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Evaluation of a SARS-CoV-2 antigen test in the rapid diagnosis of COVID-19 suspected patients

COVID-19 şüpheli hastaların hızlı tanısında SARS-CoV-2 antijen testinin değerlendirilmesi

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ABSTRACT

Aim: Diagnostic testing for SARS-CoV-2 is an important component of the overall COVID-19 prevention and control strategy. Antigen detection using lateral flow assay (LFA) platforms can be performed at the point-of-care, providing quick results while being inexpensive and simple to perform. The current study sought to determine the success of a rapid antigen test in COVID-19 suspected cases, as well as the characteristics of discrepant results.

Materials and Methods: Upper respiratory samples from 352 adult patients suspected of acute COVID-19 cases with related symptoms for <8 days, who were admitted to Ege University Faculty of Medicine Department of Infectious Diseases and Department of Emergency Unit were tested routinely for SARS-CoV-2 RNA and evaluated with TOYO COVID-19 antigen test (Türklab-Turkey).

Results: There were 164 females and 184 males among the 352 adult patients (>18 years old) suspected of acute COVID-19 cases. The patients ranged in age from 18 to 88 years old, with a median age of 41.25 years. A routine test for SARS-CoV-2 RNA found positive results in 127 (37.1%) of the patients and negative results in 225 (63.9%). The COVID-19 Ag test was positive in 116(33%) of these patients' nasal swab samples and negative in 236 (67%). The sensitivity and specificity of the COVID-19 Ag test was 89.7% and 99%, respectively.

Conclusion: A rapid SARS-CoV-2 antigen test, which will be tested easily and supervised by medical personnel, could help decide for immediate isolation for patients or asymptomatic individuals that are shedding large number of viruses. In this study, lower viral loads can be strongly linked to the false negative antigen test results.

Keywords: SARS-CoV-2, antigen-antibody reactions, COVID-19 testing.

ÖZ

Amaç*: SARS-CoV-2 ile enfekte olmuş bireyler için erken teşhis, izolasyon ve tedavi stratejileri kritik öneme sahiptir. Lateral akış testleri (LFA) kullanılarak antijen tespiti, ucuz ve basit olmakla birlikte hızlı sonuçlar sağlar. Bu çalışmada, COVID-19 şüpheli vakalarda hızlı antijen testinin başarısını ve ayrıca tutarsız sonuçların özelliklerini belirlemek hedeflenmiştir.*

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***Gereç ve Yöntem**: Ege Üniversitesi Tıp Fakültesi Hastanesi Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği ile Acil Servis ünitesine yatırılan, 8 günden daha kısa süredir COVID-19 ilişkili semptomları olan 352 yetişkin hastadan alınan üst solunum yolu örnekleri; rutin olarak kullanılan SARS- CoV-2 RNA testi ve TOYO COVID-19 (Türklab-Türkiye) antijen testi ile değerlendirildi .*

***Bulgular**: Akut COVID-19 vakalarından şüphelenilen 352 yetişkin hasta arasında 164 kadın ve 184 erkek mevcuttu. Hastaların yaşları 18 ile 88 arasında olup ortanca yaş 41.25 idi. Rutin olarak çalışılan SARS-CoV-2 RNA PCR testi hastaların 127'sinde (%37.1) pozitif, 225'inde (%63.9) negatif olarak saptandı. Bu hastaların nazal sürüntü örneklerinin 116'sında (%33) COVID-19 antijen testi pozitif ve 236'sında (%67) negatif saptandı. COVID-19 hızlı antijen testinin duyarlılığı ve özgüllüğü sırasıyla %89.7 ve %99 olarak saptandı.*

***Sonuç**: Sağlık personeli için kullanımı kolay ve hızlı bir SARS-CoV-2 antijen testi, semptomatik hastalar veya çok sayıda virüs saçan asemptomatik bireyler için acil izolasyon kararı vermede yardımcı olabilir. Bu çalışmada, düşük viral yükler, yanlış negatif antijen testi sonuçlarıyla daha yakın olarak ilişkilendirilmiştir.*

***Anahtar Sözcükler:** SARS-CoV-2, antijen-antikor testleri, COVID-19 testleri.*

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is a severe viral disease caused by a novel RNA virus that originated in China and has now spread over the world, resulting in a pandemic (1).

