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ORIGINAL ARTICLE

Comparative Assessment of Microscopy and RDTs in Diagnosing *Plasmodium vivax*

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Abstract

Plasmodium vivax is the primary cause of malaria in Indonesia in 2023, particularly in areas with low to moderate endemicity. To reduce malaria transmission, rapid and accurate diagnostic tools are essential for early detection. Microscopic examination remains the gold standard, but the limited availability of trained microscopists and facilities hinders its effectiveness in endemic areas. The Rapid Diagnostic Test (RDT) based on pLDH, such as SD Bioline® Malaria Ag Pf/Pan®, offers rapid results and ease of use, which are beneficial in resource-limited settings. However, performance data for this RDT in Indonesia remains limited. This study evaluates the field performance of SD Bioline® compared to microscopic examination as the reference standard for P. vivax diagnosis. The study involved 62 EDTA blood samples from suspected malaria patients at the Parasitology Laboratory of the Faculty of Medicine, Sam Ratulangi University, from July 2022 to December 2024. Microscopic examination revealed 54 positive cases (87.1%), while SD Bioline® detected 50 positive cases (80.6%). The sensitivity and specificity of SD Bioline® were 92.6% and 100%, respectively, with a negative predictive value (NPV) of 66.6% and a positive predictive value (PPV) of 100%. The results suggest that SD Bioline® demonstrates good sensitivity and specificity for detecting P. vivax in endemic areas. However, false negatives require microscopic confirmation. Combining RDT with microscopy can improve diagnostic accuracy and support Indonesia's malaria elimination efforts by 2030.

Introduction

Malaria is a disease caused by the protozoan *Plasmodium* sp., transmitted via the bite of infected female *Anopheles* mosquitoes. One of the species responsible for malaria, *Plasmodium vivax* (P. vivax), causes tertian malaria [1]. In 2022, malaria infected approximately 249 million people and resulted in 608,000 deaths globally [2,3]. In Indonesia, there were 418,546 reported malaria cases in 2023, with *P. vivax* as the primary cause, accounting for nearly 48% of the total cases [4]. *P. vivax* dominates in areas with low to moderate endemicity [5].

To reduce malaria transmission and achieve Indonesia's target of malaria elimination by 2030, in line with the Sustainable Development Goals (SDGs), rapid and accurate diagnostic tools are essential [5,6]. Effective diagnostic tools enable faster case identification, facilitate timely treatment, and reduce the spread of disease through infected mosquito bites.

Microscopic examination is the gold standard for diagnosing malaria as it can identify *Plasmodium* species and measure parasitemia [7]. When performed by trained microscopists, this method can detect *Plasmodium* sp. at densities as low as <50 parasites/μL of blood [8]. However, a main challenge in implementing microscopic examination in Indonesia is the limited availability of resources. Of the 4,018 healthcare facilities in 2022, only 3,603 were equipped with microscopes. Additionally, there were only 1,996 trained microscopists registered for malaria diagnosis, with only 24 individuals across 20 provinces meeting the Level 1 criteria,

defined as microscopists with a diagnostic accuracy of greater than 90%, which limited the widespread effectiveness of malaria diagnosis [5].

As an alternative, Rapid Diagnostic Tests (RDTs) based on lactate dehydrogenase (pLDH) offer the advantage of faster results (15-20 minutes) and can directly identify *Plasmodium* species. Furthermore, their ease of use without specialized expertise makes RDTs a potential alternative for diagnosing malaria [9]. pLDH is a glycolytic enzyme produced by *Plasmodium* sp. during the intra-erythrocytic stage, and its presence indicates parasite metabolic activity [10]. RDTs use immunochromatographic techniques, where pLDH antigens bind to specific antibodies on the test strip, resulting in a red line as an indicator of *P. vivax* infection [11]. Additionally, cost-effectiveness analyses, such as one conducted in China, a Country that successfully eliminated malaria, have shown that RDTs (USD 2.19/test) are more cost-effective than microscopy (USD 6.98/test) [12].

