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Immunochromatographic detection of SARS CoV-2 antigen in comparison with the polymerase chain reaction for laboratory diagnosis of Covid-19 in Karachi, Pakistan

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ABSTRACT

Introduction: The Covid-19 pandemic raised the question of which laboratory test was relevant for a rapid and accurate diagnosis of the disease. The current two methods for the detection of SARS CoV-2 are the ICT (Immunochromatographic Technique) and the PCR (Polymerase Chain Reaction) tests; therefore comparative analysis of test performance has to be done.

Objective: To perform a comparative analysis of two different techniques (Immunochromatographic technique for Antigen and Polymerase Chain Reaction for RNA) which are implicated for the detection of SARS CoV-2.

Materials & Methods: The Rapid Antigen Test (RAT) and Polymerase Chain Reaction (PCR) were performed for the detection of Covid-19 on individuals having the signs and symptoms of SARS Covid-19. Immunochromatographic technique (ICT) was performed by SD Biosensor kits, while for Polymerase Chain Reaction RNA extraction was performed by Liferiver® auto extraction analyzer, and amplification was performed by SLAN®-48P Real-Time PCR System.

Results: Among of 100 samples tested, 62 (23.56%) were positive on ICT while PCR had 66 (22.44%) and 38 were negative on ICT while PCR had 34. Thus the sensitivity of ICT was 90.77% (95% CI, 80.98% - 96.54%) and specificity was 91.43% (95% CI 76.94%-98.20%). The Positive Predictive Value was 95.16% while the Negative Predictive Value was 84.21%. However the Cohen's Kappa Index Value was recorded as 0.806.

Conclusion: The clinical performance of SD Biosensor Roche kits was excellent. On the basis of sensitivity and specificity it was concluded that RAT have comparable results with RT PCR.

Keywords: Antigens, Viral; COVID-19; Diagnostic Techniques and Procedures; Immunoassay; Polymerase Chain Reaction; Severe Acute Respiratory Syndrome.

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

The coronavirus is a group of viruses that causes diseases in animals, as well as causing mild to severe respiratory infection in human. Coronavirus disease 19 (COVID-19) is caused by a novel coronavirus designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Like the other coronaviruses (order Nidovirales, family Coronaviridae, subfamily Coronavirinae), SARS-CoV-2 is an enveloped virus with a positive-sense, single-stranded RNA genome of 30 kb. SARS-CoV-2 belongs to the genus beta coronavirus, together with SARS-CoV-2 and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (with 80% and 50% homology, respectively).[2]

SARS-CoV-2 is an enveloped β- coronavirus, with a genetic sequence very similar to SARS-CoV-1 (80%) and bat coronavirus RaTG13 (96.2%).[2] The viral envelope is coated by spike (S) glycoprotein, envelope (E), and membrane (M) proteins. The S protein mediates the host cell for binding and entry. The first stage /step in the infection in virus is to the binding with host cell by the target receptor. The S1 subunit of the S protein contains the receptor binding domain that binds to the peptidase domain of angiotensin-converting enzyme 2 (ACE 2). In SARS-CoV-2 the S2 subunit is highly preserved and is considered a potential antiviral target. Coronaviruses have the capability for proofreading during replication, and therefore mutation rates are lower than in other RNA viruses. As SARS-CoV-2 has spread globally it has, like other viruses, accumulated some mutations in the viral genome, which contains geographic signs. In the years 2002 and 2012 coronavirus caused Severe Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), in humans and produced disastrous respiratory illness, making coronaviruses a new public health alarm in the twenty-first century.[3] At the end of 2019, a novel coronavirus labeled as SARS-CoV-2 appeared in the city of Wuhan, China, and caused an outbreak of unusual viral pneumonia. Being significantly contagious, this novel coronavirus disease, also known as coronavirus disease 2019 (COVID-19), has spread fast all over the world.[4,5]

The population affected by the COVID-19 had showed the same sign and symptoms that were identified in the SARS and MERS like Cough and chest discomfort, fever, viral pneumonia, and in severe cases dyspnea and bilateral lung infiltration.[6,7]

Although the diagnosis of SARS-CoV-2 is based on clinical, epidemiological and some radiological and laboratory findings, for example the chest X-ray and especially the chest tomography (CT-scan) revealing the characteristic images of ground glass, that are also seen in asymptomatic patients. However the gold standard for COVID-19 diagnosis is through the analysis of nucleic acids, (Quantitative Reverse-Transcription Polymerase Chain Reaction (RT-PCR) that is the demonstration of SARS-CoV-2 RNA in respiratory samples.[8,9,10] Sidewise from the quantitative RT-PCR, the immunochromatographic tests have also been debated in the context of COVID-19. It is a rapid test performed by the use of a drop of the patient's sample (whole blood, serum or plasma) and a specific buffer on an immunochromatographic stick. By capillary attraction, the analyte of interest (SARS-CoV-2 protein or peptide) binds to its specific antibody in a reaction zone and the antigen-antibody reaction is evidenced by the formation of a colored band.[11-13] Immunochromatographic tests are a best option for diagnosing a large number of samples, as it is fast, stress-free to perform, presenting sensitive results, allowing the identification of suspicious cases, as well as the screening and monitoring of COVID-19 progression in populations.[14]

