comparable to those of the real-time RT-PCR assay. As a result, the rapid and easy SARS-CoV-2 antigen detection test could be used as a screening assay.

Keywords: Rapid antigen test, reverse transcription-polymerase chain reaction, severe acute respiratory syndrome coronavirus 2

Introduction

Since its first confirmed incidence in the Chinese city of Wuhan in December 2019, the coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread around the world. The cumulative number of cases and deaths reported globally is about 194 million and over 4 million, respectively, according to the WHO weekly epidemiological bulletin published on July 27, 2021. A total of 200 countries have been afflicted with the virus. These figures are likely to continue to climb, particularly in populous countries such as the United States, Brazil, and India. As of July 27, 2021, there have been 31,695,958 confirmed COVID-19 cases with 424,773 deaths.[1,2] The first known instances of COVID-19 infection were reported in Kerala, India. On January 27, 2020, a 20-year-old female presented to the Emergency Department in General Hospital, Thrissur, Kerala, with a 1-day history of dry cough and sore throat.[3]

Coronaviruses are single-stranded enclosed RNA viruses with helical capsids that infect humans, bats, other animals, and birds.[2] The transmission of SARS-CoV-2 is thought to occur mainly through respiratory droplets. Prolonged exposure to an asymptomatic infected person (within 6 feet for at least 15 min) and briefer exposures to symptomatic individuals (coughing) are associated with a higher risk of transmission than shorter exposures to asymptomatic individuals. Viral shedding appears to start 2 to 3 days before the onset of symptoms and peaks around the time

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Submitted: 20-Feb-2022 **Revised:** 25-Mar-2022
Accepted: 28-May-2022 **Published:** 27-Sep-2022

Access this article online

Quick Response Code:

Website: www.journaldmims.com

DOI: 10.4103/jdmimsu.jdmimsu_72_22

How to cite this article: Mantur PG, Bagali S, Sani G, Shahapur P, Honnutagi R, Patil MM. Evaluation of rapid antigen test for detection of SARS-COV2 Virus in comparison with real-time reverse transcription-polymerase chain reaction assay. J Datta Meghe Inst Med Sci Univ 2022;17:354-7.

of symptom onset and has been shown in asymptomatic individuals.[2,3]

Like pulmonary epithelial cells, vascular endothelial cells express angiotensin-converting enzyme 2, and SARS-COV-2 has been found inside endothelial cells in pulmonary capillaries, leading to inflammatory cytokine production, endothelial cell death, and endothelial barrier disruption. As the inflammation progresses, pulmonary edema and hyaline membrane formation occur and cause acute respiratory distress syndrome, which interferes with oxygen diffusion.[4,5]

The real-time reverse transcription-polymerase chain reaction (RT-PCR), which is currently the gold standard for laboratory diagnosis of SARS-CoV-2 infection, has a long turnaround time and is only conducted by highly skilled people.[6] As a result, SARS-CoV-2 screening assays that are accurate and timely are crucial for disease prevention and control. If lateral flow immunoassays using monoclonal anti-SARS CoV-2 antibodies that target SARS-CoV-2 antigens are as accurate as real-time RT-PCR tests, they can be utilized as a supplement to real-time RT-PCR assays.[7,8] Hence, the present study was conducted to evaluate the rapid antigen test (RAT) for the detection of SARS COV-2 in comparison with a real-time RT-PCR assay.

Materials and Methods

Sample collection

This was a cross-sectional observational study carried out at our tertiary care hospital, South India. The study protocol was approved by the Ethical Committee of our Institute. During the study from April to June 2021, respiratory samples, primarily nasopharyngeal (NP) and throat swabs, were taken from 185 probable COVID-19 patients who visited or were admitted to the hospital. The sample was collected after obtaining informed consent from participating individuals. Demographic and relevant clinical data were collected from each participant. 2 mL of viral transport medium was used to collect the samples. The samples were transported to the microbiology laboratory at 2°C–8°C and processed in a matter of hours. All of the samples were processed in biosafety level 2 laboratories with full personal protective equipment.

Viral RNA extraction

Viral RNA extraction was done manually using TRUPCR® VIRAL RNA EXTRACTION KIT (Kilpest India Limited) from 200 µL of NP and oropharyngeal swabs. Extraction was performed according to the manufacturer's instructions. Viral RNA was eluted with 40 µL buffer and used for RT-PCR assay.

