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Sensitivity and Specificity of Rapid Diagnostic Tests (RDTs) in Detecting Plasmodium Vivax

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Abstract
Introduction: *Malaria is an infectious disease caused by the Plasmodium genus of parasites. Plasmodium vivax is currently the most reported type of Plasmodium in Indonesia, accounting for 47.9% of cases. To reduce the incidence of malaria cases, a rapid, efficient, and affordable diagnostic method is needed, such as the Rapid Diagnostic Test (RDT).* ***Objective:*** *This study conducted laboratory tests on the RDT to evaluate its sensitivity and specificity in diagnosing malaria compared to the microscopic method, which is considered the gold standard.* ***Method:*** *This research employed an analytical study design using a cross-sectional approach. The data collected were processed and organized into contingency tables, calculating sensitivity, specificity, positive predictive value, and negative predictive value.* ***Result and Discussion:*** *The sensitivity of the RDT was found to be 92.59%, with a specificity of 100%. The positive predictive value was 100%, while the negative predictive value was 66.66%.* ***Conclusion:*** *This study concludes that the RDT diagnostic method is both sensitive and specific in detecting Plasmodium vivax when compared to the microscopic method as the gold standard.*
Keywords: Malaria; Microscopy; Plasmodium Vivax; Rapid Diagnostic Test;

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Introduction

Malaria is an infectious disease caused by the Plasmodium parasite, with primary symptoms including high fever, which may be accompanied by chills, headache, and muscle pain (Riskesdas, 2018) (Arief, Arif, & Erlani, 2020). Malaria is a communicable disease caused by parasites of the Plasmodium genus, transmitted to humans through the bite of infected female Anopheles mosquitoes (Talapko, Skrlec, Alebi, Juki, & V ev, 2019),(Avichena & Anggriyani, 2023).

In Indonesia, malaria remains a significant public health issue, with an increasing prevalence in recent years. Four Plasmodium species are known to infect humans: Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae, and Plasmodium ovale (Purwanto & Ottay, 2011), (Sazqia, 2024). To detect malaria infection, commonly used diagnostic methods include microscopic examination and Rapid Diagnostic Tests (RDT).

Microscopic examination is performed using two techniques: thick blood smear and thin blood smear analysis (Setiadi et al., 2014), (Wulandhani & Mudrika, 2023). This method is considered the gold standard for malaria diagnosis, although it requires specific expertise and adequate laboratory facilities (Ritung, Pijoh, & Bernadus, 2018). RDT, on the other hand, utilizes antigen detection, including Histidine-Rich Protein II (HRP-II) for identifying Plasmodium falciparum and lactate dehydrogenase (LDH) antigens for other Plasmodium species (Kusuma, Lestari, Herawati, & Yasa, 2006), (Atun & Rohima, 2024), RDT offers simplicity and rapid results, making it especially useful in resource-limited areas (Ministry of Health of the Republic of Indonesia, 2023)

The malaria control program in Indonesia has been established under Ministry of Health Regulation Number 293/Menkes/SK/VI/2009 concerning Malaria Elimination (Ministry of Health of the Republic of Indonesia). With the increasing incidence of malaria over the years, having a rapid and effective diagnostic method is crucial. RDT, which operates on the principle of immunochromatography to detect specific malaria parasite antigens in the blood, has become an attractive alternative due to its ability to be performed quickly and without the need for advanced laboratory equipment (Organization, 2018) (Aryani, 2023)

According to data from the World Health Organization (WHO), an estimated 249 million malaria cases occurred globally in 2022, with 608,000 deaths reported (Sari, Andayani, & Endarti, 2024). In Indonesia, the number of malaria cases increased from 304,607 in 2021 to 443,530 in 2022 (Tagora, Rahajeng, Windiyaningsih, & Prameswari, 2023), however, in 2023, there was a decrease to 418,546 cases, representing approximately 5.64%. This indicates that malaria control efforts still need to be strengthened. *Plasmodium vivax is one of the most common species causing malaria in Indonesia* (sitta Dewi, Gustawan, Utama, & Arhana, 2019). Based on data from the Ministry of Health of the Republic of Indonesia in 2023, *Plasmodium vivax* accounted for 47.9% of all positive malaria cases, making it the most prevalent species compared to other *Plasmodium* species (Ministry of Health of the Republic of Indonesia, Malaria Cases in Indonesia, 2024)

This study aims to compare the sensitivity and specificity of microscopic methods and Rapid Diagnostic Tests (RDT) in diagnosing *Plasmodium vivax* in febrile patients. By understanding the effectiveness of RDT compared to microscopic methods, this research is expected to contribute to the development of more effective malaria control strategies in Indonesia and improve diagnostic accuracy to reduce morbidity and mortality rates associated with the disease.

Method

The study was conducted at the Parasitology Laboratory, Faculty of Medicine, Sam Ratulangi University, from August to December 2024. This research is an analytical study carried out cross-sectionally, aimed at comparing the sensitivity and specificity of microscopic methods with RDT in diagnosing *Plasmodium vivax* infections in febrile patients. Blood samples were collected in EDTA tubes from 62 patients exhibiting clinical symptoms of fever.

Data collection in this study followed several stages. First, blood samples were taken from patients presenting with fever complaints. The samples were then examined using microscopic methods (thin and thick blood smears) and RDT. Once data were collected, data processing was performed using Microsoft Office Excel. The collected data were analysed to calculate sensitivity, specificity, Negative Predictive Value (NPV), and Positive Predictive Value (PPV)

Results and Discussion

Results of the study on 62 blood samples from patients with clinical symptoms of fever at the Parasitology Laboratory, Faculty of Medicine, Sam Ratulangi University:

Table 1

Frequency Distribution Based on Microscopic Examination and RDT

Microscopic Examination			**RDT Examination**		
Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**
54	8	62	50	12	62

Based on Table 1, it can be observed that microscopic examination showed 54 positive samples (87.09%) and 8 negative samples (12.90%). Meanwhile, RDT results showed 50 positive samples (80.64%) and 12 negative samples (19.35%).

