"Evaluation of Rapid Antigen Test (RAT) and Real-Time Polymerase Chain Reaction (RT-PCR) for detection of COVID-19 in Kanpur UP"

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Abstract:

Introduction: Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), which emerged as a novel human pathogen in China at the end of 2019, is responsible for corona virus disease 2019 (COVID-19), which causes symptoms such as cough and fever, severe pneumonia, and death. The WHO reported that more than 29 million cases of COVID-19, including approximately 55, 40,000 deaths, have occurred as of 17 January 2022

Aim: To Evaluation of Rapid Antigen Test (RAT) and Real-Times Polymerase Chain Reaction (RT-PCR) for detection of COVID-19 in kanpur UP.

Materials and Methods: This study was be conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur. Total 100 known samples, out from 50 positive by RAT patient and 50 negative patients already tested by RAT kit for Covid -19 test. For the present study, Tru PCR SARS-CoV-2 RT-q PCR Kit (Kilpest India Ltd., India) were selected on the basis of multiple SARS-CoV-2 specific gene and Standard Q COVID-19 Ag kit (SD Biosensor, Healthcare Pvt. Ltd Guru gram Haryana India) is a rapid chromatographic immunoassay for the detection of SARS-CoV-2 nucleocapsid (N) antigen in respiratory specimen.

Result: In this study100 respiratory samples collected from individuals living in a shared housing were analyzed head to head by Rapid antigen test and RT-PCR using CFX-96 real-time thermal cycler. Out of 100, 50 negative samples by RAT, 21 (10.5%) of the samples were found positive by SARS-CoV-2 by rRT-PCR with cT values ranging between 17.32–32.91 and 50 positive samples by RAT, they were all 50 (100%) samples found positive by SARS-CoV-2 by rRT-PCR with cT values ranging between 16.62–33.91 The antigen test diagnosed the infection status with a sensitivity of 79.0 % (79/100) and a specificity of 100 %.

Conclusion: All six COVID-19 RT-PCR kits included in this study demonstrated satisfactory performance and can be used for the routine molecular diagnosis of COVID-19 disease.

Keywords: Corona virus disease 2019, Genes, Ribonucleic acid, severe acute respiratory syndrome coronavirus-2, cyclic threshold.

Introduction

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), which emerged as a novel human pathogen in China at the end of 2019 [1], is responsible for corona virus disease 2019 (COVID-19), which causes symptoms such as cough and fever, severe pneumonia, and death. The WHO reported that more than 29 million cases of COVID-19, including approximately 55, 40,000 deaths, have occurred as of 17 January 2022 [2]. To control the spread of SARS-CoV-2 infections, rapid identification and isolation of patients are required.

Rapid detection, effective isolation of symptomatic cases and systematic tracing of close contacts are paramount to blunt the community spread of severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infections. Nowadays, Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) is the diagnostic reference standard for corona virus disease 2019 (COVID-19) [3]. However, specialized instruments and. Expertise are required to conduct RT- PCR assays In addition, many countries have encountered supply shortages of RT-PCR reagents. Rapid antigen Test immunoassays (RAT) are particularly suited for point-of-care testing, as they can easily be performed and interpreted without equipment, are inexpensive, and improve turnaround times. Moreover, results returned by a recently launched antigen assay appeared to correlate better with patient infectiousness than RT-PCR results [4]. For rapid diagnosis of SARS-CoV-2 infection, rapid antigen detection (RAT) tests for qualitative determination of SARS-CoV-2 antigen are available. RAT tests detect viral antigen by the immobilized coated SARS-CoV-2 antibody on the device. The test results of RAT can be interpreted without specialized instrument and available within 30 min.[5]. Center for Disease Control and Prevention (CDC) [6] protocols, and a number of commercial assays [7]. The SARS-CoV-2 RT-PCR has high specificity and sensitivity [8, 9]. However, the type and quality of the patient specimen [10, 11], stage of the disease, and the degree of viral replication and/or clearance have an impact on the test outcome [12]. These factors are critical not only for PCR-based but also for other diagnostic test systems aiming to detect

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the presence of the virus. Hence interpreting a test result for SARS-CoV-2 depends on the accuracy of the test, but the prevalence and the estimated risk of disease before testing should also be taken into consideration.

In many countries SARS-CoV-2 testing is extended to asymptomatic population, e.g. in schools, airports, nursing-homes, and workplaces. This leads to a growing gap between the large number of demand and the laboratory capacities to perform rRT-PCR tests, especially in developing Countries. Despite high specificity and sensitivity, rRT-PCR has a disadvantage in point of care testing, because it usually requires professional expertise, expensive reagents and specialized equipment. Therefore, alternative assays, such as rapid antigen detection tests, which can also detect the presence of the virus directly in respiratory samples, have been developed [7] and tested by different groups [13–17]. However, it is vital to determine the sensitivity, specificity of such tests relative to standard rRT-PCR in order to identify the ideal circumstances that their application would be beneficial.