The RT-PCR assay, which is the current standard test for laboratory diagnosis of SARS-CoV-2 infection, requires at least four hours of procedure performed by expert technicians. Therefore, rapid and accurate tests for SARS-CoV-2 screening are needed to expedite disease prevention and control, as well as screening during pre-operative management for invasive procedures.[15,16] Lateral flow immunoassays using monoclonal anti-SARS-CoV-2 antibodies, which target SARS-CoV-2 antigens, can be the corresponding screening tests if their accuracy will comparable to that of the real- time RT-PCR assays.[17,18]

In this study, the comparative analysis was carried out between the immunochromatographic technique (ICT) in contrast to the Polymerase chain Reaction (PCR) for the detection of SARS-CoV-2, to detect antigen in immunochromatographic technique and RNA in PCR.

MATERIALS & METHODS

The study was approved by the Ethics Committee of Baqai Medical University Karachi, with reference letter (BMU-EC/01-2021) based on the basic international ethics guidelines laid down in the declaration by the World Medical Association at Helsinki (2008).

Respiratory samples, mainly nasopharyngeal swabs, were collected from 100 suspected COVID-19 cases from different area of East Karachi, Pakistan from June 2021 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 Muhammadi Laboratory and Diagnostic Center within a few hours. All specimens were processed in a Biosafety Level-3 (BSL-3) laboratory with full personal protective equipment.

Liferiver® preloaded automated extraction 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 600 μL buffer and used for RT-PCR assay. Viral RNA isolation kit (preloaded for Auto-Extraction) utilizes magnetic particles technology for isolation and purification of pathogenic nucleic acids from biological specimens. The kits can be used in combination with auto nucleic acid extraction system.

Liferiver® 2019-nCoV Assay (Shanghai, China), which targets envelope gene (*E*) of Coronavirus, FAM gene and nucleocapsid (*N*) genes of SARS-CoV-2, was used for SARS-CoV-2 RNA detection according to the manufacturer's instructions. Briefly, 20 μL of extracted RNA was added to 20 μL of master mix. The master mix contains 18 μL of PCR assay, 3 μL Enzyme Assay and 2 μL of Cov-19 Assay. SLAN®-48P Real-Time PCR System, Thermal Cycler (Sansure) was used for amplification. The conditions consisted of 1 cycle of 20 min at 50°C, 1 min at 95°C and followed by 45 cycles of 15s at 94°C, 30s at 58°C. The result was analyzed using Salan Real-Time PCR System Viewer (Sansure), in which a cycle threshold value (Ct value) < 40 for all three target genes was defined as a positive result.

STANDARD Q COVID-19 Ag Test has two pre-coated lines, "C" Control line, and "T" Test line on the surface of the nitrocellulose membrane. Both the control line and test line in the result window are not visible before applying any specimens. Mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region and mouse monoclonal anti-Chicken IgY antibody is coated on the control line region. Mouse monoclonal anti-SARS-CoV-2 antibody conjugated with color particles are used as detectors for SARS-CoV-2 antigen device. Its result is generating a colored red-purple line. The appearance of a red-purple line on the membrane indicates the presence of antigen of interest in the sample.

The data were statistically analyzed by SPSS version 22 and MedCalc Software for statistical analysis. The data of the screening techniques was assembled categorically on Microsoft Office Excel 2007. Results were compared on the basis of various diagnostic values. The Sensitivity, Specificity, Positive Predictive Value (PPV) Negative Predictive value (NPV), True Positive (TP), True Negative (TN), False Positive (FP), False Negative (FN), Positive Likelihood Ratio, Negative Likelihood Ratio, and Accuracy were calculated.

RESULTS

Characteristics of Covid-19 Cases

Characteristics of Covid-19 cases shown in Table 1. Suspected cases and contact individuals were laboratory-confirmed by the gold standard RT-PCR assay as a national guideline for laboratory diagnosis of COVID-19. The number of negative samples were 36 on Rapid Antigen Test (RAT) and 34 on PCR. The frequency of severe acute respiratory syndrome was 64% on the RAT, and 66% on the PCR.

Table 1: Characteristics of the Covid-19 cases (n=100).

