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RESEARCH ARTICLE



Comparison of performance of LIAISON SARS-CoV-2 antigen assay with RT-PCR during the Omicron wave

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ABSTRACT

Due to the newly emerging Omicron variant, there is a need to re-evaluate the performance of automated antigen tests. Our study aim was to evaluate the performance of the automated Liaison SARS-CoV-2 antigen assay against reverse transcriptase polymerase chain reaction (RT-PCR) in samples with Omicron variant.

A prospective study was performed on 373 combined oro-nasopharyngeal samples (NPS) randomly collected from symptomatic patients. NPS were tested with Liaison SARS-CoV-2 Ag test (DiaSorin, Italy) and DS Coronex COVID-19 Multiplex RT-PCR Diagnosis Kit (DS BioTechnology, Ankara, Turkey).

Of 373 samples, 124 (33.2%) were found to be RT-PCR positive and 249 (66.8%) RT-PCR negative. Taking RT-PCR as a reference, the sensitivity and specificity of the Liaison SARS-CoV-2 Ag assay were found as 84.6% (95%CI 77.3%–90%) and 100% (95%CI 98.5%–100%), respectively. For samples with a cycle threshold (Ct) value <25 (high viral load), the sensitivity increased to 100%. When antigen concentration and Ct values were compared, a strong negative correlation between antigen and Ct values was determined ($P < 0.001$).

The Liaison antigen test met the performance criteria recommended by the WHO for samples with the Omicron variant. In addition, it showed excellent sensitivity and specificity in patients with high viral load. Therefore, Liaison antigen test can be a reliable and useful alternative in the diagnosis of SARS-CoV-2 infection, particularly in resource-constrained laboratories.

KEYWORDS

SARS-CoV-2, Liaison antigen test, RT-PCR, Omicron variant

INTRODUCTION

The key to prevent spread of SARS-CoV-2 infection in the community and early detection of new cases is an accurate and sustainable testing strategy [1]. Although hospitalizations are low due to the last reported variant, the Omicron variant, its more rapid spread than previous variants poses an important public health problem [2]. Therefore, rapid and accurate detection of infected persons is still up-to-date [3]. Although the gold standard in the diagnosis of SARS-CoV-2 infection is the real-time reverse transcriptase-polymerase chain

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reaction (RT-PCR) method, the test has some difficulties due to the need for specially equipped laboratory and time-consuming nature in large-scale population screenings [1, 4, 5]. Therefore, the World Health Organisation (WHO) has published a guidance on the potential use of antigen tests to avoid delays in diagnosis and to reduce the burden on molecular biology laboratories and recommends working with antigen tests with high specificity ($\geq 97\%$) and acceptable sensitivity ($\geq 80\%$) [6]. Subsequently, different commercial tests have been included in national testing strategies in many countries [7]. The Liaison SARS-CoV-2 Antigen test (DiaSorin, Saluggia, Italy) is a CE-IVD (Conformité Européenne for *in vitro* diagnostics) approved, chemiluminescence-based quantitative automated antigen test developed for this purpose that detects the SARS-CoV-2 nucleocapsid protein [8]. Working with fully automated systems of antigen tests reduces manual errors, allows large-scale samples to be run in a shorter time, and being integrated with the laboratory information system facilitates result follow-up [9, 10]. It is more relevant to perform antigen tests on individuals who show signs and symptoms of SARS-CoV-2 infection and those who have come into contact with infected patients [11, 12]. A positive result from these patients indicates infection with a high probability, whereas a negative result does not exclude the possibility of infection [4, 13].

Due to the new variants with various spike and nucleocapsid protein mutations, antigen tests used in the diagnosis of SARS-CoV-2 require constant close monitoring [4]. It has also been reported by the FDA that some rapid antigen tests give false-negative results, especially in detecting the last reported variant, the Omicron variant [14]. Moreover, it was also stated in a recent review that most of the first analytical performance studies on antigen tests were conducted before the emergence of the Omicron variant, emphasizing the need for studies evaluating the reliability of these assays in detecting of new variants [11]. Since its introduction, there have been no data investigating the effect of the Omicron variant on the performance of the Liaison antigen test. In addition, there are many publications on this assay in Europe however, there is no study on the general performance of this test in non-European countries [4, 11, 12]. The present study is the first in the literature in terms of these two features. Our study aims to determine the diagnostic performance of the Liaison SARS-CoV-2 Antigen test by comparing it with the SARS-CoV-2 RT-PCR (DS Coronex, Turkey) during the Omicron wave.