One pLDH-based RDT authorized for use in Indonesia and pre-qualified by the WHO is the SD Bioline Malaria Ag Pf/Pan (hereafter referred to as SD Bioline). This test claims a sensitivity of 95.5% and a specificity of 99.5% for detecting pLDH [13]. Studies in malaria-endemic areas, such as French Guiana, have shown that SD Bioline® has a sensitivity of 93% and a specificity of 99.4% for detecting *P. vivax* [14]. Despite these promising claims, field performance data for SD Bioline® in Indonesia, a malaria-endemic country, remains limited. Therefore, this study aims to assess the field performance of SD Bioline® to ensure it provides consistent and reliable results in the field, aligned with the malaria epidemiological conditions in Indonesia.

Materials and Methods

Materials

The materials used for microscopic examination included glass slides, sterile tissue and gauze, buffer solution, EDTA tubes, dropper pipettes, medical gloves, Giemsa solution, methyl alcohol, staining racks, cool boxes, and a microscope. For the RDT examination, the SD BIOLINE Malaria Ag Pf/Pan® (05FK60 and 05FK63, Lot No. 05EDJ007A and 05EDI010A, Standard Diagnostics Inc., Korea) was used. Each kit contained a package of buffer solution, sterile lancets, alcohol swabs, capillary pipettes, and the RDT device [15].

Sampling

This study used EDTA blood samples from suspected malaria patients who had a history of fever within the last 48 hours and a history of travel to malaria-endemic areas. The samples were obtained from the Parasitology Laboratory at the Faculty of Medicine, Sam Ratulangi University (FK-UNSRAT). Sample selection was performed by reviewing patients' medical records, which included personal information, examination dates, and the results of the microscopic malaria examination. Only records authorized by the hospital where the patient was treated were included, according to the policies and procedures of each healthcare facility. Samples with complete medical records were separated from those with incomplete records and were obtained from the healthcare facilities between July 2022 and December 2024. Additionally, selection was based on the quality of the sample. Only samples with a minimum volume of 3 mL and in good condition (i.e., non-hemolyzed) were used in this study. After selection, 62 EDTA blood samples meeting the inclusion criteria were identified for further analysis.

Microscopic Examination

Two to three drops of blood were collected using a dropper pipette from the EDTA tube and spread onto a glass slide to prepare a thin blood smear. The smear was then fixed with methyl alcohol, stained with Giemsa stain solution, and rinsed with water. After staining, the slide was examined under a microscope with a 100x objective lens to detect the presence of P. vivax. The initial microscopic examination was conducted at the healthcare facility where the patient

received treatment. The samples were then re-examined at the Parasitology Laboratory, FK Unsrat, by an expert examiner who was blinded to the previous examination results to avoid bias and ensure objectivity.

SD BIOLINE® RDT Examination

The SD BIOLINE RDT was performed according to the manufacturer's instructions to minimize technical errors. Blood samples were collected using a capillary pipette (5 µL) or a single-use inverted cup (5 µL), then transferred into the specimen well. The test buffer solution was added to the test well, and the results were interpreted after 15-30 minutes had passed. A positive result for P. vivax was indicated by the appearance of both the pan and control lines. In contrast, a negative result was indicated by the presence of only the control line.

Data Analysis

The analysis was conducted to evaluate the performance of the SD BIOLINE® RDT, using microscopic examination as the reference standard. The performance assessment was determined by calculating Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) [16].

