EVALUATE THE SARS- COV-2 DIAGNOSTIC VALUE OF QUANTITATIVE ANTIGEN TEST

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One of the most successful strategies to halt the spread of the COVID-19 pandemic is to quickly screen by testing to identify infected individuals, leading to effectively isolating patients. However, in order to ensure the test's value, the diagnostic value must be thoroughly analyzed before using it. In this research, we used a cross-sectional descriptive study to evaluate the diagnostic efficacy of the Lumipulse G SARS-COV-2 Ag test on salivary and nasopharyngeal swab specimens. The results showed that at the manufacturer's recommended cut-off of 0.67 pg/mL, the sensitivity and specificity were 70.00% and 68.87% on saliva samples, respectively. The test's sensitivity and specificity for nasopharyngeal swab samples were 100% and 95.9%, respectively, at the 1.34 pg/mL cut-off, and it could achieve high sensitivity of 99.4% and specificity of 99.3% at the cut-off value of 17.66 pg/mL. With nasopharyngeal swab samples, we found that the Lumipulse G SARS-COV-2 Ag test achieved the WHO testing standards for the diagnosis and screening of COVID-19. The sensitive diagnostic of this test in nasopharyngeal swab samples is high even the viral load is low and higher than in saliva swab samples.

Keywords: COVID-19, SARS-CoV-2, lumipulse G SARS-COV-2 AG, quantitative antigen test.

I. INTRODUCTION

In December 2019, the World Health Organization (WHO) received a report of pneumonia with an unknown origin in Wuhan, China (Hubei, China). A novel coronavirus, distinct from SARS-CoV and MERS-CoV, was rapidly identified as the causal agent. The International Committee on Virus Taxonomy (ICTV) designated this virus SARS-CoV-2 on February 11, 2020, and the World Health Organization (WHO) named the novel coronavirus pneumonia COVID-19 on that day.[1,2] Immediately after the first reported case, COVID 19 spread rapidly around the world. As of 24 April 2022, according to WHO [3] over 500 million confirmed cases and over six million deaths have been reported globally in 223 countries. In Vietnam, the latest updated data shows that Vietnam is experiencing a fourth wave epidemic, for a total of over 10 million infected cases and 43090 death cases.[4] To prevent the spread and limit the consequences of the COVID-19 pandemic, the deployment of tests to quickly screen and identify infected cases, thereby isolating patients to limit the risk of infection is always considered one of the most effective measures.

In the run up to the epidemic, many tests have been researched and developed to detect people with COVID-19, but as each test has its pros and cons, we must consider making the right choice, with different purposes and circumstances. Real-time RT-PCR tests used to detect viral genetic material have high sensitivity and specificity, but these tests require highly specialized personnel and equipment. Modern equipment, high costs and long waiting times for results make large-scale use in primary

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care wards difficult.[5,6] Another group of tests commonly used in screening for COVID-19 cases are rapid antigen tests. These tests typically use immunochromatographic techniques to detect SARS-CoV-2 virus antigens. In addition to advantages such as ease of implementation, saving time and money, the rapid antigen test also has disadvantages such as sensitivity, low specificity, the results are easily influenced by many factors such as sampling technique, time of disease progression, etc.[7] Recently, a technique with many advantages such as sensitivity, high specificity, fast implementation time on the automated immune system and reasonable cost have been developed and applied in some COVID-19 tests, i.e. chemiluminescent enzyme immunoassay (CLEIA) typically representative of the Lumipulse G SARS-COV-2 Ag test.[8]

The Lumipulse G SARS-COV-2 Ag assay is a test implemented on automated immunoassay systems such as Lumipulse G600II, G1200II. The test helps detect and quantify the SARS-CoV-2 virus Nuclecapsid protein antigen so that it can screen and diagnose patients with COVID-19. Additionally, the correlation between the nucleocapsid protein antigen levels obtained from the Lumipulse G SARS-COV-2 Ag assay and the viral load or cut-off Cycle threshold (Ct) value from the RT-PCR assay in time real has been demonstrated.[9,10] In Vietnam, this test has been included in the list of those approved by the Ministry of Health; however, little attention has been paid to evaluating the diagnostic value of this test. Therefore, we conducted this study with the following objective: to evaluate the value of the Lumipulse G SARS-COV-2 Ag immunoassay in the diagnosis of SARS-COV-2 infection.

II. METHODS AND MATERIALS

1. Subjects

Research subjects

Salivary and nasopharyngeal swab samples from participants confirmed infected or non-infected SARS-CoV-2 by realtime RT-PCR.