Diagnostic testing for SARS-CoV-2 is an important part of the overall COVID-19 prevention and control plan, and early detection, isolation, and treatment options for SARS-CoV-2 infected individuals are key to the pandemic response. The ongoing COVID-19 pandemic has reaffirmed diagnostic testing's important role in outbreak control (2). All currently available technologies have been used to generate extremely sensitive and specific detection assays for SARS-CoV-2 in the last year. Testing for SARS-CoV-2 should be linked to public health strategies for appropriate clinical care and contact tracking to interrupt transmission chains (3).

The identification of SARS-CoV-2 RNA in respiratory specimens using reverse transcriptase polymerase chain reaction (RT-PCR) is the optimum specimen and the most reliable diagnostic procedure for a conclusive diagnosis (4). Antibody assays are insensitive in the early stages of acute infection, which necessitates quick diagnosis and intervention to contain the epidemic. Because they allow for widespread testing, rapid antigen tests employing salivary and nasal swabs are gaining popularity. These tests, which can be performed in various locations and have quick turnaround times, have made widespread COVID-19 testing possible. Some antigen tests, according to new research, can detect infectious cases with appropriate sensitivity, particularly in patients with high viral loads (5).

Antigen detection using lateral flow assay (LFA) platforms can be used as point-of-care testing, with advantages like quick results, low cost, and ease of use. Because these assays' sensitivity is often lower than that of nucleic acid detection tests, to boost testing capacity, new methods were created, such as an inkjet-printed assay with a non-fouling polymer brush to increase signal-to-noise ratio and test-zone pre-enrichment processes.

In upper respiratory samples of probable acute COVID-19 patients, we compared a rapid COVID-19 antigen (Ag) test produced in Turkey with our routine SARS-CoV-2 RNA PCR test, which has a result time of approximately 25 min and six hours, respectively.

MATERIALS and METHODS

Patients and samples

Upper respiratory samples from 352 adult patients (> 18 years old) suspected of acute COVID-19 were admitted to the Ege University Faculty of Medicine Department of Infectious Diseases and Department of Emergency Unit for the first time between 09.11.2020 and 13.06.2021, with related symptoms for <8 days, were tested routinely for SARS-CoV-2 RNA and were screened with the SARS-CoV-2 antigen test. Related symptoms included fever, chills, congestion or runny nose, body aches, fatigue, headache, sore throat, nausea, vomiting, diarrhea, cough, shortness of breath, difficulty breathing, loss of smell/taste, or severe

respiratory illness. Test results were compared for their performance and result reporting time.

SARS-CoV-2 RT-PCR test

Nasopharyngeal samples were transported to the Virology Laboratory in less than an hour in viral nucleic acid tampon solution (VNat, Bioeksen, Turkey) that was used for transport and RNA release. Three SARS-CoV-2 RT-PCR test kits authorized by the Turkish Ministry of Health (Bio-Speedy® SARS-CoV-2, Bioeksen/Diagnovital SARS-CoV-2 RT-PCR, RTA/Coronex RT-qPCR, Gensutek, Turkey) were used according to the manufacturer's recommendations. Amplification procedures and analyzes were performed using Rotorgene (Qiagen, Luxemburg).

SARS-CoV-2 antigen test

Nasal swab samples were tested for COVID-19 antigen (SARS-CoV-2 antigen) (TOYO COVID-19 antigen test, TürkLab, Turkey). This assay targets the nucleocapsid protein (N) in clinical samples. Swab samples were rotated for 10 seconds in the tube that contained the reagent, and afterwards, the swab was removed out from the tube by pressing the swab along one side of the tube. Two drops were added to the sample well of the flow assay test cartridge. After 20 min with a visible control line, the test was evaluated as positive and negative for SARS-CoV-2 antigen by visually assessing the visible test lines (Figure-1).