Here, we evaluated a rapid SARS-CoV-2 antigen detection test, Standard Q COVID-19 Ag kit (SD Biosensor, Healthcare Pvt. Ltd Gurugram Haryana India) using 100 respiratory specimens. The performance of this lateral flow immunoassay was compared with the SARS-CoV-2 RT-PCR for viral gene detection assay, Tru PCR SARS-CoV-2 RT-q PCR Kit/Kilpest India Ltd. India. This evaluation is critical before the implementation of the rapid antigen test for screening of SARS COV- 2 infected individuals.

Material and Methods

Study Setting: This study was being conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur.

Samples from already known patient related to covid-19 disease were being collected from Rama Medical College Hospital and Research Centre as the source of the sample for the study.

Study Design: Prospective study.

Type of Study: Observational study.

Study Period: This study will be conducted from 2021 to 2022.

Size of Sample: Total 100 known samples, out from 50 positive by RAT patient and 50 negative patients already tested by RAT kit for Covid -19 test.

Incision Criteria: All the patients who were having symptoms related to covid-19 disease and confirm by RAT either or positive or negative.

Excision Criteria: Asymptomatic patient were excluded from the study.

Ethical Consideration: Ethical clearance will be taken from the institutional ethical committee.

Selection of Rapid Antigen Test Kit Standard Q COVID-19 Ag kit (SD Biosensor, Healthcare Pvt. Ltd Gurugram Haryana India) is a rapid chromatographic immunoassay for the detection of SARS-CoV-2 nucleocapsid (N) antigen in respiratory specimens. This rapid antigen test device has two precoated lines on the result window: control (C) and test (T) lines. The control (C) region is coated with mouse monoclonal anti-chicken Igγ antibody; the test (T) region is coated with mouse monoclonal anti-SARS-CoV-2 antibody against SARS-CoV-2N antigen. The result was read as positive, negative, invalid (if no control line was shown).

Selection of RT-PCR Kit

For the present study, TruPCR SARS-CoV-2 RT-qPCR Kit (Kilpest India Ltd., India) were selected on the basis of multiple SARS-CoV-2 specific gene targets in a single tube with simultaneous detection of each target on different detection channel. This kit was 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. A Cycle threshold (Ct) value of less than 35 was reported as positive The CFX-96 real-time thermal cycler (Bio-Rad Laboratories, USA) was used for amplification.

Sources of Sample

Respiratory samples, mainly nasopharyngeal and throat swabs were collected from 100 suspected COVID-19 cases, at Rama Medical College Hospital, & University, U.P, India, from January to December 2021. Samples were mixed in 2 ml of viral transport media (VTM), consisting of Hanks' balanced salt, 0.4% fetal bovine serum, HEPES, antibiotic and antifungal agents. Samples were transported at 2–8 °C to the Microbiology laboratory, Rama Hospital, for processing within a few hours. All specimens were processed in Bio safety level-3 (BSL-3) and Bio safety level-2 enhanced (BSL-2 +) facilities with full personal protective equipment.

Statistical Analysis

The numbers of positive samples were compared using two by two contingency table. The agreement between the antigen test and rRT-PCR techniques was evaluated using the Cohen's weighted kappa index (K value) [18]. Socio-demographic and clinical profile was described using percentages and mean. Sensitivity, Specificity, PPV and NPV of RAT was calculated using relevant formulas by keeping qRT-PCR as a gold standard. Cohen's Kappa was calculated. [19].

Result

Rapid antigen test sensitivity and specificity were evaluated by institutions using total 100 number of respiratory samples. Certain rapid tests may be used at the point-of-care and thus offer benefits for the

detection and management of infectious diseases. In order to assess the potential of the rapid antigen test in this context, 100 respiratory samples collected from individuals living in a shared housing were analyzed head to head by Rapid antigen test and RT-PCR using CFX-96 real-time thermal cycler. Out of 100, 50 negative samples by RAT, 21 (10.5%) of the samples were found positive by SARS-CoV-2 by rRT-PCR with cT values ranging between 17.32–32.91 and 50 positive samples by RAT, they were all 50 (100%) samples found positive by SARS-CoV-2 by rRT-PCR with cT values ranging between 16.62–33.91 The antigen test diagnosed the infection status with a sensitivity of 79.0 % (79/100) and a specificity of 100 %. Cohen's weighted kappa value of 0.511 indicated moderate agreement between rRT-PCR and the rapid antigen test. The overall concordance between the rRT-PCR and the antigen test was 21.0 % (79/100).

[Table/Fig-1]: Comparison of RT-PCR and Rapid Antigen Test (RAT).

