Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 30 (1), 2023: 14–19.
www.Ejimmunology.org

Evaluation of diagnostic performance of a rapid antigen test in diagnosing COVID-19

Eman R. Badawy, Azza M. Ezz El-Din and Randa A. El Zohne

Department of Clinical Pathology, Faculty of Medicine, Assuit University, Egypt.

Corresponding author: Eman R. Badawy, Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.
Email: e.badawy@aun.edu.eg.

Abstract

The coronavirus disease 2019 (COVID-19) pandemic is our time's major global health crisis and the greatest health challenge. Reverse transcription-polymerase chain reaction (RT-PCR) test for severe acute respiratory syndrome coronavirus (SARS-CoV-2) is the gold standard technique for diagnosis of symptomatic cases and asymptomatic carriers. By 2020, antigen rapid tests have been approved for use in Covid-19 testing by regulatory bodies all over the world owing to their benefits as they are rapid and cost effective. This work aimed to determine the diagnostic sensitivity and accuracy of the SARS-CoV-2 rapid antigen test in the detection of SARS-CoV-2 infection compared to RT-PCR data. The study included 111 symptomatic COVID-19 patients and 20 control subjects. Of the 111 study patients, 91 patients (81.98%) were positive by RT-PCR and 20 patients negative. The BIOZEK antigen COVID-19 Ag rapid test device was evaluated using sera from the 111 symptomatic COVID-19 patients. Of the 91 RT-PCR positive patients, 81 (90.1%) were positive by the antigen rapid diagnostic test (Ag-RDT). The control subjects were negative by both tests. The overall sensitivity, specificity, PPV, NPV, and accuracy of the Ag-RDT were 91.11%, 100%, 100%, 68.9%, and 91.8%, respectively and these increased as the level of viremia increased. In conclusion, the used Ag-RDT showed high sensitivity and accuracy for detecting of a SARS-CoV-2 infection, especially when the viral load was high. However, the test lacks sensitivity particularly in those with low viral load.

Keywords: COVID-19, RT-PCR, Ag rapid diagnostic tests.

Date received: 16 May 2022; **accepted:** 22 October 2022

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic dramatically affects global health and quality of life with long-lasting effects on the economy worldwide.[1,2] Identification of people infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a must for controlling the pandemic's spread. The gold standard for diagnosing SARS COV 2 viral infection is reverse transcriptase-polymerase chain reaction (RT-PCR). It is highly sensitive and accurate, and it is still the standard method to diagnose coronavirus disease 2019 (COVID-19). However, nucleotide-based viral RNA testing is costly, time-consuming, and necessitates

specialized laboratory settings in terms of personnel and instrumentation.[3]

Antigen rapid diagnostic tests (Ag-RDTs) for SARS-CoV-2, also known as antigen point-of-care tests (AgPOCT) or lateral flow devices (LFD), are regarded as an important diagnostic tool in the fight against the spread of the corona virus.[4] Rapid antigen detection (RAD) tests are useful in the context of the pandemic and may help to improve overall diagnostic ability. They provide advantages in terms of response times and costs to the healthcare system, particularly in situations where the ability to perform a molecular test on a nasopharyngeal swab may be limited.[5] Because of the lower sensitivity of the Ag-RDTs in comparison to molecular assays, they have the potential to be a highly valuable surveillance test in terms of tracking and preventing the spread of infection.[6] The antigen (Ag) tests are based on the immunochromatographic technique to detect SARS CoV-2 nucleocapsid protein (N). They provide results within a few minutes. Various commercial RAD tests (second, third, and fourth generations) are now available that meet WHO-established criteria. However, because of the method used, Ag-RDTs tests are less sensitive than RT-PCR tests, making them more susceptible to false-negative results. So, every suspected case must be affirmed by a molecular test. The purpose of this study was to determine the diagnostic sensitivity and accuracy of the SARS-CoV-2 rapid antigen test in detecting SARS-CoV-2 infection.

