SENSITIVITY OF SARS-CoV-2 ANTIGEN RAPID DIAGNOSTIC TEST (Ag-RDT) BASED ON CYCLE THRESHOLD (Ct) VALUE

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ABSTRACT

Rapid diagnostic test is an examination used to screen for COVID-19 disease. The analysis principle is immunochromatography, which detects SARS-CoV-2 antigens found in clinical specimens from the respiratory tract (nasopharynx and oropharynx). Evidence shows that using antigen rapid diagnostic tests (Ag-RDT) often gives false negative results; this is identified after a confirmation test using the real-time PCR gold standard. Despite being the gold standard, not all clinical laboratories have those facilities. Therefore, Ag-RDT remains an option. This study aims to determine the sensitivity of Ag-RDT based on the cycle threshold (Ct) value. This research is a diagnostic test with output in the form of sensitivity values. We used 200 clinical specimens at the Laboratorium Kesehatan Daerah (Labkesda) Depok West Java in January-June 2022. The results of the examination are arranged in cross-tabulations to be able to calculate the diagnostic value. The specificity of the Ag-RDT test was 98%, while the sensitivity was calculated based on the Ct value groups ≤25, 26-30, 31-35, and ≥35, which were 85.7%, 56%, 25.9%, and 15%, respectively. Ag-RDT has good specificity, but its sensitivity depends on the Ct value, which describes the viral load in the specimen. Nevertheless, Ag-RDT can still be used as a screening tool for COVID-19 disease, especially in areas with limited access to real-time PCR examination. However, a negative Ag-RDT result does not guarantee that not infected by SARS-CoV-2. Therefore, self-awareness is still needed to apply 6M to break the chain of SARS-CoV-2 transmission.

Keywords*: COVID-19; rapid diagnostic test; specificity; Ct value; Ag-RDT*

INTRODUCTION

COVID-19 has been designated a pandemic since March 2020 by the WHO (World Health Organization) (Cucinotta and Vanelli, 2020). The number of COVID-19 cases until April 2022 nationally amounted to 6,046,467 confirmed cases, with details of 5,882,062 recovered cases (97.3%), 156,240 death cases (2.6%), and 8,165 active cases (0.1%). The SARS-CoV-2 pandemic has focused attention on the important role of diagnostic technology in infectious disease control (Younes *et al.*, 2020). The threat of new variants of SARS-CoV-2 requires a rapid response to prevent sustained transmission. Therefore, strategic steps are needed to accelerate COVID-19 prevention and control by accelerating and increasing testing capacity (Cheng *et al.*, 2020). The current gold standard test is NAAT (Nucleic Acid Amplification Test), using the real-time PCR method (WHO, 2020). However, limited access to these tests has led to low testing capacity for screening purposes, close contact tracing, and diagnosis enforcement. Rapid test diagnostic antigen (Ag-RDT) is an alternative testing method in this condition.

Ag-RDT is used in early screening for COVID-19 disease due to its lower price, ease to use, and faster results. Ag-RDT is also used in close contact tracing, which is confirmed with real-time PCR if the result is positive. Real-time PCR become the second line in establishing the diagnosis of COVID-19 disease because of its limited access, especially in remote areas. Overall processes in real-time PCR take much time to declare results, especially with too many specimens queued daily. Although as the gold standard, a real-time PCR-negative result does not completely rule out disease, especially if there are signs and symptoms of disease (Zitek, 2020). So a clinical review needs to be done for further verification.

Currently, Ag-RDT self-examination is recommended by the Ministry of Health, which, if carried out routinely, is expected to help early detection of SARS-CoV-2 infection to reduce the rate of spread. However, several times false negative results were found, which will undoubtedly hamper the government's efforts to reduce the spread of COVID-19 disease. False negatives occur because Ag-RDT sensitivity is low. In contrast, Ag-RDT usually claims a high level of sensitivity and specificity to detect the presence of SARS-CoV-2 by manufacturers. Therefore, researchers are interested in determining Ag-RDT sensitivity based on cycle threshold (Ct) values.

