DOI: https://doi.org/10.54393/pbmj.v5i4.397

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
Volume 5, Issue 4 (April 2022)

Orignal Article

# Comparative field study of Rapid-Antigen Detection (RAD) with Multiplex Real Time-PCR for COVID-19 diagnosis

**Hussan[1], Fadia waheed[2*], Habib Ullah[3], Muhammad Khurram[3], Ghadir Ali[3], Maryam Shahid[2], Faisal Zaman[1], Abdullah[4], Asraf Hussain Hashmi[5]**

[1]Department of Microbiology, University of Haripur, Pakistan
[2]Institute of Microbiology and Molecular Genetics, Punjab University, Lahore, Pakistan
[3]Department of Life Sciences, School of Science, University of management and Technology Lahore, Pakistan
[4]Department of Biotechnology International Islamic University Islamabad, Pakistan
[5]Institute of Biological and Genetic Engineering, Islamabad, Pakistan

## ARTICLE INFO

***Key Words:***
COVID-19, Rapid-antigen test, Comparison, multiplex RT-PCR.

***How to Cite:***
Hussan, M., waheed, F. ., Ullah, H. ., Khurram, M. ., Ali, G. ., Shahid, M. ., Zaman, F. ., Abdullah, ., & Hashmi, A. H. . (2022). Comparative field study of Rapid-Antigen Detection (RAD) with Multiplex Real Time-PCR for COVID-19 diagnosis . Pakistan BioMedical Journal, 5(4). https://doi.org/10.54393/pbmj.v5i4.397

***Corresponding Author:**
Fadia Waheed
Institute of Microbiology and Molecular Genetics, Punjab University, Lahore, Pakistan
*fadia.waheed1@gmail.com*

Received Date: 17th April, 2022
Acceptance Date: 23rd April, 2022
Published Date: 30th April, 2022.

## ABSTRACT

RT-PCR is a gold standard test for the diagnosis of SARS-CoV2 (Covid-19) infection; however, it is an expensive, time consuming and technical demanding technique. Rapid antigen detection immunoassay (RAD) is cost-effective, quick as well as can be performed and interpreted easily. The rapid diagnosis of COVID-19 patients is essential to reduce cost and control the disease spread; however, the real world data of these tests must be validated with RT-PCR before they can be used at large scale. The objective of this study was to determine the sensitivity and specificity of Panbio™COVID-19 Ag-Rapid test device (Abbot) with multiplex RT-PCR. **METHODS:** A total of n=3509 samples were tested for SARS-CoV-2 RAD and RT-PCR at Institute of Biomedical and Genetic Engineering, Islamabad. The rapid antigen tests were performed by Panbio™COVID-19 Ag-Rapid test device (Abbott) and compared with RT-PCR performed on Thermo Fisher (ABI) Quant Studio 5 using CDC 2019-nCoV RT-PCR protocol. **RESULTS:** Total (n=3509), n=458 (7.60%) samples were reported positive by rapid antigen out of which n= 445 RT-PCR positive (13 false positive by rapid antigen), n=3051 (92.4%) were negative. True antigen negative tests n= 3051) were repeated with RT-PCR among these, n=25 were observed RT-PCR positive (rapid antigen false negative). The threshold cycle (CT) for the RT-PCR tests of these samples was >30. **CONCLUSION:** Panbio™COVID-19 Ag-Rapid test devices (Abbott) showed a sensitivity ratio 94.6% compared to RT-PCR. The Panbio™COVID-19 Ag-Rapid test device (Abbott) is reliable and can be used for screening and isolation of positive patients from the population.

