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RAPID SARS-CoV-2 ANTIGEN DETECTION ASSAY IN COMPARISON WITH REAL-TIME RT-PCR ASSAY FOR LABORATORY DIAGNOSIS OF COVID-19 IN AT JLNMC,AJMER,CENTRAL RAJASTHAN , INDIA

Microbiology

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ABSTRACT

Background: The Coronavirus disease 2019 (COVID-19) pandemic continues to spread across the world. Hence, there is an urgent need for rapid, simple and accurate tests to diagnose corona- virus-2 infection. The rapid Covid-19 antigen detection test performance should be evaluated and compared with the gold standard real-time reverse transcription-polymerase chain reaction (RT-PCR) test for diagnosis of COVID-19 cases.
Methods: The rapid COVID19 antigen detection test(Xamin , Diagnocure India)was compared with the real time RT-PCR test (Qunatiplus Multiplex Covid-19 detection kit v2.0, Huwel Lifesciences PVT.LTD. Hyderabad, India) and Covisure Covid-19 RT-PCR kit, (Genetix Biotech Asia PVT. LTD. New Delhi, India.)for detection of SARS-CoV-2 in respiratory specimens. Two hundred fifty respiratory samples (mainly nasopharyngeal and throat swabs)were obtained from Covid-19 suspected cases and contact individuals including pre operative patients at JLN MC Ajmer. during May to July 2021.
Results: Of 250 respiratory samples, 7(2.8%)were positive, and 243(97.2%)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 71.4% and 99.1% ,respectively .
Conclusion: The rapid Covid-19 antigen test showed comparable sensitivity and specificity with real time RT-PCR assay. Thus, there is a potential use of this rapid and simple Rapid Covid-19 antigen detection test as a screening assay.

KEYWORDS

INTRODUCTION

The Coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide since its first recorded case in the city of Wuhan ,China in December 2019.The first case of Covid-19 in India, which originated from China, was reported on 30 January,2020.

SARS-CoV-2 infection causes asymptomatic and mild diseases more than severe pneumonia. Severe cases may develop acute respiratory distress syndrome (ARDS) and death with an average mortality rate of 6% (range 1–14.4%).The real-time reverse transcription-polymerase chain reaction (RT-PCR) assay, which is the current standard test for laboratory diagnosis of SARS-CoV-2 infection, requires at least four hours of operation performed by skilled technicians. Therefore, rapid and accurate tests for SARS-CoV-2 screening are essential to expedite disease prevention and control, as well as screening during pre-operative management for invasive procedures.

Methods

Clinical Specimens

Nasopharyngeal and throat swabs were collected for RT-PCR and Nasopharyngeal swab only for rapid Covid-19 antigen test were collected from 250 suspected COVID-19 cases, including pre-operative patients at JLN MC Ajmer Rajasthan from May to July 2021.Samples for RT-PCR were mixed in 3 mL of viral transport media (VTM), and transported at 2–8 °C to the Microbiology laboratory, JLN MC , for processing within a few hours. All specimens were processed in biosafety level-2 enhanced (BSL-2 +) facilities with full personal protective equipment.

Viral RNA Extraction

Perkin elmer chemagic360 automated extraction System was used to extract SARS-CoV-2 RNAs from 300 µL of nasopharyngeal and throat swabs. Extraction was performed according to the manufacturer's instructions. Viral RNA was eluted with 80 µL buffer and used for RT-PCR assay.

SARS-CoV-2 RNA Detection Using Real-time RT-PCR

Qunatiplus Multiplex Covid-19 detection kit v2.0(Huwel Lifesciences PVT.LTD. Hyderabad, India)and Covisure Covid-19 RT-PCR kit(Genetix Biotech Asia PVT. LTD. New Delhi, India) 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. The result was analysed , in which a cycle threshold value (Ct- value) ≤ 35 for all three target genes was defined as a positive result.

Rapid Covid-19 Antigen Test

Xamin COVID-19 Ag test qualitatively detects antigen of novel coronavirus in human nasopharynx. This kit uses immunochromatography for detection. The specimen will move forward along the test card under capillary action. If the specimen contains a novel corona-viruses antigen, the antibody wilil bind to the colloidal gold-labeled new corona virus monoclonal antibody. The immune complex will be membrane fixed will be corona virus mono-clonal antibody capture, form the fuchsia line, display will be corona virus antigen positive; If the line does not show color, the negative result will be displayed. The test card also contains a quality control line C, which shall appear fuchsia regardless of whether there is a detection line.