MATERIALS AND METHODS

A prospective cross-sectional study was conducted from 3 March to 31 March 2022. Randomly selected 373 combined oro-nasopharyngeal swab samples (NPS) collected by healthcare professionals from patients admitted to our hospital with coronavirus disease 2019 (COVID-19)-like symptoms were included in the study. NPS were suspended in a commercially available 3 ml viral transport medium

(VTM; Liofilchem, Roseto degli Abruzzi, Italy) recommended by the manufacturers. Samples for which RT-PCR and antigen testing were incompatible, were stored at -20°C until rerun with a different RT-PCR method. Variant analysis was also performed for these samples. Because the clinical data of the patients could not be examined, the relationship between the severity of clinical findings and antigen results was not investigated.

SARS-CoV-2 antigen detection was performed using the Liaison SARS-CoV-2 Ag assay, which targets the nucleocapsid antigen. NPS samples were inactivated by adding 1,000 μL of fresh VTM into a tube containing 1,000 μL of Liaison SARS-CoV-2 sample inactivation buffer. The tubes were kept at room temperature for 120 min before testing. Samples were then stored at 4°C (5 days maximum) until the analysis. The quantification of SARS-CoV-2 Ag was determined using the Liaison SARS-CoV-2 Ag assay (DiaSorin, Saluggia, Italy) chemiluminescent immunoassay (CLIA) technology on the Liaison XL platform according to the manufacturer's instructions. SARS-CoV-2 concentrations are expressed as 50% tissue culture infectious doses per milliliter (TCID₅₀/ml). The Liaison XL instrument directly calculates SARS-CoV-2 viral concentrations from 22 up to 10^5 TCID₅₀/ml. The samples with ≥ 200 TCID₅₀/ml values are considered positive, results < 200 TCID₅₀/ml are considered negative.

Detection of SARS-CoV-2 in nasopharyngeal samples was performed by the RT-PCR method with DS Coronex COVID-19 Multiplex RT-PCR Diagnosis Kit (DS Bio and Nano Technology, Ankara, Turkey) according to the manufacturer's instructions. This RT-PCR assay targets SARS-CoV-2-specific 'Orf1ab', 'N', and human 'RNaseP (Ribonuclease P)' genes. RNaseP gene was used as internal control. Thermal cycling was performed on a CFX96 Touch™ real-time RT-PCR instrument (Bio-Rad, Italy). The cycle threshold (Ct) values ≤ 33 were defined as positive, and > 33 as negative.

For the second SARS-CoV-2 nucleic acid detection, DIAGNOVITAL® HS SARS-CoV-2 Real-Time PCR Kit targeting N and Orf1ab genes (A1 Life Sciences, Istanbul, Turkey) was used for samples with discrepant result. Seven different mutations (HV6970, Q493R, K417N, K417T, T547K, L452R, P681H) detection kits (DIAGNOVITAL SARS-CoV-2, A1 Life Sciences, Istanbul, Turkey) were used for the analysis of Alpha, Beta, Gamma, Delta, and Omicron variants.

The study was approved by the local ethics committee (approval number: HNEAH-KAEK 2022/KK/18). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Statistical analyses

The sensitivity and specificity of the antigen test were evaluated considering the RT-PCR as the gold standard. Categorical data were presented in numbers and percentages and continuous data were presented as mean, median and



range. The coefficient of determination R^2 (Spearman rho coefficient) was used to calculate the overall correlation between the methods. The level of agreement between the two assays was evaluated by Cohen's kappa coefficient. $P < 0.05$ was considered statistically significant. SPSS software version 25 (IBM, Chicago, IL, USA) was used for statistical analysis.