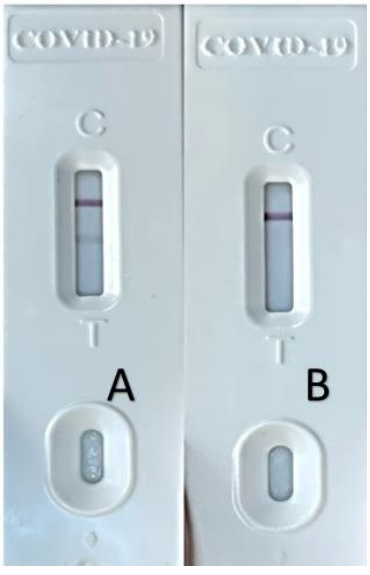

Figure-1. Visual COVID-19 antigen test (TOYO COVID_19 antigen test, TürkLab, Turkey) result. **A.** Positive results with both control and test lines visible, **B.** Negative results with only a visible control line.

Ethical approval

Ethical approval for this study was obtained from the Ege University Medical Research Ethical Committee (22-6/43) and permission for this study was obtained from the Turkish Ministry of Health.

RESULTS

Of the 352 adult patients, 168 were female and 184 were male. The age range was 18–88 years, and the median age of the patients was 41.25 years. The routine test performed for SARS-CoV-2 RNA was positive in 127 (127/352, 36.1%) and negative in 225 (225/352, 63.9%) patients. COVID-19 Ag test was positive in 116 (116/352, 33%) and negative in 236 (236/352, 67%) of the same nasal swab samples. The test result time was 5–8 hours for SARS-CoV-2 RNA test and <30 min for the COVID-19 Ag test. SARS -CoV-2 RNA and COVID-19 Ag test results are shown in Table-1.

The sensitivity and the specificity of the COVID-19 Ag test in nasal specimens collected during the first week of symptoms in acute COVID-19 infection was 89.7% and 99%, respectively compared to the SARS -CoV-2 RNA test, considered as the gold standard.

There were two SARS-CoV-2 RNA negative, COVID-19 antigen test-positive cases and 13 cases were SARS-CoV-2 RNA positive but COVID-19 antigen negative (Table-1). Of these 13 discrepant results, only one was tested for variants and delta variant was detected in that sample. The mean of Ct values was 27.48 (min: 21.06 max: 31.51) and the median value was 28.27 in those 13 patients. The mean Ct value of all positive patients (127 of 352) was 22.36 (min: 15 max: 31.51) and median Ct value was 21.91.

SARS-CoV-2 RNA negative, COVID-19 antigen positive both patients had fever and fatigue and their thorax-computed tomography (CT) imaging was reported as COVID-19 pneumonia. The clinical medical records of the 13 patients with SARS-CoV-2 RNA positive and COVID-19 antigen negative revealed that eight patients had mild, two had moderate and three had severe clinical symptoms. Two of the three patients with severe clinical manifestations had underlying malignancy, and the remaining patient had no other risk factor except old age.

Table-1. SARS-CoV-2 RNA and COVID-19 Ag results of patients with suspected acute COVID-19.

	SARS-CoV-2 RNA-Positive	SARS-CoV-2 RNA-Negative	Total
COVID-19 Ag Positive	114	2	116
COVID-19 Ag Negative	13	223	236
Total	127	225	352

DISCUSSION

The World Health Organization (WHO) interim guidance, released on November 6th, 2021, recommends antigen testing in cases with a higher viral load, early during infection, in settings where the SARS-CoV-2 prevalence is 5%, and recommends the use of antigen tests with a sensitivity of 80% and specificity of 97%. (4).

During the year our study was conducted, the mean prevalence of SARS-CoV-2 RNA positivity in our hospital was around 10%, and SARS-CoV-2 RNA was positive in 36.1% of the screened patients (5). Our findings indicate that the TOYO COVID-19 antigen test (Türklab–Turkey), with a sensitivity of 89.7 percent and specificity of 99 percent, can be used reliably in acute COVID-19 infection during the first week of infection.

SARS-CoV-2 RNA tests using upper respiratory samples produce better results within 1–8 days of the onset of symptoms because the viral load is typically at its peak during this time (6, 7). However, the nucleic acid detection test's complex procedure and long turnaround time are significant barriers in a pandemic, where rapid results are critical for clinical management of infected cases and public health measures. A test comparable to nucleic acid testing with a quick turnaround time and high sensitivity/specificity would be ideal, and a rapid and simple SARS-CoV-2 antigen test supervised by qualified healthcare workers would be an excellent candidate. This would allow for isolating patients or asymptomatic individuals who are shedding many viruses, as well as early treatment interventions, to be made in a timely and reliable manner.

