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OPTIMISATION OF 3% GIEMSA STAINING TIME IN MALARIA MICROSCOPIC EXAMINATION

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ABSTRACT
Malaria infection is a significant health issue in Indonesia, with a high prevalence in endemic areas such as Puskesmas Hanura Teluk Pandan. Giemsa 3% staining is used as the standard method for microscopic diagnosis of malaria; however, the duration of staining can affect laboratory efficiency. This study aims to optimize the staining time of Giemsa 3% in microscopic malaria diagnosis to improve efficiency without compromising diagnostic quality. The study used an experimental design with staining time variations of 30 minutes, 40 minutes, and 50 minutes on thin blood smear samples from suspected malaria patients. Staining quality was assessed using a scoring system, with a score of 1 indicating optimal staining (clear background, blue cytoplasm, and red nucleus) and a score of 0 indicating suboptimal staining (unclear background, cytoplasm, and nucleus not stained or not clearly visible). The results showed that all staining time variations produced optimal staining quality (score 1), with no samples receiving a score of 0. The data were found to be non-homogeneous and non-normally distributed, thus the Kruskal-Wallis test was employed for subsequent analysis. The Kruskal-Wallis test showed no significant differences between the three staining time variations ($p = 1.000$). The conclusion of this study is that staining durations of 30 minutes, 40 minutes, and 50 minutes with Giemsa 3% can produce equivalent staining quality, allowing operational efficiency without compromising the accuracy of malaria diagnosis.

Keywords: efficiency; giemsa staining; malaria; microscopy; staining duration

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INTRODUCTION

Malaria remains a significant public health challenge in Indonesia, with high prevalence rates observed in various endemic regions. According to Jontari et al. (2016), approximately 4.8 million individuals were estimated to be infected with malaria in 2013, with the highest concentration of cases in eastern Indonesia. Faradiba (2021) further reported 250,644 malaria cases in 2019, of which 86% occurred in endemic areas. The majority of these cases involved *Plasmodium falciparum* and *Plasmodium vivax* species, with *P. falciparum* accounting for 62% of the infections. Recent studies also highlight the increasing presence of *Plasmodium knowlesi* in areas such as Aceh, complicating the malaria control efforts (Ramadhan et al., 2021). These figures underscore the ongoing burden of malaria and the need for effective diagnostic methods.

Microscopic examination of blood smears using Giemsa staining has been a cornerstone in the diagnosis of malaria for over a century. This technique is essential for differentiating between various blood cell components and detecting malaria parasites. Giemsa staining provides detailed visualization of cell morphology, enabling the identification of different malaria species based on their unique characteristics (Hashimoto et al., 2017). Despite its effectiveness, the quality of the Giemsa stain is highly dependent on the staining duration, which can vary across different settings. Standard protocols typically recommend a staining

time of 45 to 60 minutes, but shorter or longer durations may affect the clarity of the smear and the accuracy of diagnosis (Asmawati & Kurniawan, 2023; Puasa, 2017).

The quality of the staining process directly influences the detection and identification of malaria parasites, especially in cases of low parasitemia, where parasites are difficult to distinguish from red blood cells. Optimizing the staining time is critical in ensuring that the diagnostic process is both efficient and accurate. Previous studies have suggested that variations in staining duration can significantly impact diagnostic outcomes. For example, excessive staining may obscure the parasitic structures, while insufficient staining may result in a lack of contrast, making parasite identification challenging (Pandey et al., 2023; Yağmur et al., 2015). Therefore, determining the optimal staining time for Giemsa 3% solution is crucial to enhancing diagnostic precision without sacrificing operational efficiency.

At Puskesmas Hanura Teluk Pandan, a primary healthcare facility in an endemic malaria region, diagnostic capacity is limited by the lengthy Giemsa staining process, which ranges from 45 to 60 minutes. Given the high volume of suspected malaria patients—averaging 15 to 20 per day—this extended staining duration strains laboratory resources and delays results. To address this challenge, this study aims to evaluate the impact of varying Giemsa staining times (30, 40, and 50 minutes) on the diagnostic accuracy of malaria microscopy. The findings of this study are expected to provide scientific evidence for optimizing laboratory procedures, improving both the speed and accuracy of malaria diagnosis.