RESULTS

The 373 randomly selected NPS were tested both by molecular and antigenic methods. Of 373 patients, 165 (44.3%) were male, 208 (55.7%) were female, and the mean age was 41.7 (range 13–86). Of the 373 samples included in the study, 124 (33.2%) were found to be RT-PCR positive and 249 (66.8%) RT-PCR negative. The Liaison antigen test revealed 105 positive and 268 negative samples. No false positive results were detected. When RT-PCR was taken as the reference method, the sensitivity of the antigen test was 84.6% (95%CI 77.3%–90%), and the specificity was 100% (95%CI 98.5%–100%). As a result of the concordance analysis between RT-PCR and the Liaison antigen test, it was determined that there was a level of agreement at the level of 0.881 ($P < 0.001$) (Table 1). When the cut-off value, which was determined as the gray zone in some studies, was taken as 100 TCID₅₀/ml, the sensitivity increased to 90.3% and the specificity decreased to 87.9. Ct values of RT-PCR-positive samples were between 14.8–32.3 (median 21.5). Sensitivity was found as 100% for samples with a Ct value < 20 ($n=40$), 100% for 20–24.99 ($n=49$), 55.6% for 25–29.99 ($n=27$), and 12.5% for 30–33 ($n=8$) (Table 2). Ct values of RT-PCR positive but Liaison antigen negative ($n = 19$) samples ranged from 25.7 to 32.3 (median 28.7), all above 25.

Table 1. Concordance analysis between RT-PCR and Liaison Antigen test

Liaison Antigen Test	RT-PCR		Kappa	P
	Negative <i>n</i> (%)	Positive <i>n</i> (%)		
Negative	249 (100)	19 (15.3)	0.881	$<0.001^*$
Positive	0 (0)	105 (84.7)		

* $P < 0.05$

Table 2. Sensitivity of the Liaison antigen test compared to RT-PCR Ct values

Ct	<i>n</i>	Liaison Antigen	
		Positive <i>n</i> (%)	Median (min-max)
<20	40	40 (100)	62[1]210 (2383.3–100000)
20–24.99	49	49 (100)	5224.7 (550.35–100000)
25–29.99	27	15 (55.6)	274.4 (62.8–93902)
30–33	8	1 (12.5)	86.075 (38.88–96676)
Total	124	105 (84.7)	5869.6 (38.88–100000)

When antigen and Ct values were compared, a strong negative correlation between antigen and Ct values was determined ($P < 0.001$; $r = -0.826$) (Fig. 1).

A second RT-PCR method was performed for Liaison antigen negative but RT-PCR positive samples ($n=19$). All but one of the samples were positive. Variant analysis revealed that the remaining 18 samples have the Omicron variant.

DISCUSSION

Automated antigen tests reduce the risk of biohazards in laboratories on the one hand, and on the other hand, the entire analytical and post-analytical processes can be better controlled, user dependent errors are minimized, and more consistent results are obtained in high volumes of samples [10]. Although antigen detection tests are less sensitive than molecular tests, they have advantages such as easier application, less cost and faster results [10, 12].

In this study, the performance of the automated Liaison SARS-CoV-2 antigen test on nasopharyngeal swab samples taken from symptomatic COVID-19 patients admitted to our hospital was evaluated. Compared with the reference method RT-PCR, the sensitivity of the Liaison antigen test was 84.6% (95%CI 77.3%–90%), the specificity was 100% (95%CI 98.5%–100%). These data indicate that Liaison antigen test meets the WHO-recommended criteria for at least 80% sensitivity and 97% specificity.

In studies evaluating the performance of the Liaison antigen test, the specificity of the test was found to be quite high between 99.4% - 100%, while its sensitivity was found to be in a wide range between 31% - 84.8% [1, 5, 9, 10, 13, 15–19]. In a recent meta-analysis, it was stated that the wide range of sensitivity results may be related to the variety of study methods, whether patients are symptomatic, sample types, and disease stage at the time of sample collection [4]. Therefore, both the WHO and the manufacturer have recommended the Liaison antigen test to be applied to symptomatic individuals and to asymptomatic individuals who are close contacts of a person with COVID-19 [6, 8].