Subjects and Methods

This study included 111 patients suspected of having COVID-19 infection from Assiut University Isolation COVID-19 Hospital, Assiut University during the second outbreak of the COVID-19 pandemic (the period from December 2020 to February 2021). Twenty apparently healthy subjects were also included in the study as negative controls. According to the results of SARS COV 2 RT- PCR the patients were classified into three groups (high viremia, 21 patients with a cycle threshold (ct.) value of less than 29, moderate viremia, 37 patients with a ct. value of 29-34, and low viremia, 23 patients with a ct. value of more than 34, the rest of patients were negative (Figure 1).

Ethical consideration

The Medical Ethics Committee of the Faculty of Medicine at Assiut University reviewed and approved the study protocol. (Dated October 2020). The practical part of this study was carried out in the Molecular Biology Laboratory, Immunology Unit, Clinical Pathology Department, Assiut University Hospital.

Methods

RNA was extracted from nasopharyngeal and oropharyngeal swabs (viral transport medium tube with Dacron swabs, Wellkang Ltd, England, UK). Sample collection was performed according to the Centers for Disease Control (CDC).[7] RNA isolation and purification were carried out using a commercial kit (QIAamp Viral RNA Mini Kit, lot number 52906, QIAGEN: Germany), as directed by the manufacturer' instructions. It is based on the binding of RNA to the silica membrane in a fast spin column (QIAcube Connect Automatic Nucleic Acid Extractor, QIAGEN, USA). Assessment of SARS-CoV-2 viral RNA was performed by an RT-PCR COVID-19 assay (genesig® Real-Time PCR assay supplied by Primer Design, UK, SO53 4DG), according to the manufacturer's instructions.

The SARS COV-2 antigen was detected by the BIOZEK COVID-19 antigen rapid test kit (Lot. No. BCOV5020011-2, BIOZEK Medical, The Netherland), according to the manufacturer's instructions. It is a rapid assay and color developed within 15 min.

Statistical analysis

The IBM SPSS, Version 22 software was used to analyze the data. Frequency and percentage were used to calculate descriptive statistics. The ROC curve analysis was performed to assess sensitivity, specificity, and positive and negative predictive values (PPV/NPV), which were calculated for each laboratory test.

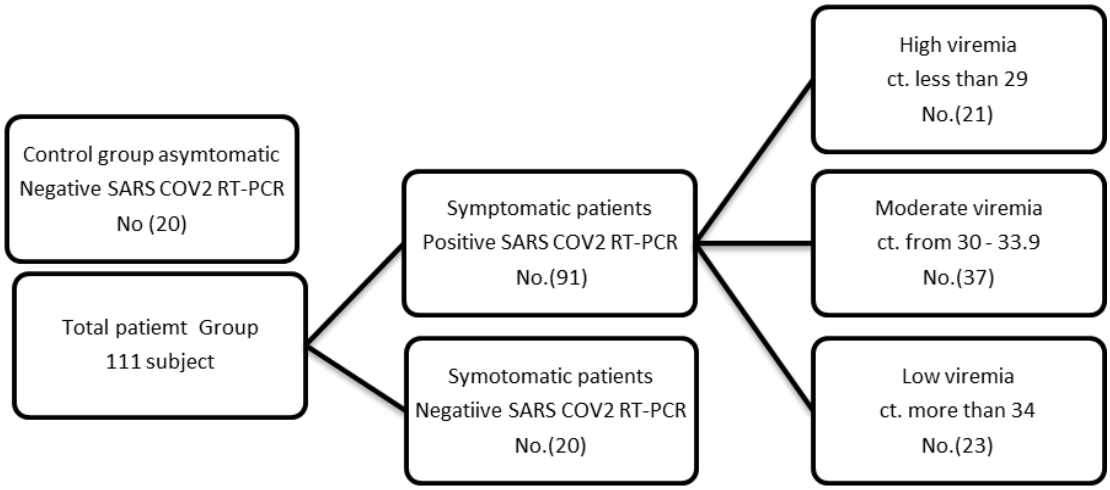

Figure 1. Classification of studied subjects according to the RT-PCR test results.

Results

RT-PCR for SARS COV 2 was performed for 111 symptomatic COVID-19 patients and showed positive results in 91 (81.98%) patients and negative in 20 patients (18.01%) (Figure 1). All 20 control subjects were negative for RT-PCR for SARS COV2.