#	Characteristics of Patients	Results
1.	**Age (years)**	
	Range	21-72
	Median	38.5
2.	**Gender**	
	Male	57
	Female	43
3.	**Results of Rapid Antigen Assay**	
	Positive	64
	Negative	36
4.	**Results of RT-PCR Detection Assay**	
	Positive	66
	Negative	34

Table 2 shows the performance of Rapid antigen test (RAT) based on the sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, disease prevalence, positive predictive value, negative predictive value, and accuracy. The sensitivity was 90.77% with 95% CI (80.98% to 96.54%) while specificity was 91.43% (95% Cl 76.94-98.20). The Positive Likelihood Ratio was 10.59 while Negative Likelihood Ratio was 0.10. Positive Predictive Value was 95.16% (95% Cl 86.92-93.31); Negative Predictive Value was 84.21% (95% Cl 71.20-92.01). The test Accuracy was found to be 91.0%.

Table 2: Performance of SARS-CoV-2 Rapid Antigen Test.

Statistic	Value	95% CI
Sensitivity	90.77%	80.98-96.54
Specificity	91.43%	76.94-98.20
Positive Likelihood Ratio	10.59	3.58-31.33
Negative Likelihood Ratio	0.10	0.05-0.22
Disease prevalence	65.00%	54.82-74.27
Positive Predictive Value	95.16%	86.92-98.31
Negative Predictive Value	84.21%	71.20-92.01
Accuracy (*)	91.00%	83.60-95.80

Real time RT PCR and SARS CoV 2 antigen assays:

The Liferiver® 2019-nCoV Assay (Shanghai, China), which targets envelope gene (*E*) of Coronavirus, FAM gene, and nucleocapsid (*N*) genes of SARS-CoV-2, was used for SARS-CoV-2 RNA detection according to the manufacturer's instructions. The average cycle threshold (Ct) values in COVID-19 positive cases were shown in Table 2 according to their Ct and sensitivity range. As the Ct value increases the sensitivity decreases and the viral load decreases as well.

The performance characteristics of SARS CoV-2 antigen detection (Standard Q COVID-19 Ag test) was evaluated. A total of 100 samples were tested serologically by RAT and then confirmed by PCR.

Among these samples 62 were found positive on RAT and 66 were positive through PCR. The number of negative samples were 38 on RAT and 34 on PCR. The frequency of severe acute respiratory syndrome was 64% on the RAT, and 66% on the PCR.

Table 03: Frequency of Severe Acute Respiratory Syndrome (SARS-CoV-2) Rapid Antigen test.

Frequency	ICT	PCR
Total number of Cases	100	100
Positive	62	66
Negative	38	34
Percentage	62%	66%

The results were interpreted as positive when both control (C) and SARS-CoV-2 antigen (T) lines appeared within 30 min, as shown in Figure1. Results were interpreted as invalid if the T line appeared but the C line was not present (false positive).

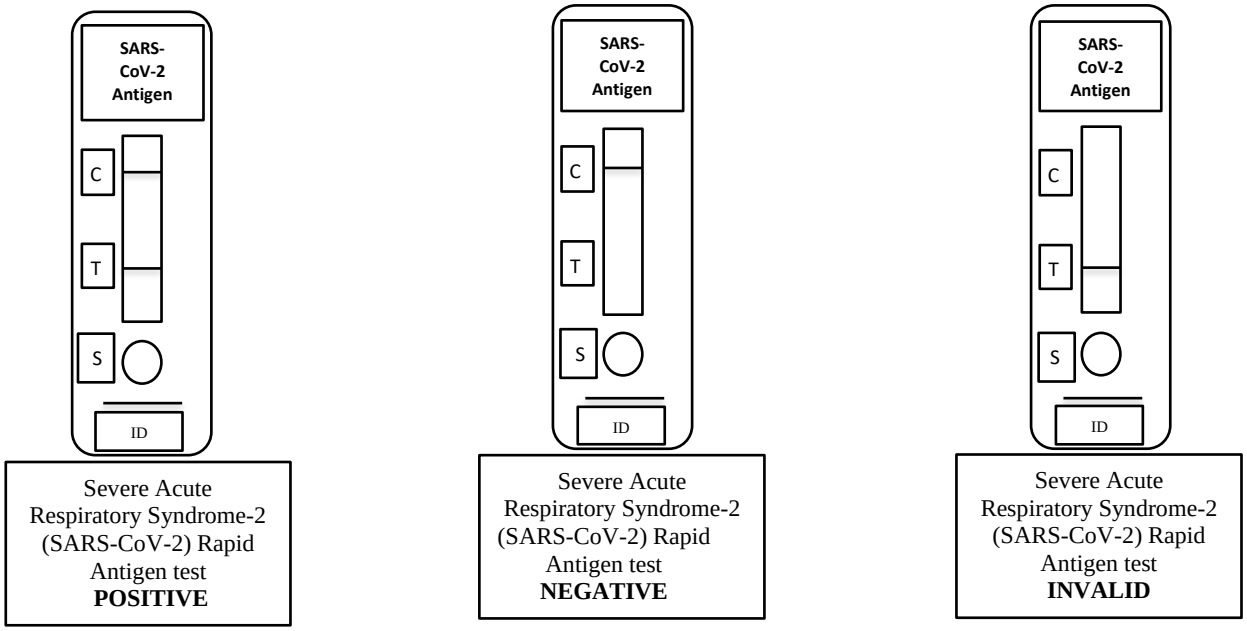

Figure 1: SARS CoV-2 antigen detection kit and interpretation.