2. Method

Research methods

A cross-sectional descriptive study.

Research period

From March 2021 to June 2022.

Research location

Laboratory, Hanoi Medical University Hospital.

Sample size

To evaluate the sensitivity and specificity of the test in the diagnosis and screening of a clinical disease, with the prevalence of COVID-19 disease at the time of the study being p = 0.113%[11] apply. Sample size formula to estimate specificity for the study:

$$n = Z^2_{\alpha} \times \frac{Sp \times (1 - Sp)}{w^2 \times (1 - p)}$$

We estimated a minimum sample size of 30 for the infected and non-infected groups. Therefore, we selected 102 saliva samples, of which 32 samples were negative and 70 samples were positive. 301 nasopharyngeal swabs, 156 negative and 145 positive.

Research flow-work

Samples from participants including nasopharyngeal and salivary samples were collected by trained healthcare staffs. For saliva samples, study participants were instructed not to eat, drink, or rinse their mouths 30 minutes before sampling. Both types of samples are transported and stored at 2-8^{o}C within1 month.

RNA from nasopharyngeal specimens from study participants were extracted by the QIAamp Viral RNA Mini Kit (Qiagen). We also amplify and detect patients in real-time RT-PCR reactions using the Lightbix® SarbecoV

E-Gene Plus EAV control kit (Roche) according to the SARS-CoV-2 viral RNA Charite Berlin Protocol of WHO.[12] This kit uses a positive control sample and a control sample for RNA extraction, and the SARS-CoV-2 virus E gene is the target gene for the PCR reaction. According to the manufacturer's report, the detection limit was 5.2 copies/response. Samples with standard curve and cycle limit (Ct) ≤ 36 were considered positive according to the manufacturer's instructions. All experiments were conducted on the same Realtime RT-PCR test system.[13]

Simultaneously, samples of nasopharyngeal secretions or saliva of the study subjects will be tested for Lumipulse G SARS-COV-2 Ag. First, the patient sample was processed according to the manufacturer's instructions and then added to the antibody coated bead solution. The Nuclecapsid protein antigen of the SARS-CoV-2 virus in the patient sample will specifically bind to the first monoclonal antibody bound to the particles and form an antigen-antibody complex. After washing, a second monoclonal antibody specific to the SARS-CoV-2 protein Nuclecapsid protein antigen was introduced into the reaction. In particular, this second monoclonal antibody is further bound to the enzyme ALP. After subsequent washing, the substrate containing AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt) was added to the reaction. Dephosphorylation is catalyzed by the enzyme ALP. The dephosphorylation process will emit an optical signal at a maximum wavelength of 477 nm. The optical signal is processed and the results of the quantitative determination of the Nuclecapsid protein antigen of the SARS-CoV-2 virus present in the patient sample are calculated.[14] The samples will be analyzed at the manufacturer's recommended cut-off threshold (samples of nasopharyngeal secretions: 1.34 pg/mL, samples of saliva: 0.67pg/mL) and at the ideal cut-off point based on the Youden index.[14]

3. Data analysis

- Statistical analysis: data was entered using Microsoft Excel 2016 software and analyzed using STATA 16.0 software.

The sensitivity and specificity were calculated according to the formula:

$$Sensitivity = \frac{TP}{TP + FN} \times 100\%$$

$$Sencificity = \frac{Sp \times (1 - Sp)}{w^2 \times (1 - p)} \times 100\%$$

In which:

TP – true positive

FP – false positive

TN – true negative

FN – false negative

- ROC curve: Each point on the ROC curve is the coordinate corresponding to the true positive rate (Sensitivity) on the vertical axis and the false positive rate (1 - Specificity) on the horizontal axis. The accuracy of the diagnostic test is measured by the area under the ROC curve (AUC).