METHOD

This study is quasi-experimental research using a Posttest-Only Non-Equivalent Groups Design, aimed at evaluating the optimal staining duration of Giemsa 3% for malaria diagnosis at Puskesmas Hanura Teluk Pandan. The study involves comparing three groups with different staining durations (30, 40, and 50 minutes). The impact of these staining durations on the quality of microscopic examination results is measured, focusing on the differences in outcomes across the groups. The population of this study consists of all thin blood smears from malaria patients at Puskesmas Hanura during the period of January to March 2025. The sample was drawn from this population, with each malaria patient contributing one thin blood smear that was stained with Giemsa 3% for three different durations: 30, 40, and 50 minutes. A total of 10 slides were prepared for each staining duration, resulting in 30 samples for the study.

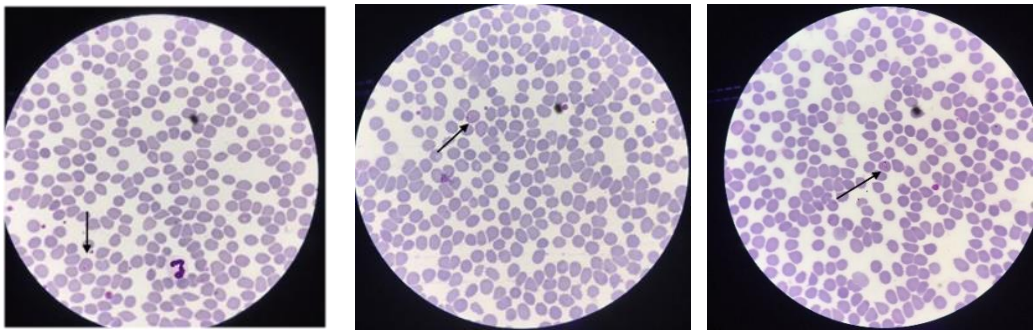
Data collection in this study was conducted using consecutive sampling, where all subjects who met the inclusion criteria were included in the study until the required sample size was achieved. The inclusion criteria included thin blood smears from patients with clinical malaria symptoms, prepared according to standard procedures without damage. The exclusion criteria consisted of blood smears from malaria-negative patients. The primary data source for this research was the microscopic examination of blood smears, with different Giemsa 3% staining durations of 30, 40, and 50 minutes. The quality of the staining was assessed by evaluating the clarity of the *Plasmodium* parasite morphology under the microscope. Each sample was assigned a score of 1 if the staining quality was considered optimal for interpretation, and a score of 0 if the staining was inadequate for interpretation. Statistical analysis was carried out to examine whether there were significant differences in staining quality across the three durations. Normality was tested using the Shapiro-Wilk test, and homogeneity of variance was tested using Levene's test. If the data were found to be normally distributed and homogenous, One-Way ANOVA was used; otherwise, the Kruskal-Wallis test was applied to determine if there were significant differences in the microscopic examination results.

RESULT

Table 1.
Respondent characteristics (n=10)

Respondent Characteristics	F	%
Age (Years)		
0-10	3	30
11-20	1	10
21-30	0	0
31-40	4	40
41-50	2	20
Gender		
Male	7	70
Female	3	30

The results of this study demonstrate that Giemsa 3% staining on malaria blood smears, using staining durations of 30, 40, and 50 minutes, all yielded optimal staining quality. Each sample across the three staining durations received a score of 1, indicating that the blood smear background remained clear, the cytoplasm was stained blue, and the nucleus was red (figure 1). These findings align with the expected standards for staining, which aim to clearly differentiate the morphology of blood cells and the malaria parasite, particularly for the detection of *Plasmodium* species. In this study, *Plasmodium vivax* was identified in all the samples.