## INTRODUCTION

Corona virus disease is known as *COVID-19* which is caused by severe acute respiratory syndrome corona virus-2 a newly discovered corona virus. SARS-CoV-2 is a type of beta-corona virus, structure of this virus is organized as an enveloped negative-sense RNA type virus and also as non-segmented[1]. Transmission of this virus is through human to human via direct body contact or droplets, and it has been estimated that this virus has an incubation period of 6.5 days. Cough, fever are common symptoms in an infected patient with SARS-CoV-2 but in severe cases, patients also have pneumonia, and the mortality rate is 6% [1-4]. There are different types of assays used for the detection of *COVID-19* including real-time-polymerase chain reaction (RT-PCR), that takes some hours and is also considered as the gold standard method [5]. Due to the cost and time-consuming patterns of RT-PCR, there are also difficulties in the management of testing facilities that have been faced. In this situation, rapid antigen tests have played an important role, which is costless and minimum time consuming (almost 30minuts) comparatively RT-PCR which takes 4hours. A rapid testing protocol can be a part of screening before invasive procedures to control and

**DOI:** https://doi.org/10.54393/pbmj.v5i4.397

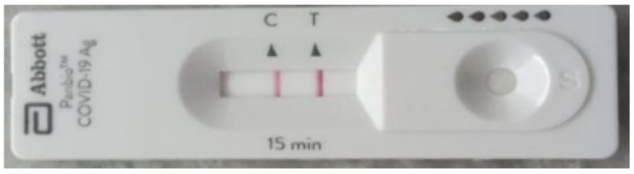
prevention from SARS-CoV-2[6,7].

## METHODS

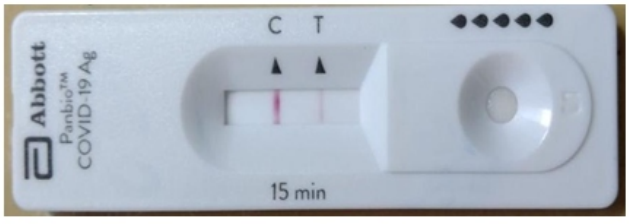
**RAPID *COVID-19* ANTIGEN TEST:** According to the manufacturer a rapid testing device for *COVID-19*-Ag consists of a small membrane like strip, which pre-coated with mouse monoclonal anti-chicken IgY on the control line and on the test line immobilized anti-SARS-CoV-2 antibody, this device was commercially prepared by (Abbott). Two types of conjugates and chicken; human CoV-2 Ag gold conjugate, chicken IgY-gold-conjugate) move up on the membrane by following chromatography techniques by reacting with anti-SARS-CoV-2 antibody and pre-coated mouse monoclonal anti-chicken IgY respectively. Both specific antigens (Human IgG specific to SRS-CoV-2 Antigen gold conjugate; anti-SARS-CoV antibody) will form a test line which indicates the positive results. This control line indicates the result is conclusive.

Nasopharyngeal samples were collected from symptomatic and non-symptomatic referral people and their antigen rapid tests (Abbott) were performed. The sample collection procedure was the same as the requirement of COVID-19 PCR, collected swab stick was allowed to dilute in a specific buffer containing tube provided with the kit. After 10 minutes diluted sample was poured on the testing device and waited for 15mint for result indication. Negative and positive results were noticed some of them showed a weak positive indication on rapid test devices which were considered as positive results as per kit protocol, which were also subjected for RT-PCR for confirmation.

a




b




c

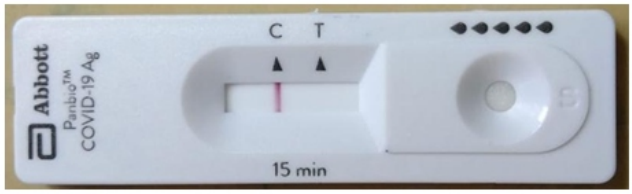



Figure 1: A) Strongly positive PanbioTM COVID-19 Antigen, B) Weakly Positive PanbioTM COVID-19 Antigen,C) Negative PanbioTM COVID-19 Antigen