The BIOZEK antigen Covid-19 Ag rapid test device was used to test sera of 111 symptomatic COVID-19 patients. Of the 91 patients with positive RT-PCR results, 81 (90.1%) patients showed positive Ag-RDT results. All RT-PCR negative samples (20 symptomatic patients and 20 controls) showed negative antigen COVID-19 Ag rapid test results (Table 1).

The ROC curve analysis revealed that the sensitivity, specificity, PPV, NPV, and accuracy of the antigen COVID-19 Ag rapid test for the overall group were 90.11%, 100.0%, 100, 68.96%, and 91.81%, respectively.

The positive RT-PCR test results were classified and analyzed based on their quantitative ct.-value. In patients with high viremia (ct.-value less than 29), this yielded a sensitivity, specificity, PPV, NPV and accuracy of 100% for each. Patients with moderate viremia (ct.-value from 29–34) had a sensitivity of 91.8%, a specificity of 100.0%, PPV of 100%, NPV of 86.95% and an accuracy of 94.74%. While patients with low viremia (ct.-value greater than 34) had a sensitivity of 73.91%, a specificity of 100.0%, PPV of 100%, NPV of 76.9 % and an accuracy of 86.05% (Table 1). In the present study, the time for releasing the results of the COVID-19 Ag rapid test was about 20 minute and that for releasing RT-PCR about 3 hours.

Table 1. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of Ag-RDT in relation to RT-PCR test.

Patients positive by RT-PCR	Sensitivity	Specificity	PPV	NPV	Accuracy
Overall Group (n=91)	90.11%	100%	100%	68.9%	91.81%
Patient with High viremia ct. less than 29 (n=21)	100%	100%	100%	100%	100%
Patient with moderate viremia ct. from 29 - 34 (n=37)	91.8%	100%	100%	86.95 %	94.74
Patient with Low viremia ct. more than 34 (n=23)	73.91%	100%	100%	76.9%	86.05%

Discussion

This study was designed to provide an independent proof of concept (POC) validation of Ag-RDT relative to RT-PCR to diagnose acute SARS-CoV2 infection in symptomatic and asymptomatic populations. Such POC would allow the use of Ag-RDT as a screening tool.

In the present study, the RT-PCR for SARS COV2 showed positive results in 81.98% of symptomatic COVID patients. This finding is in agreement with Fang et al., (2020) who reported RT-PCR of 70.58% (36/51), and suggested that false-negative results could be caused by a variety of factors such as human error when following the diagnostic kit protocol, reagent sensitivity, specimen sampling site and method, and collection times.[8] Yang et al., 2020, also reported that the overall positive rate of RT-PCR for throat swab samples was between 30 and 60%. in clinically and radiologically evaluated patients during initial presentation despite limitations in collection of sample, transportation, and kit performance.[9] Furthermore, they reported that one of the Wuhan studies revealed that a significant proportion of COVID-19 patients may have had an initial negative result for the RT-PCR test and that the positively diagnosed patients had a higher tendency to progress to more serious or severe cases. According to this study, patients with negative RT-PCR who present with typical clinical manifestations should not be ignored and should have the PCR test repeated[10]

In addition, Caturegli et al., 2020 and Hanson et al., 2020, reported that in patients with evidence of lower respiratory tract illness, sputum should be collected if they have productive cough, and lower respiratory tract specimens (tracheal aspirate or bronchoalveolar lavage), are options for symptomatic patients with negative nucleic acid amplification test (NAAT) [11, 12] and also WHO 2020a recommends reserving lower respiratory tract specimens for respiratory NAAT testing for hospitalized patients with an initial negative test on an upper respiratory tract specimen but a suspicion of lower tract SARS-CoV-2 infection persists.[13]