Severe acute respiratory syndrome coronavirus 2 RNA detection using real-time reverse transcription-polymerase chain reaction

According to the manufacturer's instructions, the TRUPCR® SARS-CoV-2 RT quantitative PCR (qPCR) Kit (V-3.2) (Kilpest India Limited) was used to detect SARS-CoV-2 RNA, which targets the envelope gene (E) of Sarbecovirus, as well as the RNA-dependent RNA polymerase and nucleocapsid (N) genes of SARS-CoV-2. In a nutshell, 10 µL of extracted RNA were mixed with 10 µL of Master mix, 0.35 µL of Enzyme mix, and 4.65 µL of Primer Probe mix. For amplification, Thermo Fisher Scientific's QuantStudioTM 5 real-time PCR System was employed. One cycle of 15 min at 50°C and 5 min at 95°C was followed by 38 cycles of 5 s at 95°C, 40 s at 60°C, and 15 s at 72°C. The result was analyzed and a cycle threshold value <35 for all three target genes was defined as a positive result.

Rapid severe acute respiratory syndrome coronavirus 2 antigen detection assay

- CIP test Plus COVID 19 Antigen Rapid Test (Cipla Limited Mumbai India) is an *in vitro* diagnostic (IVD) medical device intended for the qualitative Immunochromatographic assay for detection of nucleocapsid protein of novel coronavirus in human NP secretions
- On the result window of the RAT gadget, there are two precoated lines: control (C) and test (T). The test (T) region is coated with mouse monoclonal anti-chicken Ig antibody against SARS-CoV-2 N antigen, while the control (C) region is coated with mouse monoclonal anti-chicken Ig antibody.
- Mouse monoclonal anti-SARS-CoV-2 antibody coupled with color particles is used to detect SARS-CoV-2 N antigen in the specimen. The antigen-antibody color particle complex migrates by capillary force to the test (T) region, where it is trapped by a mouse monoclonal anti-SARS-CoV-2 antibody. The intensity of the colored test (T) line is proportional to the amount of SARS-CoV-2 N antigen present in the sample
- Three drops of the extracted sample were applied on a test device, and the test result was read in 15–30 min. For positive COVID-19 antigen results, two colored lines of control (C) and test (T) lines were presented.

Statistical methods used

- All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean ± standard deviation were used. For categorical data, the number and percentage were used in the data summaries. Data were analyzed using SPSS software. Statistical analysis considered sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results

A total of 185 suspected COVID-19 cases and contact individuals were included during the study. The respiratory samples, including NP and throat swabs, were collected from suspected COVID-19 cases and contact individuals. All the samples were tested forSARS-CoV-2 RNA detection using real-time RT-PCR and Rapid SARS-CoV-2 antigen detection assay. Out of the 185 cases, 93 were males and 92 females with a mean age of 34.8 ± 16.6 years. Majority of the cases

were in the age group of 11–30 years (47.6%) followed by 31–60 years (39.5%) [Table 1]. Most of the patients presented with complaints of sore throat and fever [Table 2].

Of the samples (n = 185) tested for SARS-CoV-2 RNA detection using real-time RT-PCR assay, 43.8% (n = 80) were positive, while 56.8% (n = 105) were negative for SARSCoV-2 RNA [Table 3]. By RAT assay, 34.1% (n = 63) were positive, while 65.9% (n = 122) were negative for SARSCoV-2 [Table 4].

The RAT assay had sensitivity, specificity, PPV, and NPV of 63.10%, 90.10%, 84.10%, and 74.60%, respectively when compared to RT-PCR [Table 5].

Discussion

SARS-CoV-2, the virus-causing COVID-19, has become a major public health concern all over the world.[1] Early diagnosis is crucial for patient management and outbreak control. Molecular tests for SARS-CoV-2 RNA identification in clinical specimens are extensively used in diagnostic laboratories, while RT-PCR techniques for SARS-CoV-2 RNA identification in clinical specimens are commonly used. Real-time PCR (RT-PCR) is used to detect SARS-CoV-2 virus RNA and takes a few hours before the results are released. As a result, very sensitive immunological diagnostic approaches that directly detect viral antigens in clinical samples would be extremely useful in detecting COVID-19 quickly and accurately.[3,4]

CIP plus test COVID-19 Antigen Rapid Test is an *in vitro* diagnostic medical device designed to detect the nucleocapsid protein of new coronavirus in human NP secretions in a qualitative immunochromatographic assay. The assay is ready to use and based on a nitrocellulose membrane technology with colloidal gold nanoparticles sensitized with monoclonal antibodies directed against highly conserved SARS-CoV-2 nucleoprotein antigens.