Table 2

Results of EDTA Blood Examination Using Microscopic and RDT Methods in a 2x2 Contingency Table

		Microscopic Examination		
Rapid Diagnostic Test Examination		**Positive**	**Negative**	**Total**
	Positive	50	0	50
	Negative	4	8	12
Total		54	8	62

Based on Table 2, the results of Microscopic and RDT examinations entered the contingency table consist of 50 true positive samples, no false positive samples, 4 false negative samples, and 8 true negative samples.

Sensitivity

$$Sen = \frac{50}{50+4}\ X\ 100\%$$
$$Sen = \frac{50}{54}\ X\ 100\%$$
$$Sen = 92.59\%$$

Specificity

$$Sep = \frac{8}{8+0}\ X\ 100\%$$
$$Sep = \frac{8}{8}\ X\ 100\%$$
$$Sep = 100\%$$

Negative Predictive Value (NPV)

$$NPV = \frac{8}{8+4}\ X\ 100\%$$
$$NPV = \frac{8}{12}\ X\ 100\%$$
$$NPV = 66.66\%$$

Positive Predictive Value (PPV)

$$PPV = \frac{50}{50+0}\ X\ 100\%$$
$$PPV = \frac{50}{50}\ X\ 100\%$$
$$PPV = 100\%$$

The management of malaria detection across different regions in Indonesia undoubtedly requires adequate and effective tools. Currently, one of the most used diagnostic tools in laboratories is the Rapid Diagnostic Test (RDT). In this study, the researchers used the RDT Egens Malaria Pf/Pv Antigen Test, with a production code according to the World Health Organization Inf-72 and LOT No. 20240504.

From the microscopic examination of 62 blood samples, 54 samples were found positive for Plasmodium vivax (87.09%), while 8 samples were negative for Plasmodium vivax (12.90%). This differs from the RDT results, where 50 samples were positive for Plasmodium vivax (80.64%) and 12 samples were negative for Plasmodium vivax (19.35%). Based on these results, the positive Plasmodium vivax cases detected using RDT were 6.45% lower compared to microscopic examination. Additionally, 8 samples were negative for Plasmodium vivax (12.90%) on microscopic examination, while 12 samples were negative (19.35%) on RDT, showing that the negative results from microscopic examination were 6.45% lower compared to RDT.

Based on the results of microscopic and RDT examinations entered the 2x2 contingency table, the findings revealed 50 true positive samples, 8 true negative samples, no false positive samples, and 4 false negative samples. True positives refer to the number of blood samples detected as positive for Plasmodium vivax in microscopic examination and confirmed positive in RDT testing.

This means that 50 samples diagnosed as positive for Plasmodium vivax by the microscopic method, which is considered the gold standard, were also identified as positive by RDT. The eight samples diagnosed as true negative indicate that these blood samples were detected as negative for Plasmodium vivax in both microscopic and RDT examinations. False negatives refer to the number of blood samples detected as positive for Plasmodium vivax in microscopic examination but identified as negative in RDT testing.

Based on the study's findings, there were 4 blood samples positive for Plasmodium vivax on microscopic examination but negative in RDT testing. This discrepancy could affect the sensitivity and negative predictive value (NPV) in this study, as evidenced by the sensitivity value of 92.59% and an NPV of 66.66%. False negative results may be attributed to low parasite density. This statement is supported by research conducted by eight researchers from Cameroon, who investigated the performance of RDT in detecting Plasmodium compared to the microscopic method.

Their study was conducted on 400 blood samples from patients, revealing that RDTs were unable to diagnose positive results when parasite density was less than 500 parasites/µL (Wardhani, Butarbutar, Adiatmaja, Betaubun, & Hamidah, 2020). In addition to this study, the WHO also stated in the book *Malaria Rapid Diagnostic Test Performance* that RDTs cannot identify positive results when parasite density is below 200 parasites/µL (*Malaria Rapid Diagnostic Test Performance WHO - Summary Results of WHO Product Testing of Malaria RDTs: Rounds 1-8 (2008-2018)*, 2018).

Apart from parasite density, the amount of pLDH antibody present in blood samples can also influence the sensitivity and negative predictive value (NPV) of RDTs. This was discussed by Jin Woo Jang and colleagues in their study on *Plasmodium vivax* pLDH levels and the detection limits of pLDH-based Rapid Diagnostic Tests (RDTs) for malaria. According to their findings, they tested three types of RDTs for detecting *Plasmodium vivax*: SD Bioline, Optimal, and Humasis. The detection limit for pLDH was found to be 25 ng/mL for SD Bioline and Humasis, while it was 50 ng/mL for OptiMAL, based on the blood sample analysis (Lee et al., 2014)

The high specificity and positive predictive value (PPV) observed in this study are greatly influenced by the false positive rate. Specificity refers to the proportion of negative subjects according to the gold standard that are identified as negative by the test tool. PPV, on the other hand, represents the probability that subjects identified as positive by the test tool are indeed positive according to the gold standard. In this study, the specificity and PPV values were 100%, indicating that the Egens RDT has an equivalent probability to microscopic examination in diagnosing negative malaria cases.

Conclusion

Based on the results of the study conducted on 62 blood samples from febrile patients, it can be concluded that the Rapid Diagnostic Test used in this study is both sensitive and specific for detecting Plasmodium vivax when compared to the microscopic method as the gold standard

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