In the present study, the overall sensitivity, specificity, PPV, NPV, and accuracy of the Ag-RDT were 91.11%, 100%, 100%, 68.9%, and 91.8%, respectively. These findings agreed with those of a study by Saeed et al., 2021, who reported that the COVID-19 Ag test had an accuracy of 94.89% and sensitivity and specificity of 85.02%. They also stated that tests with diagnostic accuracy greater than 90% have a high diagnostic value.[14] Moreover, these findings also agreed with WHO, 2021, recommendation in which, in suspected COVID-19 cases, the Ag-RDTs with minimum performance requirements of 80% sensitivity and 97% specificity when compared to a nucleic acid amplification test. These standards were developed through a formal process of developing target product profiles (TPPs) for priority SARS-CoV-2 diagnostics.[15]

The present work revealed that sensitivity, specificity, PPV, NPV and accuracy in patient with ct. value less 29 (high viral load) were 100% for each, in patient with ct. value 29 to34 (moderate viral load) were 91.8%,100%, 100%, 86.95% and 94.74%, respectively and in those with ct. value more than 34 were 73.91%,100%,100%, 76.9% and 86.05%, respectively. These findings are supported by those of a study by Baro et al., 2021, who suggested that SARS-CoV-2 antigen testing of unexposed asymptomatic individuals with specimens at ct. value<30, need to achieve sensitivity and specificity of at least 80% and 96%, respectively. While these tests may miss SARS-CoV-2 infections with low viral loads, they accurately detect individuals with high viral loads, who are therefore at greater risk of transmission.[16] These findings were also supported by Lombardo et al., 2021, who found that Ag-RDT was highly specific, but the sensitivity was acceptable only at ct. value< 25 with higher viral loads. Consequently, the test is useful in situations where AgPOCT is required or where a short-term evaluation of infectivity is needed.[17] Larremore et al., 2021, also suggested using rapid antigen in case of pandemics because it is inexpensive, rapid, and widely distributed and it may also be required in other options such as using clinical questionnaires to select higher risk patients or using repetitive sequential measures.[18]

Furthermore, Viswanathan et al., 2020, proposed using rapid antigen tests as an initial screening and that all negative results be confirmed by RT-PCR tests, which will increase test sensitivity Therefore, if used correctly, rapid antigen tests could be an effective policy. They also stated that in cases of high prevalence, a positive rapid antigen test has a high positive predictive value, whereas negative results should never be used to reduce standard protective measures.[19]

The present study revealed that the time required for the release of the results of rapid antigen tests varied from 15 to 30 minutes depending on the time of sample collection and receiving. These findings are supported by the WHO, 2020b[20] report, which indicated that because of their ease of use and quick turnaround time, Ag-RDTs have the potential to increase access to testing and reduce diagnostic delays by shifting to decentralized testing of patients with early symptoms. Furthermore, according to the report, current manufactured tests require nasal or nasopharyngeal swab samples, and many companies are conducting studies to assess the performance of their tests using alternative sample types such as saliva, oral fluid, and sample collection systems to potentially expand options for use and to facilitate safe and efficient testing. In addition, WHO, 2020b has established a sensitivity limit of >80% and specificity limit of >97% for SARS-CoV-2 antigen-based RDTs when compared to RT-PCR assay.[20]

From this study, we concluded that the used Ag-RDT had high sensitivity and accuracy for detecting SARS-CoV-2 infections, especially when the viral load is high. However, the test lacks sensitivity, or the risk of falsely positive results, particularly in those with low viral load. The accuracy attained by the best-performing Ag-RDTs, combined with the rapid turnaround time compared to RT-PCR, suggests that if used in thoughtful testing and screening strategies, these tests could have a significant impact on the pandemic.

Author Contributions

ERB, AME & RAE performed the laboratory work. AME made the statistical analysis. All authors participated in writing and reviewing the paper. N.B. Samples were sent to the laboratory from isolation hospital.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The Medical Ethics Committee of the Faculty of Medicine at Assiut University reviewed and approved the study protocol. (Dated October 2020).

Informed consent

A signed consent form was obtained from each study participant.

References

1. Johansson, M. A., Quandelacy, T. M., Kada, S., et al. (2021). SARS-CoV-2 Transmission from People Without COVID-19 Symptoms. *JAMA Netw. Open*. 2021; 4: e2035057. doi: 10.1001/jamanetworkopen.2020.35057.

