

# MALARIA DIAGNOSTIC TESTING WITH RAPID DIAGNOSTIC TEST (RDT) METHOD IN MALARIA SUSPECTS

## BUDI SETIAWAN

Department of Medical Laboratory Technology, Poltkkes Kemenkes Yogyakarta, Indonesia.  
Center of Excellent for Public Health Technology and Innovation, Poltekkes Kemenkes Yogyakarta, Indonesia. Email: budi.setiawan@poltekkesjogja.ac.id

## HADROTUL MA'WA

Kokap 2 Public Health Center, Kulon Progo, Yogyakarta, Indonesia.

## MUJI RAHAYU

Department of Medical Laboratory Technology, Poltkkes Kemenkes Yogyakarta, Indonesia.

## ANIK NURYATI

Department of Medical Laboratory Technology, Poltkkes Kemenkes Yogyakarta, Indonesia.

### Abstract

Background: Malaria is still a global health problem that can cause death, especially in high-risk groups, namely infants, toddlers and pregnant women. One of the strategies in achieving malaria elimination is the early discovery of malaria cases quickly so that transmission can be stopped. The use of malaria Rapid Diagnostic Test (RDT) in areas with minimal trained human resources is still needed so it is necessary to evaluate the accuracy of the diagnostic results of the RDT examination. Aims: to determine the diagnostic value of the Rapid diagnostic test (RDT) including sensitivity, specificity, positive predictive value, negative predictive value, accuracy and relationship to microscopic examination in malaria suspects. Method: This study is an observational analytic study with a cross-sectional design. The data obtained came from all microscopic and RDT examination results at one public health centre (puskesmas) in Kokap Regency in the period January - December 2022 with a total of 93 samples. The analysis used was chi-square analysis along with sensitivity, specificity, negative predictive value, and positive predictive value tests. Results: from this study obtained a diagnostic test value of sensitivity 100% specificity 98.11%, positive predictive value 98%, negative predictive value 100% accuracy value of 99% and from Chi square non-parametric statistical test obtained p-value 0.000 which means there is a relationship between the results of Rapid diagnostic test and microscopic examination. Conclusion: Malaria RDTs have good diagnostic test results for malaria testing in malaria suspects.

**Keywords:** Malaria, Diagnostic test, Rapid diagnostic test (RDT)

### INTRODUCTION

Malaria remains a global health challenge with 241 million malaria cases in 2020 in 85 malaria-endemic countries, up from 227 million in 2019, with most of this increase coming from African countries [1]. The World Health Organisation (WHO) recommends parasitological confirmation of suspected malaria cases before starting anti-malarial treatment and many malaria endemic countries have followed this policy [2]. The strategy in achieving malaria elimination through Early Diagnosis and Prompt Treatment, which is the early discovery of malaria cases and appropriate and rapid treatment so that transmission can be stopped [3].

Malaria diagnostic testing is largely based on Rapid Diagnostic Tests (RDTs) and examination by microscopy. Polymerase Chain Reaction (PCR) is also a sensitive detection method that can be considered a diagnostic tool [4]. Immunochromatographic antigen-based malaria RDTs detect Rich Histidine protein -2 (HRP-2) antigen or Parasite lactosadehydrogenase (pLDH) enzyme. HRP-2 antigen is specific for Plasmodium falciparum and Pan-pLDH detects all Plasmodium species [2, 5, 6].

Confirmation of malaria using RDTs in endemic areas where malaria microscopists are not available, there is growing concern about the accuracy of malaria RDT results [6]. A drawback of using RDTs in clinical settings is the problem of false positive/false negative results that can lead to misdiagnosis and overprescribing of antimalarial drugs. This makes diagnostic quality essential [5, 7]. Data on the sensitivity and specificity of RDT test kits is important information to help guide test selection by malaria control programmes [8]. Differences in sensitivity and specificity among RDTs have been reported for several possible reasons, including exposure to high temperatures and humidity that cause antibody denaturation [9]. Other variables that may cause differences in sensitivity and specificity of RDTs include high or low disease incidence, population differences, differences in characteristics, genetic variation in malaria and genetic practices and skills [10, 11].