30 minutes staining duration | 40 minutes staining duration | 50 minutes staining duration

Figure 1. Results of Giemsa staining on thin blood smear preparations for each staining duration variation, at a magnification of 100X

The normality of the data was assessed using the Shapiro-Wilk test. The p-values for all three staining durations (30 minutes, 40 minutes, and 50 minutes) were 0.00, indicating that the data did not follow a normal distribution. Consequently, non-parametric statistical analysis was applied. The Kruskal-Wallis test was used to assess the differences between the groups. The results of the Kruskal-Wallis test yielded a p-value of 1.000, which is greater than 0.05, suggesting that there were no significant differences in staining quality between the three staining durations. This indicates that the Giemsa 3% staining durations of 30, 40, and 50 minutes can be considered equivalent in terms of staining quality. These findings suggest that variations in staining duration do not affect the operational efficiency or diagnostic accuracy of malaria microscopic examination.

DISCUSSION

The quality of staining in malaria microscopy is essential for accurate detection of *Plasmodium* parasites. This study found that variations in staining duration (30, 40, and 50 minutes) did not affect staining quality, with all samples receiving a score of 1. This indicates

optimal staining, characterized by a clear background, blue cytoplasm, and red nucleus, consistent with expected standards for *Plasmodium* detection. The results align with Mohapatra et al. (2016), who highlighted that Giemsa staining facilitates detection even in low-density infections, emphasizing the importance of maintaining high staining standards for reliable diagnosis.

Staining quality also plays a crucial role in minimizing background interference, improving parasite morphology differentiation, and aiding in *Plasmodium* species identification. The study's consistent results across all durations demonstrate that high-quality staining enables clear parasite identification, essential for accurate diagnosis and appropriate treatment, particularly in mixed infections. This finding supports Kang et al. (2017), who noted that proper staining aids in differentiating *P. falciparum* from *P. vivax*, critical for correct therapeutic decision-making. Mahmoud et al. (2018) further emphasized the need for standardized staining procedures to avoid misidentification and incorrect treatment.

The relationship between staining quality and laboratory staff expertise is also critical. Although staining quality remained optimal across varying durations, accurate interpretation relies on sufficient microscopic skill. Untrained personnel may struggle with proper slide preparation and staining, leading to misdiagnosis (Odhiambo et al., 2017). This highlights the necessity of ongoing training for laboratory staff to ensure consistent and accurate diagnostic outcomes.

The scoring system used in this study provided an objective and structured method for evaluating staining quality. The consistency of the score of 1 across all samples indicates that the staining process was effectively controlled. This approach is consistent with Sori et al. (2018), who demonstrated that scoring systems help standardize procedures and ensure consistent diagnostic accuracy. This is particularly beneficial in resource-limited settings, where standardized techniques can improve diagnostic reliability.

Statistical analysis using the Kruskal-Wallis test yielded a p-value of 1.000, indicating no significant differences between the three staining durations. This result, supported by the Shapiro-Wilk test, suggests that the staining times of 30, 40, and 50 minutes provide equivalent staining quality. The findings support the conclusion that shorter durations, such as 30 minutes, can be used without compromising diagnostic accuracy, offering potential time-saving benefits in operational settings. In conclusion, staining durations of 30, 40, and 50 minutes produced equivalent results, with shorter durations (30 minutes) still yielding optimal results. This can improve efficiency in malaria microscopic examinations without affecting diagnostic accuracy.

CONCLUSION

The Kruskal-Wallis test revealed no statistically significant differences in the quality of microscopic malaria examination between the three Giemsa 3% staining durations (30 minutes, 40 minutes, and 50 minutes) at Puskesmas Hanura Teluk Pandan. A p-value of 1.000 indicates that all staining durations produced similar results, with each sample receiving a score of 1, which signifies optimal staining quality. This study concludes that the Giemsa 3% staining durations of 30 minutes, 40 minutes, and 50 minutes all yield optimal results for the detection of Plasmodium parasites. The findings indicate that all durations tested provided high-quality staining, adhering to the required standards for effective parasite visualization, thereby ensuring accurate detection in malaria microscopic examinations.

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