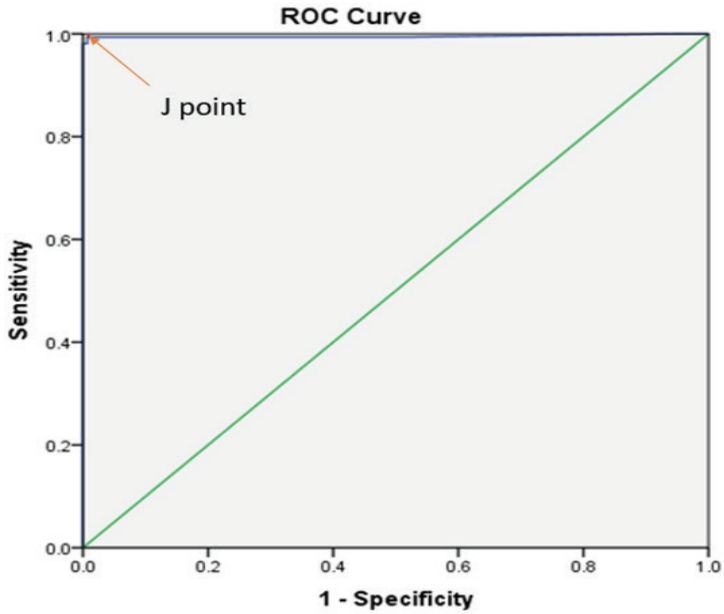
- Determine the optimal cut-off threshold calculated based on the Youden index-J.

4. Research ethics

All participants were explained and consented to the study. Information collected is secure and private. The results are used for research purposes only and do not change the clinical diagnosis of the patient. The study did not delay or change the patient's diagnosis and treatment. If the Lumipulse G SARS-COV-2 Ag test results are positive but the realtime RT-PCR results are negative, the results will be communicated to the clinician for appropriate solutions.

III. RESULTS

1. Diagnostic value of Lumipulse G SARS-COV-2 Ag test on nasopharyngeal swab specimens.



Diagonal segments are produced by ties

Figure 1. ROC curve of Lumipulse G SARS-COV-2 Ag assay on nasopharyngeal swab specimens

On nasopharyngeal swab samples, the area under the ROC curve of the Lumipulse G SARS-COV-2 Ag assay was 0.995 or 99.5% (p < 0.005). Based on the Youden index (Youden index-J), the optimal cut-off threshold was determined to be 17.66 pg/ml.

Table 1. Diagnostic value of Lumipulse G SARS-COV-2 Ag test on nasopharyngeal swab specimens

Group		Diagnosis	
		Negative	Positive
Total		**145**	**156**
Cut-off 17.66 pg/mL	**Negative**	144	1
	Positive	1	155
	Sensitivity	99.4%	
	Specificity	99.43%	
Cut-off 1.34 pg/mL	**Negative**	139	0
	Positive	6	156
	Sensitivity	100%	
	Specificity	95.9%	

On nasopharyngeal swab samples, a total of 301 people were tested for Lumipulse G SARS-COV-2 Ag, with 156 subjects proven to have COVID-19 accounting for 51.82%. The test's sensitivity and specificity were 100% and 95.9%, respectively, at the manufacturer's recommended cut-off threshold of 1.34 pg/mL. The test's sensitivity and specificity were 99.4% and 99.43%, respectively, at the ideal cut-off point of 17.66 pg/ml based on the Youden index.

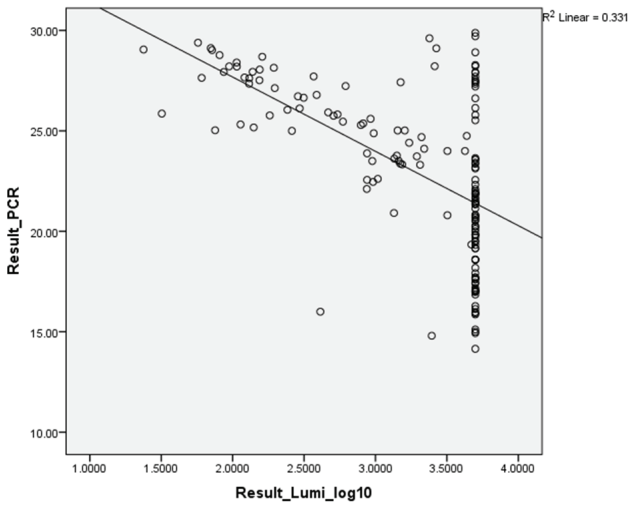
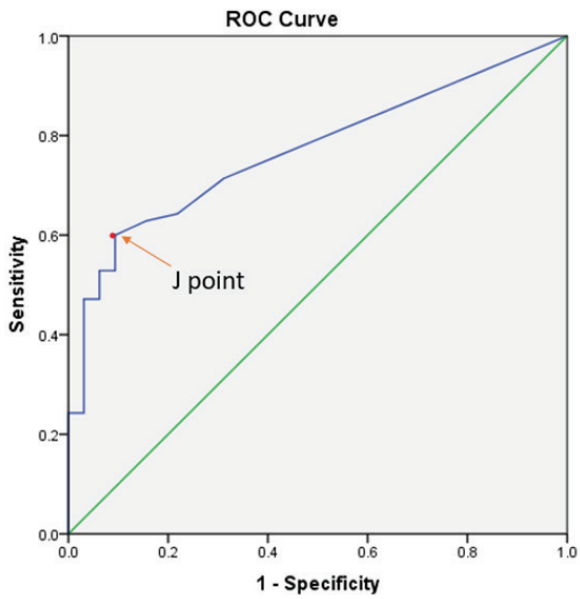