In previous studies conducted by and Bernardus et al 2017 conducted research on Comparison of the effectiveness of Rapid Diagnostic Test (RDT) with Microscopic examination in patients with Clinical Malaria and research (Wardani P et al, 2020) conducted research Comparison of the performance of two Rapid Diagnostic Tests of malaria with PCR and gold standard microscopy detection methods. In addition, research by Dozie et al (2016) and Bharti PK et al (2008) conducted diagnostic test research including sensitivity specificity accuracy positive predictive value and negative predictive value on rapid diagnostic malaria compared to microscopic examination. So this research will complement the research conducted previously.

## **METHOD**

This type of research is observational analytic research with a cross sectional approach research design. This study has received approval from the Research Ethics Commission with number e-KEPK/POLKESYO/0688/XII/2022 from the Poltekkes Kemenkes Yogyakarta. This study used primary and secondary data from the results of malaria RDT examination and the results of malaria microscopic examination in the period January - December 2022. Samples from this study were obtained from patients with symptoms of malaria who were subjected to malaria RDT sampling, making thick blood preparations and thin blood smears for microscopic examination of malaria. Malaria staining using 3% Giemsa solution for 45-60 minutes. The number of samples used was 93 samples and then the data from the Rapid Diagnostic Test (RDT) and malaria microscopic examination results were carried out diagnostic tests including sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Statistical test used chi-square.

## RESULTS AND DISCUSSION

Based on table 1, in the RDT examination there were 39 positive PAN results (42%), positive PAN Pf 2 examinations (2%), and negative 52 examinations (56%). Microscopic confirmation showed 38 positive *Plasmodium vivax* (41%) and 2 positive *Plasmodium falciparum* (2%), resulting in 52 negative RDT and microscopic results (57%). Based on table 2 of 93 examination samples there were 41 positive RDT results and 52 negative RDT results but microscopic examination found 40 positive and 53 negative with a P-value of 0.000. Besides that, the results of the malaria RDT diagnostic test showed a sensitivity of 100% specificity of 98.11% Positive Prediction Value of 98% Negative Prediction Value of 100% and Accuracy of 99%.

**Table 1: Distribution of Results and Species Based on Method Examination**

Method	Species	Total	Percentage (%)
RDT	Positive PAN	39	39
	Positive PAN <i>Plasmodium falciparum</i>	2	2
	Negative	52	52
	Invalid	6	6
microscopic	<i>Plasmodium vivax</i>	38	40
	<i>Plasmodium falciparum</i>	2	2
	Negative	52	52

**Table 2: contingency tables and diagnostic tests**

Method of Examination		Microscopic		Total	P-value
		Positive	Negative		
Rapid Diagnostic Test	Positive	40	1	41	0.000
	Negative	0	52	52	
Total		40	53	93	
Diagnostic Test		Percentage (%)			
Sensitivity		100.00			
Specificity		98.11			
Accuracy		99.00			
Positive Predictive Value		98.00			
Negative Predictive Value		100.00			

Malaria examination is carried out to find malaria parasites in malaria suspects based on clinical symptoms from patients and laboratory examinations. The gold standard of malaria examination is microscopic examination using blood preparations, RDT in certain conditions, or PCR if needed for confirmation [12]. Accurate diagnosis is very important for the management of malaria cases both RDT and Microscopic tests [13]. This study aims to determine the diagnostic test value of malaria Rapid Diagnostic Test (RDT) method with microscopic method.

The malaria RDT used is an RDT that tests for the presence of specific antigens Histidin Rich Protein 2 (HRP2) on *Plasmodium falciparum* and PAN-specific Lactate dehydrogenation (pLDH) antigens on *Plasmodium* species both *Plasmodium vivax*,

*Plasmodium falcifarum*, *Plasmodium ovale* and *Plasmodium malariae*. With specific single clone antibodies (anti-HRP-2, anti-pLDH and control), which are attached to nitrocellulose paper. If the patient's blood contains HRP-2 and or contains pLDH, the antigen will be captured by anti-HRP-2 or anti-pLDH on nitrocellulose paper, so that a positive result will cause a red colour on nitrocellulose paper [13].

