



ARTIKEL PENELITIAN—RESEARCH ARTICLE

Rapid Diagnostic Test (RDT) Prototype Validation Test for SARS CoV-2 Antigen in Comparison With RT-PCR Assay

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Abstract

Background: The diagnosis of Coronavirus Disease-2019 (COVID-19) is established based on Real-time reverse transcription-polymerase chain reaction (RT-PCR). However, due to the availability, high cost and length of time of RT-PCR, a reliable alternative is needed. Rapid Diagnostic Test (RDT) costs cheaper, near-patient use, and if performed and interpreted correctly can be used in areas without RT-PCR or if immediate diagnosis is needed.

Objective : To evaluate the quality of RDT SARS CoV-2 prototype compared to RT-PCR.

Methods: The patients in this study were patients with RDT antigen and or RT-PCR SARS-CoV-2 examination at Mataram University Hospital who had approved to be research subject. Nasopharyngeal swab sample was used for RDT Antigen SARS-CoV-2 Prototype. Whereas, nasopharyngeal and oropharyngeal swab sampel were used for RT-PCR SARS-CoV-2. The value of sensitivity, specificity and accuracy of RDT prototype were assessed based on the RT-PCR result.

Results: Total of 124 samples were included in this study. Approximately 39 (31.4%) were positive and 85 (68.5%) were negative. The sensitivity, specificity and accuracy of the SARS CoV-2 RDT prototype were 92.31% (79.13 - 98.38 %, 95% CI), 97.65% (91.76 - 99.71%, 95% CI) and 97.63 % (93.16 - 99.52 %, 95% CI), respectively. There were 5 discrepancies in RDT prototype results compare to the RT-PCR result.

Conclusion: The RDT SARS-CoV-2 Antigen prototype showed good sensitivity, specificity and accuracy. Hence, this RDT has the potential to be used for screening or diagnosis, especially in areas with high disease prevalence and low resource setting.

Key words: RDT SARS CoV-2 antigen, RT-PCR, sensitivity, specificity, accuracy

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the virus that causes Coronavirus Disease-2019 (COVID-19). The high death rate due to COVID-19 and widespread across the globe has made the WHO to declare it as a global pandemic. Based on WHO epidemiological data updates, the number of COVID-19 cases as of January 17, 2021 was 4.7 million cases, with a total number of deaths of more than 2 million cases. The total number of COVID-19 cases in Indonesia as of March 3, 2021

was 1.353.834 with a total death rate of 36,721 cases. Based on the data from NTB Health Office, the number of cases in West Nusa Tenggara have reached a total of 9,598 confirmed positive cases with 399 deaths.^{2,3}

The diagnosis of COVID-19 was established based on the results of laboratory tests using the nucleic acid amplification test (NAATs) method, one of which was the real-time reverse transcription-polymerase chain reaction (RT-PCR) which detected the SARS-CoV-2 target gene as the causative virus.^{4,5,6} The availability of RT-PCR in several countries, the high cost of the examination and the time for examination remains a challenge



particularly in low resource settings. Therefore ongoing research continues to develop tests that are reliable but with lower costs and shorter examination times.⁷ Antigen detection-based assays were developed to detect the SARS-CoV-2 protein produced by the viral replication process in respiratory secretions directly. This examination can be carried out both in the laboratory and directly at the patient's side, so it is called a rapid diagnostic test (RDTs).^{4,8} Compared with the NAAT examination of nasal or nasopharyngeal swabs, the sensitivity varies widely between 0-94% and the specificity is quite high, which is more than 97%. Although it has some limitations, Rapid Diagnostic Test Antigen (RDT-Ag) has an important role in patient management, in the decision-making process related to public health and COVID-19 surveillance if performed and interpreted correctly. The performance of RDT-Ag which can be used for the diagnosis of SARS-CoV-2 infection in areas that do not have NAAT or situations that require immediate diagnosis meets a minimum sensitivity value of 80% and a specificity value of 97%.^{4,9}

The high number of COVID-19 cases in West Nusa Tenggara with the opportunity to obtain a large number of local patient sample and the dependence on imported Rapid Diagnostic Test Antigen products which were quite difficult in the early days of the pandemic, initiated a collaboration between the Hepatika Laboratory and the Mataram University Hospital to conduct research to develop Rapid Diagnostic Test for COVID-19 Antigens. To meet the needs of the wider community, it is necessary to assess the quality of the Rapid Diagnostic Test Antigen prototype before it is mass produced, so we plan to conduct a study to evaluate the quality of the Rapid Diagnostic Test Antigen prototype.