Figure 2. Correlation between Nuclecapsid protein antigen concentration and cut-off period value (Ct)

There is a relationship between Nuclecapsid protein antigen concentration and threshold period value (Ct) with Spearman's correlation coefficient ρ = -0.594 (p < 0.0001).

2. Diagnostic value of Lumipulse G SARS-COV-2 Ag test on saliva specimens.



Diagonal segments are produced by ties

Figure 3. ROC curve of Lumipulse G SARS-COV-2 Ag Assay on saliva samples

With saliva specimens, the area under the ROC curve of the Lumipulse G SARS-COV-2 Ag assay was 0.770 or 77.0% (p < 0.005). Based on the Youden index (Youden index - J), the optimal cut-off concentration was determined to be 4.50 pg/ml.

Table 2. Diagnostic value of Lumipulse G SARS-COV-2 Ag test on salivary swab specimens

Group		Diagnosis	
		Negative	Positive
Total		**32**	**70**
Cut-off 4.50 pg/mL	**Negative**	29	28
	Positive	3	42
	Sensitivity	60%	
	Specificity	90.6%	
Cut-off 0.67 pg/mL	**Negative**	22	21
	Positive	10	49
	Sensitivity	70.00%	
	Specificity	68.87%	

A total of 102 subjects were collected saliva samples, of which 70 were confirmed to have COVID-19 accounting for 68.62%. At the manufacturer's recommended cut-off concentration of 0.67 pg/mL, the sensitivity and specificity of the Lumipulse G SARS-COV-2 Ag assay were 70% and 68.87%, respectively. At the optimal cut-off threshold based on the Youden index of 4.50 pg/ml, the sensitivity and specificity of the test were 60% and 90.6% respectively.

Table 3. The sensitivity of the Lumipulse G SARS-COV-2 Ag test on salivary specimens at the differrent threshold period value (Ct)

Lumipulse G SARS-CoV-2	rRT-PCR Ct			
	1st quartile Ct ≤ 25,68	**2nd quartile 25,68<Ct ≤ 27,92**	**3rd quartile 27,92<Ct≤31,00**	**4th quartile Ct > 31,00**
Sensitivity (cut-off 0.67 pg/mL)	94.11% (16/17)	83.33% (15/18)	72.22% (13/18)	29.41% (5/17)

According to the threshold period value (Ct), the sensitivity of the Lumipulse G SARS-COV-2 Ag test on salivary specimens at the 1st interquartile range (Ct ≤ 26.29) is 94.11%, 2nd (25.68<Ct ≤ 27.92) is 83.33%, 3rd (27.92<Ct ≤31.00) is 72.22%, and 4th (Ct > 31.00) is 29.41%.

IV. DISSCUSSION

The World Health Organization (WHO) and the Ministry of Health have established that tests for detecting SARS-CoV-2 antigens plays an important role in prevention and control to COVID-19 disease. According to the

standards of the Ministry of Health, antigen detection tests need to achieve sensitivity 80% and specificity 97% compared to Realtime RT-PCR test (as recommended by WHO) by the NIHE (National Institute of Hygiene and Epidemiology) or Pasteur Institute evaluated before use.[15] According to our findings, the sensitivity and specificity of the Lumipulse G SARS-COV-2 Ag test on nasopharyngeal swab samples were 100% and 95.9%, respectively, at the 1.34 pg/mL cut-off the same as announced by the manufacturer. This result is comparable to the research of Hirotu (2020) and Alessio Gili (2019), both of them have sensitivity and specificity of the test on nasopharyngeal swabs are greater than 95%. [9,16] The Lumipulse G SARS-COV-2 Ag assay is based on the principle of identifying the SARS-CoV-2 virus's Nucleocapsid protein antigen and foreign antigens has structure likely to the target protein antigen may exist in the patient samples. As a result, with a low cut-off, these antigens may decrease the test's specificity. According to the manufacturer recommended that the result range of 1.34 to 20 pg/mL be chosen as the "gray space" and the further evaluations be performed on patient samples in this interval.[14] Based on this recommendation, we adjusted the cycle of threshold cut-off using the Youden index and discovered that the test's sensitivity and specificity were up to 99.4% and 99.3%, respectively, at the cut-off value 17.66 pg/mL. These findings emphasize the utility of the Lumipulse G SARS-COV-2 Ag test on nasopharyngeal swab specimens in the diagnosis of COVID-19.