METHOD

This research is a diagnostic test with output in the form of sensitivity values (Dahlan, 2010). The research was conducted at Unit Pelaksana Teknis Daerah Laboratorium Kesehatan Daerah (UPTD Labkesda) Depok in January-June 2022. This research has obtained ethical approval with number 04/22.06/01889 from the UHAMKA (Universitas Muhammadiyah Prof. Dr. Hamka) ethics committee. The sample in this study amounted to 200 clinical speciments who carried out Ag-RDT and real-time PCR examinations simultaneously on the same day, consisting of 100 respondents with positive and 100 with negative real-time PCR results. Samples are taken by consecutive sampling. All data is cross-tabulated by 2x2 tables, then overall specificity, sensitivity, positive presumptive, and negative presumptive values are calculated. Furthermore, samples with positive real-time PCR results were classified again based on cycle threshold (Ct) values. There are four groups of Ct values ≤25, 26-30, 31-35, and >35, then calculated the sensitivity in each group.

RESULTS AND DISCUSSION

The total number of samples in this study amounted to 200 clinical speciments; Table 1 shows the characteristics of samples by gender and age group.

Table 1. Characteristics of samples based on gender and age group

Characteristic	Frequency (n)	Percentage (%)
Gender		
Male	98	49.0
Female	102	51.0
Ages		
≤16 y.o	47	23.5
17-59 y.o	135	67.5
≥60 y.o	18	9.0

Based on Table 1, it can be seen that the number of female and male samples is almost the same, 51% and 49%, respectively, indicating that male and female have the same risk of exposure to COVID-19 disease. The most age range is found in the age group of 17-59 years, as many as 135 people (67.5%), because this age is a productive age that triggers humans to have great mobility compared to the other two age groups.

Below is a cross-tabulation of Ag-RDT and real-time PCR test results, compiled to calculate sensitivity, specificity, positive presumptive, and negative presumptive values.

Table 2. Cross-tabulation between Ag-RDT and real-time PCR results

	real-time PCR results		
	positive	negative	total
Ag-RDT results			
positive	48	2	50
negative	52	98	150
total	100	100	200

Based on Table 2, 48 clinical speciments showed positive results in both methods (true positive), and 98 indicated negatives (true negative). In contrast, 54 test data showed discordant (Ag-RDT and real-time PCR results were different), consisting of 52 false negatives and 2 false positives. Based on these data, the calculation of sensitivity 48%, specificity 98%, positive presumptive value 96%, and negative presumptive value 68% were obtained.

48% sensitivity means that out of 100 COVID-19 patients examined using Ag-RDT, only 48 people will be identified as having COVID-19. This low sensitivity value will lead to false negative results. It relates to negative presumptive values of 65%; if the results of the Ag-RDT examination are negative, the probability of actually not having COVID-19 is only 65%. Previous research found that overall Ag-RDT sensitivity for real time PCR-positive samples ranged from 24.3% to 50% (Kohmer *et al.*, 2021). This low sensitivity is a big problem because Ag-RDT tools that already have a distribution permit should have sensitivity as recommended by WHO.

The table below shows the results of the Ag-RDT examination cross-tabulated with Ct values from 100 samples who had positive real-time PCR test results.

Table 3. Grouping of Ag-RDT results based on Ct value

	Ct *value*				Total
	≤ 25	26-30	31-35	>35	
Ag-RDT positive	24	14	7	3	48
Ag-RDT negative	4	11	20	17	52
Total	28	25	27	20	100

The data in Table 3 calculates the Ag-RDT sensitivity value based on the Ct value from the examination using real-time PCR. The calculation results found Ag-RDT

sensitivity in specimens with Ct values of ≤25; 26-30; 31-35; and >35 were 85.7%; 56.0%; 25.9% and 15.0%, respectively.