METHODS

This research was conducted at Mataram University Hospital and carried out from 1 May to 30 November 2021. The sampling method used for subject of research was convenient sampling based on the order of patient arrival with a plan to examine the Rapid Diagnostic Test Antigen and or RT-PCR SARS-CoV-2 and willingness to be the

research subject. A total of 100 patients was involved in this study.

Each subject was swabbed using a dacron swab. Swab sampling was carried out by health workers in accordance to the universal precautions and procedures recommended from the Ministry of Health. The nasopharyngeal swab sample was used for the RDT of the SARS-CoV-2 antigen prototype. The swab sample would then be inserted into a special tube containing diluent buffer before being dropped on the device then read 15 minutes after dripping. The interpretation of the Rapid Diagnostic Test Antigen results includes negative, positive and invalid results.¹⁰

The RT-PCR examination was preceded by the isolation of RNA from the nasopharyngeal and oropharynx swab samples that had been inserted into the viral transport media tube and was then taken to the Biomolecular Laboratory of Mataram University Hospital. The RNA isolation process used Patho Gene-Spin DNA/RNA Extraction Kit, Intron Biotechnology, South Korea, where the final extraction volume was 30-50 l. RT-PCR reagents using the mBioCoV-19 RT-PCR Kit, Biofarma, Bandung, Indonesia. The examination process begins with reverse transcription for 10 minutes at a temperature of 45°C, then the sample denaturation process and enzyme activation for 2 minutes at a temperature of 95°C and followed by 2 stages of cycling for 45 cycles, the first cycling within 3 seconds at a temperature of 95°C and the second in 20 seconds at 60°C. Interpretation of RT-PCR results using the CDC approach, the amplification was positive if the Ct value was < 40 and the threshold was 0.1.

Categorical data on the results of the Rapid Diagnostic Test Antigen and RT-PCR SARS-CoV-2 was presented in the form of numbers, percentages, and 95% confidence intervals (95%CI). The values of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were analyzed using MedCalc statistical software.^{11,12}



RESULT AND DISCUSSION

There were 124 participants consisting of 26 pediatric patients aged 1 to 19 years, 96 adult patients aged 20 to 63 years and 2 elderly patients aged 63 and 73 years, with total 73 male and 51 female.

The results of RDT of the SARS-CoV-2 antigen prototype obtained positive in 38 samples (30.6%) and negative in 86 samples (69.35%). While the results of RT-PCR SARS-CoV-2 in 124 samples, positive results were obtained in 39 samples (31.4%) and negative results in 85 samples (68.5%). The SARS-CoV-2 gene fragments analyzed were RdRp, Helicase, E and N genes. The results were said to be positive if there were at least two amplified fragments with a Ct value of 40 for mBioCov and Allplex reagents, and 35 for Lili Intron reagents. The comparison of the results of the Rapid Diagnostic Test of the SARS-CoV-2 Antigen prototype and the SARS-CoV-2 RT-PCR can be seen in the table below.

Table 1. The Results of the Rapid Diagnostic Test of the SARS-CoV-2 Antigen prototype and the SARS-CoV-2

	RT-PCR		
	SARS-CoV-2 Positive	SARS-CoV-2 Negative	Total
Rapid Diagnostic Test of the SARS-CoV-2 Antigen prototype			
Positive	36	2	38
Negative	3	83	86
Total	39	85	124

The performance of the SARS-CoV-2 Antigen RDT prototype compared to the results of the SARS-CoV-2 RT-PCR, showed a sensitivity value of 92.31% (79.13 - 98.38%, 95% CI), specificity 97.65 % (91.76 - 99.71 %, 95% CI). Disease prevalence data in the city of Mataram according to the time of sampling was 0.38%. Therefore, positive predictive value was 13.02% (3.65 - 37.12%, 95%

CI), negative predictive value was 99.97% (99, 91 - 99.99 %, 95% CI), with an accuracy of 97.63 % (93.16 - 99.52 %, 95% CI).

There were several unexpected examination results, 3 samples of the SARS-CoV-2 Antigen Rapid Diagnostic Test prototype had negative results with positive results of the SARS-CoV-2 RT-PCR and 2 samples of the SARS-CoV-2 Antigen RDT prototype had a positive result with a negative result of the SARS-CoV-2 RT-PCR examination (Table 2).