According to Wanja et al (2016), WHO recommends that malaria RDTs should have a sensitivity of > 95% and specificity of > 90%. Table 4 shows that the diagnostic test results of the malaria RDT brand first response are in accordance with WHO recommendations. Interpretation of diagnostic test values for Sensitivity and Specificity is 91% - 100% (very strong), 81% - 90% (strong), 71% - 80% (moderate), 61% - 70% (weak) and < 51% - 60% (very weak) [16]. From the results of this study, the sensitivity and specificity of the RDT first response brand is in the very strong category. The results of the diagnostic sensitivity and specificity test of the RDT brand first response in the tool manual book mention that from the results of the evaluation of the external testing laboratory, the sensitivity of malaria RDT in falcifarum positive malaria with a parasite density of 200 parasites / blood ul is 82% and vivac positive malaria is 91.4%. And at a parasite density of 2000 parasites / blood cell, the sensitivity of both vivax and falcifarum positive malaria is 100%. And Specificity of 98.1% in both vivac and falcifarum malaria [14]. The high Positive Prediction Value (NPV) of the RDT indicates that it is reliable for eliminating malaria. Likewise, a higher Positive Prediction Value (PPV) indicates that the patient will be accurately detected positive for malaria and will avoid overtreatment [21].

Research by Dozi et al (2016) obtained a diagnostic test on the first response brand is Sensitivity 98.6% Specificity 90% Accuracy 97% positive predictive value 95.8% negative predictive value 96.4%. Research Bharti Pk et al (2008) obtained the diagnostic test value of the first response brand Sensitivity 93% Specificity 85% Positive predictive value 79% and negative predictive value 95% accuracy 88%. The results of malaria RDT examination can show false positives or false negatives, RDT examination is easy, fast and relatively simple in conducting malaria examinations, but RDT has the disadvantage that it cannot detect low parasite densities. RDTs can only read the presence of 40-100 parasites per microlitre of blood while microscopic examination can read parasites in the blood up to 5-10 parasites /ul blood [15].

One of the false positive results on RDT in this study can be attributed to antigenemia proteins that can still be detected in the patient's blood or rheumatoid factor and heterophile antibodies in the patient's blood [4, 17, 20]. Other causes of false positive results are persistent asexual stage parasitaemia, below the microscope detection limit and antigen persistence due to absorption. The absorption of malaria parasites at the time of blood collection is quite an interesting factor. There was evidence of parasitaemia clinically, the RDT kit also tested positive whereas microscopy could not detect parasites at any stage. Subsequently it was found by thorough microscopic examination that malaria pigments were seen in the peripheral blood leucocytes of this patient [2, 19].

The malaria parasite HRP2 antigenic protein could still be identified in the patient's blood up to 30 days after antimalarial therapy, and the presence of gametocytes in the blood still produced all three proteins HRP2, p-LDH and aldolase [18]. These findings add weight to the utility of RDTs, their advantages over microscopy when parasites appear in cerebral and placental malaria. Detection of both RDTs as negative with HRP-2 and HRP-2/pLDH with later had higher negative values whereas corresponding microscopy positives were found. False-negative results have also been encountered in other studies where this is due to small levels of parasitaemia that fall below the microscopic threshold.

## CONCLUSION

Malaria RDT diagnostic test shows good results in testing for possible malaria infection in individuals suspected of having the disease. The test provides a quick and effective solution in identifying the presence of malaria parasites in the patient's body. This helps in early diagnosis and appropriate treatment, and enables more efficient management in preventing the spread of the disease. Malaria RDTs are thus an important and reliable tool in malaria treatment and control.