The spread of SARS-CoV-2 can be prevented by early detection and isolation of infected individuals. Studies have shown that low cycle threshold (Ct) values (usually ≤ 25) obtained by RT-PCR correlate with high viral load, playing a key role in the spread of the virus [11]. In recent meta-analysis studies, it has been emphasized that there is a clear relationship between the sensitivity of SARS-CoV-2 antigen tests and the Ct values of RT-PCR. It has been reported that the sensitivity of the tests is higher in patients with a Ct value of ≤ 25 [20, 4]. Consistent with these data, some authors [15, 16] found the sensitivity of the Liaison antigen test for samples with a Ct value < 25 to be between 93% and 94.3%, whereas Fernandez-Rivas et al. [10] and Hartard et al. [13] found the sensitivity to be 100%. Similarly, we found that the sensitivity reached 100% in samples with a Ct value of < 25 (Table 2). These data suggest that the Liaison antigen



assay can be a rapid and reliable alternative to RT-PCR-based methods for detecting SARS-CoV-2 in symptomatic individuals with high viral load, in providing effective epidemiological control, as noted by many authors [5, 9, 11, 12, 16, 17]. Besides, repetitive testing strategy or confirmation with RT-PCR should be performed when a negative result was obtained by antigen-based testing in person with a high suspicious SARS-CoV-2 infection, in order to avoid false negativity in individuals with lower viral load ($Ct > 25$).

The relationship between antigen concentration and Ct values was investigated in previous studies and a strong correlation was found [1, 5, 16, 18, 20]. Accordingly, lower Ct values (high viral load) correspond to higher antigen concentrations. Similarly, we also found a strong negative correlation between antigen and Ct values (Fig. 1).

In studies on the Liaison antigen test, the effect of different cut-off values (especially the gray zone between 100–200) on the sensitivity and specificity results other than 200 TCID₅₀/ml was also evaluated. Fernandez-Rivas et al. [10] found that the sensitivity increased from 84% to 93.6%, and the specificity decreased from 100% to 99.3% for samples with $Ct < 30$, when the threshold value was 100 TCID₅₀/ml. According to these results, they stated that the manufacturer's threshold value (200 TCID₅₀/ml) is more suitable for the accuracy of the test. Salvagno et al. [9] showed that the sensitivity increased to 94% for samples with a $Ct < 29.5$ at a similar threshold (106 TCID₅₀/ml). Apart from this, in some recent studies, researchers analyzed the optimal cut-off value of the test using receiver operating characteristic (ROC) analysis and obtained different results such as 22, 57, and 60 TCID₅₀/ml. Using these threshold values, they achieved specificity values ranging from 95.2% to 100%, with sensitivity ranging from 72.1% to 95.8% [13, 15, 16]. When the samples were examined in our study, considering the threshold value of 100 TCID₅₀/ml, it was found that the sensitivity increased from

84.6% to 90.3%, but the specificity decreased from 100% to 87.9%, so that the specificity result did not meet the WHO criteria. Therefore, considering all the Ct values, we think that the cut-off value of 200 TCID₅₀/ml determined by the company, as emphasized by Fernandez-Rivas et al. [10], is more suitable for the accuracy of the test.

Since the first molecular diagnosis of SARS-CoV-2, many variants of the virus have emerged so far. The WHO recently named variant B.1.1.529 (Omicron) as the sixth variant of concern (VOC) [2]. The Omicron variant carries one or more mutations in the NP gene that may impact the sensitivity of antigen detecting tests. Therefore, there is a need to investigate the effect of these newly emerged variants on the performance of automated antigen tests. However, few studies have been published on this subject until now, many of them rapid antigen tests. Although Bayart et al. [3] reported that some point-of-care rapid antigen tests are less sensitive in detecting the Omicron variant in samples with low viral load; some authors stated that antigen tests are still reliable in detecting this variant [21, 22]. On the other hand, it was pointed out that the nucleocapsid protein is a stable protein and that antigen-based tests, which detect this protein are slightly affected by the new variants [4, 12, 19, 22]. Until now, no publication has been found examining the effect of the Omicron variant on the performance of the Liaison antigen test. In the city of Istanbul, where we conducted the study, all cases have the Omicron variant since February 2022 (one month before the start of the study) according to official health authority data [23]. In the variant analysis performed for the samples with discordant results between RT-PCR and antigen test, we found that all samples have the Omicron variant. Here, even if the discordant results are thought to be due to the Omicron variant, the overall test sensitivity (84.6%) to detect this new variant shows that it is still reliable in the current diagnosis of SARS-CoV-2.