Fig.No.1: "The first Antigen Test has two pre-coated lines, "C" Control line, "T" Test line on the surface of the nitrocellulose membrane. Both the control line and test line in the result window are not visible before applying any specimens. Mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region and mouse monoclonal anti-Chicken IgY antibody is coated on the control line region. Mouse monoclonal anti-SARS-CoV-2 antibody conjugated with color particles are used as detectors for SARS-CoV-2 antigen device.

During the test, SARS-CoV-2 antigen in the specimen interacts with monoclonal anti-SARS-CoV-2 antibody and conjugated with color particles making antigen-antibody color particle complex. This complex migrates on the membrane via capillary action until the test line, where it will be captured by the mouse monoclonal anti-SARS-CoV-2 antibody. A colored test line would be visible in the result window if SARS-CoV-2 antigens are present in the specimen.

The intensity of colored test line will vary depending upon the amount of SARS-CoV-2 antigen present in the specimen. If SARS-CoV-2 antigens are not present in the specimen, then no color appears in the test line. The control line is used for procedural control, and should always appear if the test procedure is performed properly and the test reagents of the control line are working".

DISCUSSION

The standard laboratory diagnosis for Covid-19 is the Nucleic Acid Test (NAT) to confirm SARS-CoV-2 infection. The widely used testing is RNA detection in clinical specimens for diagnostic laboratories. Study was aimed to evaluate the diagnostic performance of Rapid Antigen Test (RAT) to detect SARS-CoV-2 antigens from a nasopharyngeal swab directly after sampling and to provide the result within 15 min. In our study we have observed that out of 100 samples of symptomatic patients 62 were positive on the RAT, and 38 were found negative on RAT. On the other hand 66 samples were recorded positive on the gold standard method PCR and 34 were negative. In a study conducted in Rahman Medical Institute Peshawar, Pakistan, Bilal Iqbal et al. concluded 72% sensitivity and 95% specificity. While sensitivity trend of antigen test progressively declined from 94.3% in Ct<25 to 70.8% in Ct 26-29 and then to 47.2% in Ct 30-35.[19]

The sensitivity and specificity was calculated, and the sensitivity of our samples was 90.77% while the specificity was 91.43%. The Positive Predictive Value was calculated as 95.16% and the Negative predictive value was 84.21%. Similar finding was reported by Chutikarn Chaimayo et al in Thailand, and in another similar study that was conducted by the Paloma Merino-Amador et al and they also reported the comparative results in their finding.[20]

A study conducted by J. Agarwal et al (in Uttar Pradesh, India)[21] where the overall sensitivity was 89.7% and specificity was 97.5%, showing strong agreement among Rapid Antigen test and PCR test. The Positive Predictive Value was 95.16% in our study, and the Positive Likelihood Ratio was 10.59, while the Negative Predictive Value in our study was 84.21%, and the Negative Likelihood Ratio was 0.10. A similar study conducted by Umar Saeed et al.,[22] in Islamabad Diagnostic Center Pakistan, where the observation remains lower from our study; their Positive Predictive Value was 67.82%, while the Negative Predictive Value was 64.40%. Rapid Diagnostic Tests (RDTs) showed limited sensitivities and specificities compared to gold standard RT-PCR. False negative rate (FNR) was calculated as 0.0923. The reasons reported to be associated with FNR are that the infected individuals may be in the window period or low viral load, that remains undetectable on PCR, and most of the false negative results were noted in the ICT method, because it detects only the infection that are fully developed and spread in the whole body, but the PCR detects each and single strain of the virus present in the sample.

Based on the results of this study, it can be stated that Rapid Antigen Test (RAT) shows comparable results for Severe Acute Respiratory syndrome (SARS-Cov-2) with Polymerase Chain Reaction (PCR), but it is only recommended in the resource constrained areas due to its False Positive (FP) and False Negative (FN) results.

CONCLUSION

It is concluded that RAT (Rapid Antigen Test) have comparable results with RT-PCR, with 90.7% sensitivity and 91.43% specificity; moreover, being cheaper and quicker, they help to save costs and time for patients and their later management.

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