## Reference

1. World Health Organisation, world malaria report, Geneva, 2021
2. Dozie UW, Chukwuocha UM (2016) *Comparative Evaluation of Malaria Rapid Diagnostic Test Kits Commercially Available In Parts of South Eastern Nigeria*. J Trop Dis 4: 201. doi:10.4172/2329-891X.100020.
3. Kementerian Kesehatan Republik Indonesia. 2017. Pedoman Teknis Pemeriksaan Parasit Malaria. Dirjen P2TVZ: Jakarta
4. Wardhani P, Verawaty T, Butarbutar, Adiatmaja C.O, Betaubun A. M, Hamidah N, Aryati, 2020. *Performance comparison of two malaria rapid diagnostic test with real time polymerase chain reaction and gold standard of microscopy detection method* Infectious Disease Reports 2020; volume 12(s1):8731
5. Plucinski M, Aidoo M, Rogier E, 2021. *Laboratory Detection of Malaria Antigens: Strong Tool for Malaria Research, Diagnosis, and Epidemiology*, <https://doi.org/10.1128/CMR.00250-20>
6. Bharti PK, Silawat N, Singh PP, Singh MP, Shukla M, Chand G, et al. *The usefulness of a new rapid diagnostic test, the first response® malaria combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India*. Malar J. 2008;7(1):126
7. Shittu O, Opeyemi O. A. Ajibaye O. Omotesho B. O. Fakayode O. 2018. *Evaluation of Clinical Proficiency of RDTs, Microscopy and Nested PCR in the Diagnosis of Symptomatic Malaria in Ilorin, North-central, Nigeria*. a. Access Maced J Med Sci. 2018 Jun 20; 6(6): 1032–1040. doi: 10.3889/oamjms.2018.218.
8. Wanja E, Nickline Kuya<sup>1</sup>, Collins Moranga<sup>1</sup>, Mark Hickman<sup>2</sup>, Jacob D. Johnson. Carolyne Moseti<sup>1</sup>, Lalaine Anova<sup>2</sup>, Bernhards Ogutu<sup>1</sup> and Colin Ohrt<sup>4</sup>, 2016. *Field evaluation of diagnostic performance of malaria rapid diagnostic tests in western Kenya*. Malaria journal 15:456 <https://link.springer.com/article/10.1186/s12936-016-1508-y>
9. Mouatcho, J.C. and Goldring, J.P.D, 2013. Malaria Rapid Diagnostic Tests: Challenges and Prospects. Journal of Medical Microbiology, 62, 1491-1505

10. Leeflang MM, Bossuyt PM, Irwig L, Leeflang MM, Bossuyt PM, Irwig L, 2009, Diagnostic test accuracy may vary with prevalence: implications for evidence-based diagnosis. *J Clin Epidemiol.* 2009; 62:5–12.
11. Murray CK, Gasser RA Jr, Magill AJ, Miller RS, 2008. Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev.* 2008; 21:97–110.
12. Kementerian Kesehatan Republik Indonesia, 2020, Modul Pelatihan Mikroskopis Malaria bagi tenaga ATLM (Ahli teknologi Laboratorium Medik. Dirjen P2TVZ : Jakarta
13. Iwuofor A ita, O I, Chimereze A OU, Amajor A, 2018. *Evaluation of Diagnostic Accuracy of Rapid Diagnostic Test for Malaria Diagnosis among Febrile Children in Calabar, Nigeria.* *Journal of the Nigeria Medical*
14. Manual Book RDT first response Combo Card Test, 2017, diakses tanggal 03 September 2022
15. Bernadus.B.B Jano, Pijoh D Victor, Ritung N. 2018. Perbandingan efektifitas Rapid Diagnostic Test (RDT) dengan pemeriksaan Mikroskopis pada penderita Malaria Klinis di puskesmas Mubene kecamatan Likupang. *Jurnal e-biomedik.* Volume 6. Nomer 2. (2018)
16. Dahlan, MS. 2009. *Statistik untuk Kedokteran dan Kesehatan Edisi 4.* Jakarta : Salemba Medik
17. Gatton L. M, Ciketic S, Barnwell W J, Cheng Qin, Chiodini L Peter, Incardona S, Bell D, Cunnungham J, 2018. An assessment of false positive rates for malaria rapid diagnostic tests caused by non-Plasmodium infectious agents and immunological factors. doi: 10.1371/journal.pone.0197395
18. Mogeni P, Williams TN, Omedo I et al. detecting malaria hotspots: A comparison of rapid diagnostic test, microscopy, and polymerase chain reaction. *J Infect Dis.* 2017; 216:1091–8
19. Sardjono T W, Fitri L E, 2019. *Kupas Bahas Ringkas tentang Malaria.* Malang. UB Press
20. Orimadegun AE, Funwei RI, Michael OS, Ogunkunle OO, Badejo JA, Olusola FI, Agede O, Anjorin OE, Ajayi IO, Jegede AS, Ojurongbe O, Falade CO, 2021. *Comparative Evaluation of Three Histidine-Rich Protein-2 Based Rapid Diagnostic Tests, Microscopy and PCR for Guiding Malaria Treatment in Ibadan, Southwest Nigeria.* *Nigerian Journal of Clinical Practice,* DOI: 10.4103/njcp.njcp\_491\_20
21. Balaghaleh MR, Zarean M, Aghaee MA, et al. Comparison of PfHRP-2 / pLDH RDTs with Light Microscopy in a Low Prevalence Setting in Southeastern Iran, Sistan and Baluchestan: Due to Implementation of Malaria Elimination Program. 2018. doi: 10.5812/iji.12286