Besides nasopharyngeal swab specimens, the Lumipulse G SARS-COV-2 Ag test can also be performed on saliva specimens. The results of this study showed that the sensitivity and specificity of the Lumipulse G SARS-COV-2 Ag test on saliva samples were 70% and 67.78%, respectively. This result is lower than the manufacturer's announcement (sensitivity 76.4% and specificity 99.2%) as well as the results of the study by Daniela Basso et al (2020) with sensitivity and specificity were 72% and 92%. [10,17] Many studies show that the sensitivity and specificity of the antigen test depend on many factors, in particular on the specimen collection and storage technique.[10,16,17] In our study, donors were instructed not to eat, drink, or use mouthwash chemical for 30 minutes before collecting the sample. However, it is difficult to control and completely removed inhibitor factor in the saliva collection process. In many cases, the sample is mixed with sputum and food debris, which affected to the test results.[16] These limitations may be the mainly reason which make saliva samples not commonly used in the COVID-19 diagnostic test.

Many studies around the world have shown that the sensitivity of some antigen tests has good value on patient samples collected from patients with high viral load, corresponding to the stage strong virus transmission.[8,16] According to the guidelines of the Ministry of Health, the transmission period of a COVID-19 case is counted from 2 days before onset until the RT-PCR test result is negative or positive with Ct value ≥ 30. Therefore, the analysis model use combination of the antigen detection test and the viral load through the cycle of threshold (Ct) value of the RT-PCR test is very important. The sensitive of Rapid Antigens Testing (RAT) decrease in the samples with Ct value above 25 and strongly drop when Ct value above 30.[18] Similar these results, in our study, the sensitivity of the Lumipulse G SARS-COV-2 Ag test for saliva samples at the 1st interquartile range (Ct ≤ 26.29) was 94,11%, 2nd interquartile

range (25.68<Ct ≤ 27.92) was 83,33%, 3rd interquartile range (27.92<Ct ≤31.00) was 72.22%, and 4th interquartile range (Ct > 31,00) was only 29,41%. For nasopharyngeal swab samples, we found that the sensitivity is very high in all group of threshold period value (Ct). Furthermore, we also found a relationship between the Nucleocapsid protein antigen levels obtained from the Lumipulse G SARS-COV-2 Ag test and the cycle of threshold value (Ct) of Realtime-RT-PCR assay with Spearman`s correlation coefficient ρ = -0.594 (p < 0.0001). This result is similar to the study of Menchinelli et al with a correlation coefficient from -0.72.[10] These findings emphasize the utility of the Lumipulse G SARS-COV-2 Ag test on nasopharyngeal swab specimens in the diagnosis of COVID-19.

Recently, new variants of SARS-CoV-2 virus such as Delta, Omicron... have appeared. With the principle based on detecting the antigen component is the viral protein, mutations in new strains of the SARS-CoV-2 virus can affect the diagnostic value of antigen tests in general and Lumipulse G SARS-COV-2 Ag test in particular. In addition, a measure to reduce costs and increase the efficiency of testing and screening for COVID-19 cases is also of great concern, which is sample pooling. However, this measure is not currently used in tests for the detection of SARS-COV-2 antigens due to concerns about decreased sensitivity and specificity when pooling is applied. With very high sensitivity on nasopharyngeal swab samples, the Lumipulse G SARS-COV-2 Ag test has great potential for implementation on pooled samples. However, in this study, we have not been able to evaluate the diagnostic value of the Lumipulse G SARS-COV-2 Ag test for each variant of the SARS-CoV-2 virus as well as performed on samples. therefore, further studies are needed to address these issues.

V. CONCLUSIONS

The sensitivity and specificity of the Lumipulse G SARS-COV-2 Ag test on nasopharyngeal swab samples met the Ministry of Health recommendations on requirements for the test in the diagnosis of COVID-19. The sensitive diagnostic of this test is high even the viral load is low. For saliva samples, it is necessary to consider in the selection of test subjects and focus on sampling to ensure the validity of the test.

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