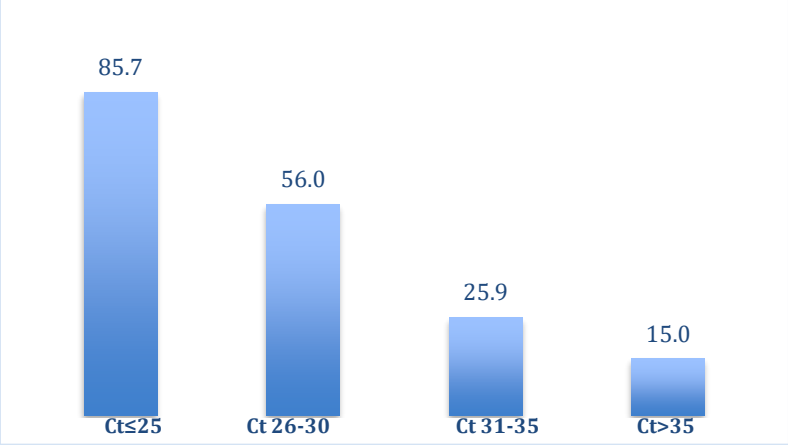

Figure 1. Ag-RDT sensitivity based on Ct value

Figure 1 shows that the sensitivity of SARS-CoV-2 Ag-RDT can vary depending on the viral load present in the specimen. Viral load can be described through the Ct value. The lower the Ct value, the more viral load on the sample, and vice versa. Research by Karon *et al.*, (2021) proves that viruses RNA copy number determines the sensitivity level of Ag-RDT tools in detecting the presence of SARS-CoV-2. Ag-RDT sensitivity value for specimens with high viral load is very good, at 85.7%, following WHO criteria of ≥80% sensitivity (Berger *et al.*, 2021).

98% specificity means that out of 100 healthy people examined using Ag-RDT, 98 will be identified as healthy. A presumptive positive value of 96% means that if the Ag-RDT method test results are positive, then the probability of really suffering from COVID-19 is 96%. These calculations show exceptionally few false positives, so someone with a positive Ag-RDT test result can be 96% confirmed to have COVID-19 disease.

The sensitivity and specificity values claimed by Ag-RDT manufacturers are usually close to 100%, but there is a significant gap in practice. It happens if Ag-RDT is applied to patients who are still in the virus incubation period, so the viral load has not reached the device limit of detection (LoD). Samples with a viral load of more than 6 $\log_{10}$ RNA copies/mL, Ag-RDT sensitifity was between 81.8% and 100% (Kohmer *et al.*, 2021). Previous research by Kittel *et al.*, (2020) proves that Ag-RDT from

several different vendors can have different sensitivity values, but in the study did not calculate sensitivity values based on Ct values.

The real-time PCR method is used as the gold standard because it detects the genetic material (RNA) of the virus, wherein in the PCR process, there is an exponential amplification of the viral RNA target until millions of copies. Therefore, detecting such RNA using real-time PCR is possible despite the low viral load. False negatives can also occur due to the specimen collection process that does not comply with standard operating procedures (SOPs). False negative results occur due to improper timing of specimen collection and shortcoming in sampling proficiency (Shrestha and Pokharel, 2020).

However, the Ag-RDT examination method has effectiveness in terms of speed of results, ease of use, and affordable price to use as an alternative examination method, and this is very necessary, especially in areas where access to real-time PCR examination is limited. The possibility of false negative results makes it essential to carry out periodic or routine checks. Ultimately, it is crucial to guard against SARS-CoV-2 infection. Each individual should apply 6M as one of the measures to prevent transmission. 6M stands for washing hands (mencuci tangan), using masks (memakai masker), keeping distance (menjaga jarak), avoiding crowds (menghindari keramaian), avoiding eating together (menghindari makan bersama), and reducing mobility (mengurangi mobilitas).

CONCLUSION

Ag-RDT's sensitivity, specificity, and positive presumptive and negative presumptive values were 48%, 98%, 96%, and 68%, respectively. Low sensitivity and negative presumptive values make Ag-RDT tools often give false negative results. Therefore, a negative Ag-RDT result does not guarantee to be free of SARS-CoV-2 infection. This study concluded that the sensitivity of the Ag-RDT test can vary depending on the SARS-CoV-2 viral load depicted in the Ct value of the real-time PCR test results. Ag-RDT sensitivity in specimens with Ct value ≤25; 26-30; 31-35; and >35 were 85.7%; 56.0%; 25.9% and 15.0%, respectively.

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