**RNA EXTRACTION AND MULTIPLEX RT-PCR:** Covid-19 RNA was extracted from the nasopharyngeal swabs using a commercially available QIA-amp Viral RNA Mini Kit (Qiagen Cat. # 52906) after diluting them in 140µl of viral transport medium (VTM). To summarize, 140µl of each sample were combined with 560µl of lysis buffer (AVL) which contains carrier RNA and incubated at room temperature for 10 minutes. Then samples were centrifuged after adding 560µl of 100% ethanol. The columns were then sequentially washed in 500µl with washing buffers AW1 and AW2. Then RNA was eluted in 60µl of TE buffer [8]. Purified RNA of each sample (10µl) was subjected to reverse transcription and amplification by using multiplex real-time PCR assay for detection of SARS-CoV2by CDC 2019-Novel Corona virus (2019-nCoV) Real-Time -PCR Diagnostic Panel on Thermo Fisher (ABI) Quant Studio 5[9].

## RESULTS

In the study duration total of n=3509, rapid antigen tests were collected and performed at IBGE of (random) hospital visitors. From n=3509 about n=458 (7.60%) reported rapid antigen-positive, and 3051 (92.4%) were negative. Rapid antigen-positive cases were further tested through multiplex RT-PCR for conformation and comparative analysis. In RT-PCR 445 (97.17%) showed positive results and 13 (2.83%) as negative out of 458 rapid antigen-positive cases as shown in Fig 4. Rapid antigen-negative samples also tested with RT-PCR out of n=3051 (rapid antigen negative) n=25 were observed RT-PCR positive (rapid antigen false negative) and n=3026 were negative.

| Test | Positive | Negative | Total |
|---|---|---|---|
| Rapid antigen | 458 (7.60 %) | 3051(92.4 %) | 3509 |
| Confirmative RT -PCR | 445 (97.17 .6%) | 13 (2.83 %) | 458 |

**Table 1:** Comparative results of rapid antigen with RT-PCR.

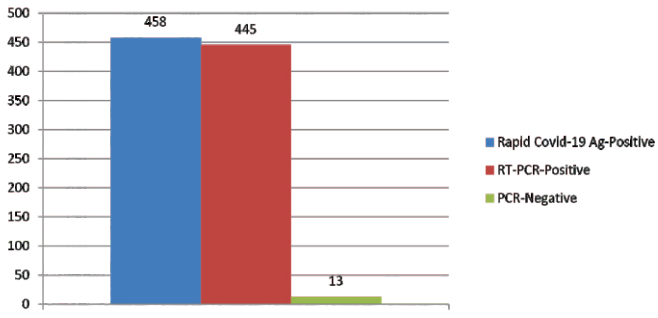



**Figure 4:** Positive rapid COVID -19 amtigen results reconfermation with RT-PCR analysis

## DISCUSSION

In COVID-19 diagnostic laboratories, RT-PCR techniques for

**DOI:** https://doi.org/10.54393/pbmj.v5i4.397

SARS-CoV-2 RNA detection in clinical specimens are commonly employed [6]. The performance parameters of the RAT test for detecting SARS-CoV-2 virus in nasopharyngeal samples were determined in this study, and the results were compared to those of the gold standard, RT-PCR. The RAT test for rapid detection of SARS-CoV-2 antigen had a sensitivity of 94.6% (total n = 3509; positive n = 458; negative n = 3051). Thus at the Institute of Biomedical and Genetic Engineering in Islamabad, the sensitivity of this test was further verified by Multiplex RT-PCR of n=458 RAT positive nasopharyngeal swabs (Table 1).

| | | Real Covid -19 cases |
|---|---|---|
| Rapid antigen tested | True P ositive | 458 |
| | False Negative | 25 |
| | Total | 470 |

Sensitivity= 445/470= 94.6%

| | | Without Covid -19 |
|---|---|---|
| Rapid antigen tested | False Positive | 13 |
| | True Negative | 3026 |
| | Total | 3039 |