COVID-19 CIP Plus Antigen test for quick detection of SARS-CoV-2 antigen (total n = 185; positive n = 63; negative n = 122), has a sensitivity of 63.1% and a specificity of 90.1% (total n = 185; positive n = 63; negative n = 122). COVID-19 CIP Plus Kit Antigen test has the advantage of being a straightforward procedure with quick results and a high PPV, but it has the disadvantage of low sensitivity. As a result, the nucleic acid test for detecting the SARS-CoV-2 gene, which is more sensitive and specific than this lateral flow immunoassay, is still used to diagnose COVID-19. Despite its flaws, the quick SARS-CoV-2 antigen test can help all healthcare staff manage sick patients more efficiently in a timely manner, especially in rural and outbreak locations. The results obtained in our study are comparable with Lambert-Niclot *et al*.[9] who have reported sensitivity of 50% for RAT when compared to RT-PCR. In a meta-analysis of 83 studies that compared SARS-CoV-2 rapid antigen-based lateral flow testing (RALFT) to RT-qPCR for SARS-CoV-2 conducted by Parvu *et al*.[10] the overall sensitivity for RALFT was determined to be 75%.

Table 1: Distribution of cases according to age

Age (years)	*n* (%)
≤10	9 (4.9)
11-30	88 (47.6)
31-60	73 (39.5)
>60	15 (8.1)
Total	185 (100)

Table 2: Distribution of cases according to complaints

Complaints	*n* (%)
Fever and breathlessness	22 (11.9)
Sore throat and fever	163 (88.1)
Total	185 (100)

Table 3: Distribution of cases according to severe acute respiratory syndrome coronavirus 2 RNA detection using real-time reverse transcription-polymerase chain reaction

RT-PCR	*n* (%)
Negative	105 (56.8)
Positive	80 (43.2)
Total	185 (100)

RT-PCR: Reverse transcription-polymerase chain reaction

Table 4: Distribution of cases according to rapid severe acute respiratory syndrome coronavirus 2 antigen detection assay

Rapid antigen test	*n* (%)
Negative	122 (65.9)
Positive	63 (34.1)
Total	185 (100)

Table 5: Association of rapid severe acute respiratory syndrome coronavirus 2 antigen detection assay with severe acute respiratory syndrome coronavirus 2 RNA detection using real-time reverse transcription-polymerase chain reaction

Rapid antigen test	RT-PCR	
	Positive	Negative
Positive	53	10
Negative	31	91
Total	84	101

RT-PCR: Reverse transcription-polymerase chain reaction

In a study conducted by Berger *et al*., a total of 1064 patients were enrolled with 106 positive Ag-RDT and 124 positive RT-PCR individuals. The PanbioTM COVID-19 Ag Rapid Test device (Abbott) showed a sensitivity of 85.5% (95% confidence interval [CI]: 78–91.2) which is higher compared to the result of our study.[11]

Ampuero *et al*. compared a SARS-CoV-2 RAT and RT-PCR in 842 asymptomatic individuals and SARS-CoV-2 RAT showed

a sensitivity of 69.86% which is comparable to our study but showed high specificity of 99.61%.[12]

This SARS-CoV-2 antigen detection test may be indicated for individuals who present early after symptom onset and are expected to have greater virus loads.[9] Other factors, such as clinical manifestation, time from onset of disease to laboratory test, specimen type, and how specimens were gathered and processed (sample handling and processing techniques), may have an impact on the interpretation of the results.[10,13]

Conclusion

The sensitivity and specificity of the fast assay for SARS-CoV-2 antigen detection were comparable to those of the real-time RT-PCR assay. We accept there is a likely utilization of this fast and straightforward SARS-CoV-2 antigen recognition test as a screening measure, particularly in a high commonness region.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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