Rapid Antigen Test (Rat) Result	RT-PCR Test		Total
	Positive	Negative	
Positive	50	0	50
Negative	21	29	50
Total	71	29	100

[Table/Fig-2]: Socio-demographic and clinical profile of study subjects

Variables	N - %
Gender	
Male	64%
Female	36%
Age (in years)	
18 to 40	41%
41-60	45%
≥61	14%
Residence	
Urban	38%
Rural	42%
Symptomatic at testing	
Yes	100%
No	0
Type of symptoms in those symptomatic cases	
Fever	68%
Cough	71%
Sore throat	69%
Myalgia	33%
Diarrhea	11%
Anosmia	5%
Primary reason for testing	
Severe Acute Respiratory Infection (SARI)	33%
Symptomatic Influenza-Like Illness (ILI)	24%
High risk contact	22%
Low-risk contact	16%
Voluntary testing	3%
Surgical clearance	2%
Past history of COVID-19	
No	100%
Yes	0
Pre-existing medical conditions	
Hypertension	56%
Diabetes	39%
Chronic lung diseases	21%
Chronic Kidney diseases	9%
Malignancies	7%
Others*	8%

[Table/Fig-3]: Statistics for Rapid Antigen Test (RAT) in comparison with RT-PCR.

Statistic	Value
Sensitivity	77.00%
Specificity	100%
Positive Predictive Value (PPV) (*)	100%
Negative Predictive Value (NPV) (*)	58%

Discussion

S.No.	Study	Year	Results
1	**E. Albert et al[20]**	2021	Between 2nd September and 7th October 2020 this prospective study enrolled 412 patients with clinical suspicion of COVID-19 of whom 327 were and 85 children, attending primary care centers of the Clínico-Malvarrosa Health Department in Valencia (Spain).
2	**Seema Aleem et al[21]**	2022	A cross-sectional study was conducted by Government Medical College, Srinagar; The sample size was estimated at 359. A total of 473 were included in the study.
3	**In the present study**	2022	This study was be conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur. Total 100 known samples, Out from 50 positive by RAT patient and 50 negative patients already tested by RAT kit for Covid -19 test.
4	**Seema Aleem et al[21]**	2022	A total of 473 subjects were included in the final analysis. The selection of study subjects in depicted in [Table/Fig-2]. The subjects comprised of 277 (58.6%) males and 196 (41.4) females. The mean age of subjects was 38.4±12.2 years and 57.29% of subjects belonged to urban areas. A total of 124 subjects (26.2%) had any symptom at the time of testing. The most common presenting symptom was fever reported by 71 subjects (15.01%). Loss of smell was reported by seven (1.5%) subjects. A 13% of subjects had a previous history of COVID-19. The primary reason for testing included a positive contact history 221(47%) subjects, symptoms 124(26%) and voluntary testing 116 (24.5%). A total of 1/5th of subjects had any concomitant co-morbidity.
5	**In the present study**	2022	A total of 100 subjects were included in the final analysis. The subjects comprised of 64 males and 36 females. The mean age of subjects was 38 years and 38 of subjects belonged to urban areas. A total 100 subjects had any symptom at the time of testing. The most common presenting symptom was cough reported by 71 subjects and fever was reported by 68 subjects. None of any subjects had a previous history of COVID-19. The primary reason for testing included a Severe Acute Respiratory Infection (SARI) 33% subjects and Symptomatic Influenza-Like Illness (ILI) 24%.
6	**Chutikarn Chaimayoet al22**	2020	The results were interpreted as positive when both control (C) and SARS-CoV-2 antigen (T) lines appeared within 30 min, as shown in Fig. 1. Comparing SARSCoV-2 antigen detection to RNA detection by RT-PCR assay, the sensitivity and specificity of rapid SARS-CoV-2 antigen detection to identify COVID-19 were 98.33% (59/60; 95%CI, 91.06–99.96%) and 98.73% (389/394; 95%CI, 97.06–99.59%), respectively,
7	**Seema Aleem et al[21]**	2022	The present study estimated the sensitivity and specificity of RAT to be 54.43% (42.83% to 65.69%) and 99.24 (97.79% to99.84%), respectively. The overall accuracy was estimated at 91.75%..
8	**In the present study**	2022	The results were interpreted as positive when both control (C) and SARS-CoV-2 antigen (T) lines appeared within 30 min. Comparing SARSCoV- 2 antigen detection to RNA detection by RT-PCR assay, the sensitivity, specificity PPV and NPV of rapid SARS-CoV-2 antigen detection to identify COVID-19 were 77.%, 100%,100% and 58% respectively,

Conclusion

The rapid assay for SARS-CoV-2 antigen detection showed comparable sensitivity (77 %) and specificity (100%) with real-time RT-PCR assay. We believe 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. Using both of these tests together and following up a RAT negative person with qRT-PCR will enhance the overall sensitivity.

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