Specificity= 3026/3039=99.5%

With a comparison of RT-PCR, it has been discovered that there is good sensitivity and specificity ratio (99.5%). Several rapid antigen-positive samples yielded RT-PCR negative findings, re processing of samples were used; after retesting, some samples became RT-PCR positive n=25, while others yielded negative results n=3026. The viral load in the test sample is one reason for a negative RT-PCR result of a positive fast antigen sample; this is because the patient could be in the recovery or early stages of infection. As a result, the amount of virus in the sample for RT-PCR will be undetectable [5,10-12]. Rapid antigen cannot be replaced with standard gold test real time PCR because rapid test is beneficial in onset of disease first five to seven days but cant after that due to the borderline or decrease in viral load[13-15]. Increasing demand for COVID-19 fast results due to flights timing or move towards abroad countries invent the COVID-19 Antigen test made by different countries with same technical approach. To check the sensitivity, specificity as well as reliability we compared results of COVID-19 antigen kit's results with the Real time PCR. Samples were retested from both kits and evaluated the results. We find 458 (7.60%) were rapid antigen positive while 470 were Real time PCR positive out of total 3509 samples. Among these twelve were observed Rapid antigen false negative. When reason of this false negativity was traced it was observed that for testing a sample results depends upon the viral load or onset of disease as with the rapid antigen case results reliability is 5-7 days of onset of disease with the lowest Ct-value as the disease move towards its end (last incubation days) Rapid antigen test is not recommendable because it was weak to detect borderline cases either they are true positive. We used different studies to justify our research. As in previously conducted study it was found that out of 14,188 patient's samples rapid antigen and real time sensitivity and specificity was 0.68%, 0.99% respectively. Results variation followed the viral load with Ct-value ≤25 or onset of disease (5 days of symptoms onset)[16]. In another study out of 412 patients 43 were tested positive, 358 were negative by both rapid antigen (Panbio™) COVID-19 and RT-PCR, 2.7% (11 Patients) showed false negative results due to disease onset or viral load when asked for patients history. Rapid antigen Panbio™ Covid-19 performed well as compared to other kits [17]. Same results were observed 824 individuals with 2425 repeated tests 52 individuals (6.3%) were RT-PCR positive with sixteen inconclusive Panbio screening approach but specificity was 99% [18]. Study conducted in Netherlands, Utrecht (1367 subjects) and Aruba (208 subjects) specificity of Panbio ™ COVID-19 Ag was 100% but sensitivity was 72.6%. False negative probability was associated with Ct-values but not with symptoms duration [19-21]. The ECLIA based Elecsys antigen test of Roche was compared to Real time PCR with the outcome of specificity (95%), sensitivity (72%) and accuracy (94.9%). Antigen sensitivity was noted as inversely proportional to the Ct-value so we concluded that antigen test cannot be replaced standard real time polymerase chain reaction [22]. The Rapid Ag test has the advantage of a simple method and quick results with a high negative predictive value (NPV), but it has the drawback of a poor positive predictive value (PPV) in a low prevalence area. As well as, the rapid antigen test can help all healthcare staff manage infected patients more efficiently in a timely manner, especially in rural and outbreak locations. Thus this rapid and easy SARS-CoV-2 antigen detection test could be used as a screening assay, particularly in high-prevalence areas[5].

## CONCLUSIONS

Panbio™COVID-19 Ag Rapid test device (Abbott) showed good sensitivity and specificity ratio compared to multiplex RT-PCR. Though, multiplex RT-PCR is gold standard and confirmative test for Covid-19 but given the pandemic situation and unavailability of RT-PCR facilities, Rapid antigen-positive test can be used for screening and isolation of positive patients from the population.