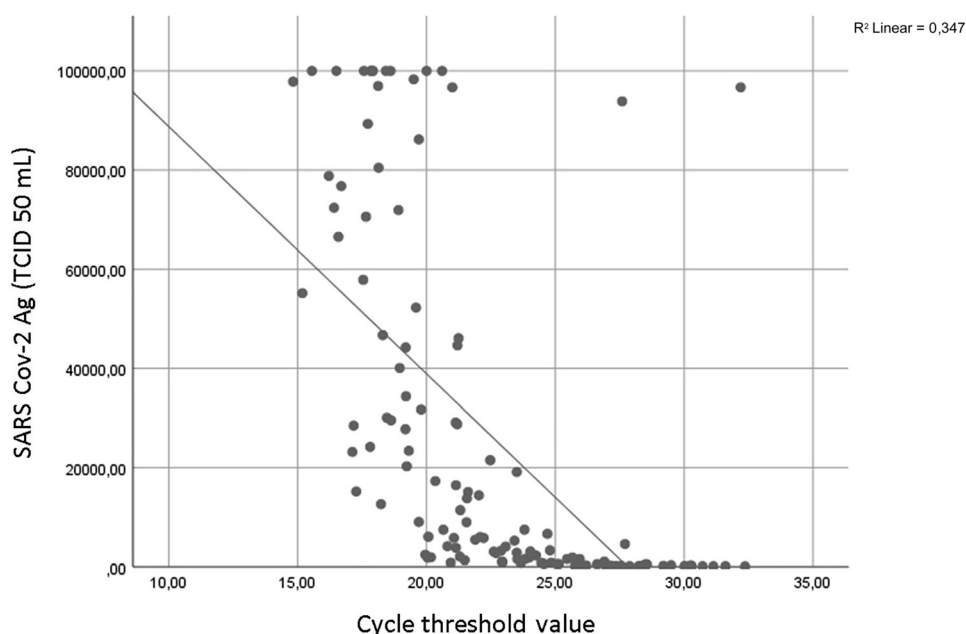


Fig. 1. Correlation analysis between antigen and Ct values

Our study has some limitations. The first was the RT-PCR test was performed based on the patients' statements that they were symptomatic. The second is that the relationship between the accuracy of the test and the duration of the disease could not be investigated since we do not have any data on time between the day of the test and the onset of symptoms.

CONCLUSION

The Liaison antigen test showed excellent sensitivity and specificity in patients with high viral load, and also met the WHO-recommended performance criteria even for samples with the Omicron variant. Therefore, the Liaison antigen test will contribute to controlling the spread of the virus by providing fast and reliable results in the early detection of SARS-CoV-2. However, we should particularly emphasize that molecular methods should be used to prevent false-negative results in symptomatic patients. In conclusion, we consider that the Liaison antigen test can be a reliable and useful alternative in the diagnosis of SARS-CoV-2 infection, especially in laboratories where molecular testing capacity is limited. We also consider that our study will provide data for future studies that will investigate the performance of the automated antigen test in detecting different variants in both European and the non-European countries.

AUTHOR CONTRIBUTIONS

Concept – N.A., N.K., S.A.; Design – N.A., N.K., T.Ş., K.B., S.A.; Supervision – N.A., N.K., T.Ş., S.A.; Data Collection and/or Processing – N.A., N.K., T.Ş.; Analysis and/or Interpretation – N.A., N.K., T.Ş., K.B., S.A.; Literature Search – N.A., N.K., T.Ş.; Writing – N.A., N.K., S.A.; Critical Reviews – N.A., N.K., T.Ş., K.B., S.A.

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