Package was opened and the test card was taken out. Extraction tube was placed on the workbench. The swab Antigen extraction buffer bottle was pressed vertically downward to allow the solution to drip freely into the extractor tube without touching the edge of the tube 10 drops of Antigen extraction buffer were added to the extractor tube. The swab specimen was put into the extraction tube, the swab was rotated for about 10 seconds,and the swab head was pressed against the tube wall to release the antigen in the swab.The swab was squeezed over the head to remove the swab so as to remove as much liquid as possible from the swab.The materials of swabs were disposed according to biohazard waste disposal method.The nozzle was installed on the extraction tube, three drops were put into the specimen hole of the test card and results were read within 20 minutes.

RESULT

A total of 250 samples of nasopharyngeal (NP) swabs and throat swab were collected from suspected COVID-19 cases and pre-operative patients at JLNMC & Hospital from May to July 2021. Of the samples tested for COVID-19(n=250) by real-time RT-PCR assay 2.8%(n=07)were positive, while 97.2% (n=243) were negative for SARS-CoV-2 RNA.

We evaluated the performance characteristics of Covid-19 antigen detection test. The results were interpreted as positive when both control (C) and antigen (T) lines appeared within 20 min. Comparing Covid-19 antigen detection to RNA detection by RT-PCR assay, the sensitivity and specificity of rapid Covid-19 antigen detection to

identify COVID-19 were 71.4% (5/7) and 99.1% (241/243) respectively, as shown in Table 1. Of four samples discordant with RT-PCR results two were false negative, and two were false positive. The false negative sample's Ct-values were : 26.61 for *E* gene, 26.63 for *RdRp* gene; 26.26 for E gene, 30.47 for *RdRp* gene , as shown in Table 2.

DISCUSSION

RTPCR assay is the standard laboratory diagnosis to confirm SARS-CoV-2 infection; The rapid Covid-19 antigen detection kit was compared with the RT-PCR assay for detection of SARS-CoV-2 infection.

The sensitivity and specificity of the Xamin Covid-19 Antigen Test for rapid detection of SARS-CoV-2 antigen reported by the manufacturer were 95.00% and 100.00% ,respectively. Our results showed lower sensitivity (71.4%) & less specificity 99.1% than the manufacturer's results. The difference in our test performance from the manufacturer could be due to various factors, including the batch of kit reagents, the sample quality and level of extracted antigen, and sample handling and processing techniques.

Table 1: The Sensitivity And Specificity Of The COVID-19 Ag Test

RT-PCR assay			
	Positive	Negative	Total
Rapid Covid-19 antigen test			
Positive	5	2	7
Negative	2	241	243
Total	7	243	250
Sensitivity	71.4% (5/7)		
Specificity	99.1% (241/243)		

[#1] Negative RT-PCR is defined as having Ct-values of *E*, *RdRp*, and *N Gene*>35

Of 7 RT-PCR positive samples in our study, the two showed false negative results by rapid Covid-19 antigen test which could be due to lower levels of extracted antigen than the test's detection limit. These two RT-PCR samples had relatively high Ct-values: 26.61 for *E* gene, 26.63 for *RdRp* gene; 26.26 for E gene, 30.47 for *RdRp* gene, which may explain the negative result of the COVID-19 Ag test. Of 243 RT-PCR-negative samples, two NP swabs were tested positive for SARS-CoV-2 antigen using the rapid COVID-19 Ag test. Although it is unclear what caused the discordant result, we observed that thick and highly viscous mucous tended to yield false positive results when tested with the antigen detection kit. For patients with negative SARS-CoV-2 detection by RT-PCR, clinical data (such as underlying diseases or infection with other pathogens) were not included in the study. Therefore, the possibility of cross-reactivity with other antigens cannot be excluded.

Table 2 Cases With Discordant Results Between The Rapid COVID-19 Ag Test And RT-PCR Assays

S No.	Specimen type	Ct value of RT-PCR			Rapid antigen test result	Interpretation
		E	RdRp	N		
1	NP Swab , Throat Swab	26.61	26.63	-	Negative	False Negative
2	NP Swab , Throat Swab	26.26	30.47	-	Negative	False Negative
3	NP Swab , Throat Swab	38.96	-	35.17	Positive	False Positive
4	NP Swab , Throat Swab	40.44	Not Detected	-	Positive	False Positive

The advantage of the Rapid COVID-19 Ag test as a screening for COVID-19 is its simple procedure and quick results and thus, the nucleic acid test (NAT) for SARS-CoV-2 gene detection, which is more sensitive and specific than this rapid Ag test, is still a standard test for COVID-19 diagnosis.Even with its limitations, the rapid SARS-CoV-2 antigen test can benefit all healthcare workers in managing infected individuals in time effectively, especially in rural and outbreak areas.

CONCLUSIONS

The Rapid COVID-19 antigen detection test showed comparable sensitivity (71.4%) and specificity (99.5%) with real-time RT-PCR assay. We believe there is a potential use of this rapid and simple Rapid COVID-19 antigen detection test as a screening assay, especially in a high prevalence area.

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