2. Chen, C. C., Lu, S. C., Bai, C.H., et al. (2021). Diagnostic Accuracy of SARS-CoV-2 Antigen Tests for Community Transmission Screening: A Systematic Review and Meta-Analysis. *Int, J. Environ. Res. Public Health*, 18, 11451.

3. Wiersinga, W. J., Rhodes, A., Cheng, A. C., et al. (2020). Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* Aug 25; 324(8):782-793.

4. World Health Organization (2021). Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: interim guidance, 11 September 2020. WHO/2019-nCoV/Antigen Detection/2020.1. *Geneva: World Health Organization*; 2020.

5. Fenollar, F., Bouam, A., Ballouche, A., et al. (2021). Evaluation of the Panbio COVID-19 rapid antigen detection test device for the screening of patients with COVID-19. J Clin70.5 *Microbiol*. 59(2): e02589.

6. Mina, M. J., Parker, R., & Larremore, D. B. (2020). Rethinking Covid-19 test sensitivity—a strategy for

containment. *New England Journal of Medicine*, 383(22), e120.

7. Kline, A., Putnama, N. E., Youn, J., et al. (2020). Dacron swab and PBS are acceptable alternatives to flocked swab and viral transport media for SARSCoV-2. *Diagnostic Microbiology and Infectious Disease 99* (2021) 115209.

8. Fang, Y., Zhang, H., Xie, J., et al. (2020). Sensitivity of chest CT for COVID-19: comparison to RT-PCR. *Radiology*. 296(2): E115-7.

9.Yang, Y., Yang, M., Yuan, J., et al. (2020). Laboratory diagnosis and monitoring the viral shedding of SARS-CoV-2 infection. The Innovation, 1(3), 100061.

10. Huang C., Wang Y., Li X., et al. (2019). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. www.thelancet.com Vol 395 February 15, 2020.

11. Caturegli, G., Materi, J., Howard, B. M., et al. (2020). Clinical Validity of Serum Antibodies to SARS-CoV-2: A Case–Control Study. *Annals of internal medicine*, 173(8), 614-622.

12. Hanson, K. E., Caliendo, A. M., Arias, C. A., et al. (2020). Infectious Diseases Society of America 2020 (IDSA) Guidelines on the Diagnosis of COVID-19: Serologic Testing.

13. World Health Organization (WHO). (2020a). Antigen-detection in the Diagnosis of SARS-CoV-2 Infection Using Rapid Immunoassays: *Interim Guidance* (2020)

14. Saeed, U., Uppal, S. R., Piracha, Z. Z., et al. (2020). Evaluation of SARS-CoV-2 antigen-based rapid diagnostic kits in Pakistan: formulation of COVID-19 national testing strategy. *Virol. J.*, 18 (1) (2021), p. 34.

15. World Health Organization. (2021). Interim Guidance Antigen-detection in the diagnosis of SARS-CoV-2 infection 6 October 2021.

16. Baro, B., Rodo, P., Ouchi, D., et al. (2021). Performance characteristics of five antigen-detecting rapid diagnostic test (Ag-RDT) for SARS-CoV-2 asymptomatic infection: a head-to-head benchmark comparison. *Journal of Infection*, *82*(6), 269-275.

17. Lombardo, F., Triolo, G., Yang, B., et al. (2021). Diagnostic Performance of a Rapid Antigen Test Compared with the Reverse Transcription Polymerase Chain Reaction for SARS-CoV-2 Detection in Asymptomatic Individuals Referring to a Drive-in Testing Facility. *COVID 2021*, 1, 784–789.

18. Larremore, D. B., Wilder, B., Lester, E., et al. (2021). Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. *Science advances*, *7*(1), eabd5393.

19. Viswanathan, M., Kahwati, L., Jahn, B., et al. (2020). Universal screening for SARS- CoV-2 infection: a rapid review. *Cochrane Database of Systematic Reviews*, (9).

20. World Health Organization: (2020b). Diagnostic testing for SARS-CoV-2. Interim guidance. https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2 (Accessed on September 21, 2020).