Sensitivity measures the proportion of individuals who are truly malaria-infected and are correctly identified as positive by the test, as shown in Equation 1:

$$Sensitivity = \frac{TP}{TP + FN} \times 100\% \quad (1)$$

Specificity quantifies the proportion of individuals who are not malaria-infected and are correctly identified as negative by the test, as expressed in Equation 2:

$$Specificity = \frac{TN}{FP + TN} \times 100\% \quad (2)$$

PPV represents the proportion of patients who test positive and are clinically confirmed to have malaria, as calculated in Equation 3:

$$PPV = \frac{TP}{TP + FP} \times 100\% \quad (3)$$

NPV denotes the proportion of patients who test negative and are clinically confirmed to be malaria-free, as given in Equation 4:

$$NPV = \frac{TN}{TN + FN} \times 100\% \quad (4)$$

Where True Positive (TP) refers to a sample that is positive for *P. vivax* based on both microscopic examination and the SD BIOLINE® RDT, True Negative (TN) refers to a sample that is negative for *P. vivax* based on both microscopic examination and the SD BIOLINE® RDT, False Positive (FP) refers to a sample that is negative for *P. vivax* based on microscopic examination but tests positive on the SD BIOLINE® RDT, and False Negative (FN) refers to a sample that is positive for *P. vivax* based on microscopic examination but tests negative on the SD BIOLINE® RDT.

Results and Discussion

Microscopic Examination and SD BIOLINE® RDT Results

Based on Table 1, the microscopic examination showed that 54 out of 62 (87.1%) of the samples were positive for P. vivax. The results from the SD BIOLINE® Malaria Ag Pf/Pan® RDT showed that 50 out of 62 (80.6%) samples were positive. Among the RDT tests, four samples were initially negative by RDT but subsequently found to be positive by microscopic examination, classified as false negatives.

Table 1. Frequency distribution based on examination results.

Method	Number of Samples	Positive Pv	Negative Pv
Microscopic Examination	62	54 (87.1%)	8 (12.9%)
SD BIOLINE Malaria Ag Pf/Pan® RDT	62	50 (80.6%)	12 (19.4%)

Pv = *Plasmodium vivax*

The performance evaluation of the SD BIOLINE® RDT is shown in Table 2. The results indicate that the sensitivity and specificity of the SD BIOLINE® RDT were 92.6% and 100%, respectively, while the Negative Predictive Value (NPV) and Positive Predictive Value (PPV) were 66.6% and 100%, respectively.

Table 2. Diagnostic value analysis of the SD Bioline RDT using microscopic examination as the reference standard.

Diagnostic Value	Result
TP	50
TN	8
FP	0
FN	4
Sensitivity (%)	92.6
Specificity (%)	100
NRP (%)	100
NRN (%)	66.6

In this study, the sensitivity of the SD BIOLINE® was found to be 92.6%, which is not significantly different from the manufacturer's claim. This sensitivity value aligns with the findings of Tadesse (2016) in Ethiopia, a country with high malaria endemicity, which reported a sensitivity of 92.6% for the SD BIOLINE®, including for mixed *P. vivax* infections [17]. Among the 62 samples tested using the SD BIOLINE®, four showed false negative results, which lowered both sensitivity and NPV. These false negatives indicate limitations in the RDT's ability to detect malaria infections. Diagnostic errors, such as false negatives, can lead to delayed treatment and increase the risk of severe malaria [5]. Several factors can influence the occurrence of false negatives, including parasitic factors such as low parasitemia, as well as RDT-related factors, including test line visibility and the type of monoclonal antibodies used [18–22].

One of the false negative samples in this study was from a patient with typical malaria symptoms, including periodic fever and chills. Despite the patient being suspected of having malaria and undergoing three tests using different RDT brands, all results were negative. However, after microscopic examination, *P. vivax* infection was confirmed. This case demonstrates that, despite strong clinical evidence supporting a malaria diagnosis, the RDT may fail to detect the infection, likely due to low parasitemia. A decrease in the sensitivity of the SD BIOLINE at low parasitemia levels has been reported in several studies in Southeast Asia, including those in Myanmar and Thailand. A study in Myanmar using the SD BIOLINE® RDT showed a significant decrease in sensitivity, reaching 69.8%, for parasitemia levels between 100 and 1,000 p/µL [19]. A similar decrease in sensitivity (71.43%) was observed in Thailand for parasitemia levels ranging from 501 to 1,000 parasites per microliter. These findings confirm that, at lower parasitemia levels, the SD BIOLINE® RDT's ability to detect malaria infections is limited, thus increasing the potential for false negatives [18].