**DOI:** https://doi.org/10.54393/pbmj.v5i4.397

## REFERENCES

[1] Su, S., et al., Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends in microbiology, 2016. 24(6): p. 490-502.
[2] WHO, C.O., World health organization. Responding to Community Spread of COVID-19. Reference WHO/COVID-19/Community_Transmission/2020.1, 2020.
[3] Florez, H. and S. Singh, Online dashboard and data analysis approach for assessing COVID-19 case and death data. F1000Research, 2020. 9.
[4] Sohrabi, C. and Z. Alsafi, O Neill N., Khan M., Kerwan A., Al-Jabir A., Iosifidis C., Agha R. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). International Journal of Surgery, 2020.
[5] Chaimayo, C., 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. Virology journal, 2020. 17(1): p. 1-7.
[6] Tang, Y.-W., et al., Laboratory diagnosis of COVID-19: current issues and challenges. Journal of clinical microbiology, 2020. 58(6): p. e00512-20.
[7] van Kasteren, P.B., et al., Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. Journal of Clinical Virology, 2020. 128: p. 104412.
[8] Graham, T.G., et al., Open-source RNA extraction and RT-qPCR methods for SARS-CoV-2 detection. PloS one, 2021. 16(2): p. e0246647.
[9] Randazzo, W., et al., SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water research, 2020. 181: p. 115942.
[10] Mak, G.C., et al., Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. Journal of Clinical Virology, 2020. 129: p. 104500.
[11] Lambert-Niclot, S., et al., Evaluation of a rapid diagnostic assay for detection of SARS-CoV-2 antigen in nasopharyngeal swabs. Journal of clinical microbiology, 2020. 58(8): p. e00977-20.
[12] Zou, L., et al., SARS-CoV-2 viral load in upper respiratory specimens of infected patients. New England journal of medicine, 2020. 382(12): p. 1177-1179.
[13] Cattelan, A.M., et al., Rapid Antigen Test LumiraDxTM vs. Real Time Polymerase Chain Reaction for the Diagnosis of SARS-CoV-2 Infection: A Retrospective Cohort Study. International Journal of Environmental
[14] Research and Public Health, 2022. 19(7): p. 3826.
Vojtkovská, V., et al., Direct Detection of Feline Coronavirus by Three Rapid Antigen Immunochromatographic Tests and by Real-Time PCR in Cat Shelters. Veterinary Sciences, 2022. 9(2): p. 35.
[15] Baldanti, F., et al., Choice of SARS-CoV-2 diagnostic test: challenges and key considerations for the future. Critical Reviews in Clinical Laboratory Sciences, 2022: p. 1-15.
[16] Albert, E., et al., Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres. Clinical Microbiology and Infection, 2021. 27(3): p. 472. e7-472. e10.
[17] Torres, I., et al., Evaluation of a rapid antigen test (Panbio™ COVID-19 Ag rapid test device) for SARS-CoV-2 detection in asymptomatic close contacts of COVID-19 patients. Clinical Microbiology and Infection, 2021. 27(4): p. 636. e1-636. e4.
[18] Winkel, B., et al., Screening for SARS-CoV-2 infection in asymptomatic individuals using the Panbio COVID-19 antigen rapid test (Abbott) compared with RT-PCR: a prospective cohort study. BMJ open, 2021. 11(10): p. e048206.
[19] Gremmels, H., et al., Real-life validation of the Panbio™ COVID-19 antigen rapid test (Abbott) in community-dwelling subjects with symptoms of potential SARS-CoV-2 infection. EClinicalMedicine, 2021. 31: p. 100677.
[20] Treggiari, D., et al., SARS-CoV-2 rapid antigen test in comparison to RT-PCR targeting different genes: A real-life evaluation among unselected patients in a regional hospital of Italy. Journal of Medical Virology, 2022. 94(3): p. 1190-1195.
[21] Linares, M., et al., Panbio antigen rapid test is reliable to diagnose SARS-CoV-2 infection in the first 7 days after the onset of symptoms. Journal of Clinical Virology, 2020. 133: p. 104659.
[22] Iqbal, B., et al., Comparison of SARS-CoV-2 antigen electrochemiluminescence immunoassay to RT-PCR assay for laboratory diagnosis of COVID-19 in Peshawar. Diagnosis, 2021.