Table 2. Discrepancy of the Rapid Diagnostic Test of the SARS-CoV-2 Antigen prototype and the SARS-CoV-2

Sample number	Sex	Age (year)	RT-PCR Result		Ct
			RDT	RT-PCR	
44	Female	31	Negative	Positive	22.75
50	Female	11	Negative	Positive	23.59
52	Female	8	Negative	Positive	27.83
74	Male	28	Positive	Negative	
80	Female	23	Positive	Negative	

In this study, it was found that the prototype RDT of the SARS-CoV-2 antigen met the WHO criteria, which had a sensitivity value of 80% and a specificity value of 97% compared to the results of the NAAT examination, which can be used to diagnose SARS-CoV-2 infection. With a note that there were differences in the results of the RDT of the SARS-CoV-2 Antigen prototype in the sample compared to the results of the RT-PCR, there were 3 samples with low CT values with negative results and 2 samples with positive results which read negative by RT-PCR. False negative results could be caused by an inappropriate sampling time. Viral replication in the pharynx is highest at the beginning of the disease and decreases over time, but it can also be caused by variations in sampling. This is in accordance with a study conducted by Porte et al in 2020, which evaluated the Bioeasy SARS-CoV-2 antigen test, from 82 positive samples (127 samples in total) 5 false negative results were obtained with a Ct value



> 26. The samples was appropriate, namely in the early stages of the disease, but some sample CT values with low viral concentrations were obtained which may be due to the sampling technique or the inaccuracy of the data regarding the onset of the disease. In addition, it should be considered that the Ct value that describes the concentration of target RNA varies greatly between the reagents used for RT-PCR examination and also does not fully assess the viral load quantitatively.^{4,13} False positive results can be caused by the presence of thick mucus in the respiratory tract. This was similar with the study conducted by Chaimayo et al in 2020, where the results obtained from 60 positive samples (a total of 454 samples) which were tested for RDT with the SD Biosensor kit, there were 5 samples with false positive results. Several factors that could affect the performance of the SARS-CoV-2 Antigen Rapid Diagnostic Test include: (1) Patient factors: immune status and examination time from disease onset, (2) Sample type (upper or lower respiratory tract), sample quality and handling, storage and dilution of viral transport media, (3) viral factors: concentration and duration of viral antigen shedding, structural variations of target antigens, cross-reaction with other viruses, (3) specific target proteins: some antigens are produced at higher concentrations than others, examples of nucleocapsins versus spike proteins, (4) Product design and quality: lack of antibody quantity or affinity for target antigens, poor packaging and exposure to heat and moisture during transport or storage, incorrect or inappropriate instructions, and (5) Lack of training or competence of inspection operators.^{4,14}

The results of low PPV and high NPV correspond to the prevalence of the disease. In general, the higher the prevalence rate in the population being examined, the more likely it is that someone tested positive for COVID-19. With a low prevalence of disease at the time of the study, namely 0.38%, a low PPV value was obtained. Hypothetically, at a 10% COVID-19 prevalence rate, PPV and NPV values was 81.34 % (52.49-94.50%, 95% CI) and 99.13% (97.47-99.71%)., 95% CI), hence the prototype Rapid Diagnostic Test

Antigen SARS-CoV-2 is acceptable for use in areas with a high prevalence of COVID-19. The advantages of the RDT antigen test for screening COVID-19 are that it is easy to use and the results are fast in areas with high NPV, but the disadvantage is that the PPV value is low in areas with low prevalence, so that NAAT examination, one of which is RT-PCR, is more sensitive and specific to serve as a reference for the diagnosis of COVID-19.^{15,16} In this study, the results obtained accuracy of 97.63% (93.16 - 99.52%, 95% CI), the results are satisfactory as the accuracy value is also strongly influenced by the prevalence of the disease. With the same sensitivity and specificity, the diagnostic accuracy of the examination can increase with a decrease in disease prevalence.¹⁷

CONCLUSION AND SUGESSTIONS

The prototype RDT for the SARS-CoV-2 antigen showed a sensitivity of 92.31%, specificity of 97.65% compared to the results of the SARS-CoV-2 RT-PCR examination that met the WHO criteria. The prototype Rapid Diagnostic Test Antigen SARS-CoV-2 showed an accuracy of 97.63% compared to the results of the RT-PCR examination of SARS-CoV-2. The rapid and easy use of the RDT Antigen SARS-CoV-2 prototype has the potential to be used for screening or diagnosing COVID-19, especially in areas with a high prevalence of the disease. Observations need to be made by paying attention to the time of sampling, especially with the results of a positive SARS-CoV-2 RT-PCR examination. Further research needs to be done by classifying the results of RT-PCR SARS-CoV-2 based on the Ct value. Further research is needed in areas with a high prevalence of COVID-19.

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