The pLDH concentration significantly affects the visibility of test lines in RDTs in blood samples. P. vivax infections typically present with low parasitemia because the parasite infects reticulocytes and migrates more widely, thus not concentrating in the peripheral blood. As a result, the pLDH levels in peripheral blood tend to be low, affecting the RDT test reaction [23]. Low pLDH levels result in weak or unclear test lines, potentially leading to false negative results. To achieve clear visibility of the Pan line in the RDT, a minimum of 45 ng/mL of pLDH is

required. Additionally, pLDH has only one epitope, limiting the number of colored antibodies that can bind, thereby reducing the intensity of the test line [21]. The use of monoclonal antibodies in RDTs may also affect the intensity of the test line, depending on the Plasmodium species detected [24].

Furthermore, the difference in the sensitivity of the SD BIOLINE® in this study may be related to the type of blood sample used. Sujariyakul's study, which achieved a sensitivity of 95%, utilized fresh blood samples obtained through finger pricks [18]. While this study only used EDTA blood samples. According to the manufacturer's instructions, using EDTA blood samples stored for more than three days can lead to non-specific reactions [15]. Some samples in this study were stored for over three days. Moreover, blood samples in Sujariyakul's study were collected during the peak malaria transmission season [18]. This may have affected parasitemia and could explain the presence of false negatives in this study [25].

In addition to the false negatives, the low NPV (66.6%) was also influenced by the small number of TN samples, which are both the numerator and denominator in the NPV calculation. In Sujariyakul's study, the NPV was 98.35%, based on 815 samples, of which 665 were true negatives. In contrast, this study had only 8 TN samples, which affected the NPV calculation.

The sensitivity and specificity of the SD BIOLINE® RDT in this study were excellent, at 92.6% and 100%, respectively. The combined sensitivity and specificity exceeding 1.5 (92.6% + 100%) indicates that this test meets the criteria for use in diagnostic practice [26]. The SD BIOLINE® offers advantages in terms of ease of use and rapid results, making it ideal for use in healthcare settings with limited resources. Its high specificity (100%) indicates that negative results are highly reliable. However, the test also has some limitations, particularly lower sensitivity compared to microscopic examination, which can detect even low parasitemia. The false negative results found in this study suggest potential limitations in detecting P. vivax infection. Therefore, although the RDT provides convenience and speed, further confirmation through microscopic examination is still required in certain cases, especially when the RDT result is negative. Still, the clinical symptoms strongly suggest the presence of malaria.

The limitations of this study include the small sample size (62 samples), the lack of parasitemia data, the absence of information regarding the patients' malaria treatment history and medical history, and the prolonged storage of EDTA blood samples, all of which could affect RDT sensitivity, sample quality, and the validity of the study results.

Conclusions

This study demonstrates that the SD BIOLINE RDT exhibits good sensitivity and specificity in detecting P. vivax, although its performance differs slightly from the manufacturer's claims. Despite this, it remains a viable alternative for malaria diagnosis in endemic regions with limited resources. However, the occurrence of false negatives in several samples highlights the limitations of this tool in detecting *P. vivax* infections, which could lead to missed malaria cases. Therefore, microscopic confirmation, particularly in patients with clinical malaria symptoms but negative rapid diagnostic test (RDT) results, remains necessary. This study supports Indonesia's malaria elimination goals for 2030 by emphasizing the importance of combining RDT and microscopy to enhance diagnostic accuracy and reduce the risk of detection errors. In the future, further studies with larger sample sizes and multi-center research are strongly recommended to comprehensively assess the performance of the SD BIOLINE® RDT and identify factors that affect result accuracy under various field conditions in Indonesia.

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