Previous research on viral load in upper respiratory specimens suggests that antigen tests perform well during the first week of symptoms (8-10). Compared to the gold standard SARS-CoV-2 RNA test, 36.1% of the 352 patients with symptoms suggestive of acute COVID-19 for 8 days tested positive for SARS-CoV-2 RNA and 33% tested positively for COVID-19 antigen with very high sensitivity (89.7 percent) and specificity (99 percent). Another study from Turkey reported a sensitivity of 61.8 percent and specificity of 100 percent using a different rapid antigen test (11).

Corman et al. found that the cumulative specificity of seven different antigen tests ranged from 88.24 to 100 percent (when the test with the lowest specificity was excluded, the specificities were greater than 94.85 percent) (6). A cross-sectional study evaluating two rapid antigen tests in asymptomatic and pre-symptomatic close contacts of individuals with confirmed SARS-CoV-2 infection found that sensitivities in confirmed cases were 63.9 percent and 62.9 percent, respectively, and 58.7 percent and 59.4 percent in pre-symptomatic close contacts. Sensitivities increased to 82.4 percent and 73.3 percent, respectively, in close contacts who developed symptoms later (10).

In the pediatric group, the sensitivity and specificity of antigen detection tests for SARS-CoV-2 were reported to be 85% and 91%, respectively. The lateral flow assay test used by Shaik et al. had low sensitivity and specificity in the pediatric group. One of our study's limitations was the lack of pediatric patients (12).

Current LFA procedures developed for COVID-19 antigen diagnosis are highly specific, but their variable sensitivity precludes their use as a primary detection method (13). These low-cost assays that can test multiple samples at the same time are critical for a quick response to the pandemic, proper patient management, developing clinical algorithms, and implementing isolation procedures.

Previous research has shown that the S1 domain of the spike (S) protein has very low cross-reactivity between epidemic coronaviruses and common human coronaviruses, and the S2 domain of the S protein and the nucleocapsid protein (N protein) have low-level cross-reactivity (14). The high specificity demonstrated by the COVID-19 antigen test, which targets the N

protein and was used in our study, correlates with this finding.

Corman et al. reported that the rate of false positive antigen tests is higher than that of RT-PCR, and we had two patients who were SARS-CoV-2 RNA-negative but COVID-19 antigen positive, which could be interpreted as false positive results. Due to their lower sensitivity compared to the SARS-CoV-2 RNA test, antigen tests may miss the infection in the very early and late stages of COVID-19 (6). The 13 patients who were SARS-CoV-2 RNA-positive (median Ct value of 28.27) but COVID-19 antigen test negative were likely false negatives; however, medical records of those patients did not reveal any underlying factor that would account for the false negative result. Low-viral load titers were a likely cause of the false negative result, but a viral load assay was impossible in those patients (15).

According to viral load studies, antigen tests in upper respiratory tract samples should perform best during the first 8 days of symptoms (8-10, 14). He et al. reported a significant decrease in viral load 8 days after symptom onset, when the virus could no longer be cultured and transmission would be significantly lower (7). The analytical sensitivity of point-of-care antigen tests was reported to be 2 million to 9 million copies per swab by the researchers (6).

More research is needed to clinically validate these findings, confirm the observed sensitivity and specificity, and incorporate them into clinical guidelines.

The major limitations of our study were the lack of variant data for most of the patients and the small number of participants, which prevented precise projection of sensitivity and specificity rates to the overall population. Finally, because of the small number of participants, we were unable to evaluate the success of this test for rare variants and cross reactions.

CONCLUSION

SARS-CoV-2 antigen tests that meet WHO's minimum requirements can be used in symptomatic COVID-19 patients and asymptomatic individuals at high risk of COVID-19. Antigen tests should be reliable, affordable, and accessible, with results available quickly, to ensure appropriate clinical care and to inform actions to prevent the spread of SARS-CoV-2. According to this study, antigen detection with the TOYO COVID-19 antigen (Türklab-Turkey) test is a good option for acute COVID-19 diagnosis.

Declaration of competing interest

TOYO Rapid antigen test cartridges and their solutions were donated by TurkLab (İzmir, Turkey) for use in this study.

Conflict of interest: The authors declare no conflict of interest.

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