Indonesian Journal of Global Health Research
Volume 7 Number 4, August 2025
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

COMPARATIVE ANALYSIS OF STAINING TIME USING GIEMSA 10% ON THE RESULTS OF MALARIA BLOOD PREPARATIONS

M. Richo Realdy Wirawan, Dwi Haryatmi*
Sekolah Tinggi Ilmu Kesehatan Nasional, Jl. Raya Solo-Baki, Kwarasan, Grogol, Sukoharjo, Central Java 57552. Indonesia
*dwiharyatmi@stikesnas.ac.id

ABSTRACT
Malaria is a parasitic infectious disease that remains a significant public health problem in Indonesia, particularly in endemic areas. Rapid and accurate diagnosis of malaria is essential to prevent serious complications. The 10% Giemsa staining method is a rapid technique for microscopic examination of malaria, but the staining time standard needs to be validated for each batch of staining solution used in local laboratories. This study aims to analyze the comparison of malaria blood smear staining results using 10% Giemsa with different staining durations of 10 minutes, 20 minutes, and 30 minutes at Natar Medika Hospital. This research employed a cross-sectional approach with observational analysis of thin blood smears from 15 malaria-positive blood samples. The blood smear evaluation used a scoring system: a score of 0 was given for poor staining results (unclear background, unstained or non-blue cytoplasm, and unstained or non-red nucleus), and a score of 1 for good staining results (clear background, blue cytoplasm, and red nucleus). The results showed that staining for 20 minutes produced the best quality thin blood smears, characterized by a clear background, blue cytoplasm, and red nucleus. Statistical analysis using the Kruskal-Wallis test indicated significant differences between staining durations ($p < 0.05$). Therefore, staining for 20 minutes using 10% Giemsa is recommended to obtain optimal microscopic results in malaria diagnosis at Natar Medika Hospital.

Keywords: giemsa staining; malaria; microscopy; staining time; quality

How to cite (in APA style)
Wirawan, M. R. R., & Haryatmi, D. (2025). Comparative Analysis of Staining Time Using Giemsa 10% on the Results of Malaria Blood Preparations. Indonesian Journal of Global Health Research, 7(4), 323-328. https://doi.org/10.37287/ijghr.v7i4.6347.

INTRODUCTION

Malaria remains a major public health problem in Indonesia, particularly in endemic regions such as South Lampung Regency (WHO, 2023; Open Data Lampung, 2023). According to the Indonesian Ministry of Health (2024), there were 418,546 confirmed malaria cases out of 3,464,738 examinations nationwide. In Lampung Province, South Lampung ranked third for the highest number of malaria cases in 2022, with 946 laboratory-confirmed cases among 3,845 suspected cases (Open Data Lampung, 2023). Malaria infection is caused by Plasmodium parasites transmitted through the bites of female Anopheles mosquitoes (Hassor, 2023). Five Plasmodium species are responsible for human malaria infections: P. falciparum, P. vivax, P. ovale, P. malariae, and P. Knowlesi (Kemenkes RI, 2023). Microscopic examination of blood smears using Giemsa staining remains the gold standard for malaria diagnosis, allowing for species identification based on the morphological characteristics of the parasite (Pramudiyatika, 2022). The accuracy of diagnosis heavily depends on the quality of the staining process, especially in cases with low parasitemia (Pandey et al., 2023; Yagmur et al., 2015).

Giemsa 10% staining is widely applied due to its rapid and efficient staining process. However, the staining quality may vary depending on the batch of staining solution and the staining duration. An optimal staining time is critical; overstaining can obscure the morphological details of the parasite, while understaining may lead to poor contrast, making parasite identification difficult (Sathpathi et al., 2014; Worung et al., 2020). According to

WHO guidelines (2016), rapid Giemsa staining recommends an 8–10 minutes duration, but local validation of staining time is advised for laboratory-specific conditions.At Natar Medika Hospital, a type C hospital in South Lampung Regency, malaria diagnosis is routinely performed using 10% Giemsa staining with durations between 20 and 30 minutes. However, given the high workload and limited number of trained laboratory personnel, there is a need to optimize the staining time to achieve faster and more accurate diagnosis, especially for severe cases such as cerebral malaria caused by P. falciparum.Due to the absence of a validated standard operating procedure for staining time at Natar Medika Hospital, this study aims to compare the microscopic results of malaria blood smears stained with 10% Giemsa at different staining times—10, 20, and 30 minutes. The findings are expected to provide scientific evidence for establishing an optimal staining protocol to enhance the accuracy and efficiency of malaria diagnosis in this setting.

METHOD

This study was a quantitative research with an analytic observational design using a cross-sectional approach (Sugiyono, 2018; Notoatmodjo, 2012), conducted at the Laboratory of Natar Medika Hospital, South Lampung. The study involved patients with positive malaria laboratory results, using 10% Giemsa staining at three different staining durations: 10 minutes, 20 minutes, and 30 minutes. The study population consisted of residents within the working area of Natar Medika Hospital who underwent malaria examinations between December 2024 and February 2025. Samples were selected through quota sampling until 15 positive malaria samples were obtained, with inclusion criteria of positive laboratory diagnosis for malaria and willingness to participate, and exclusion criteria of negative malaria results.Primary data were collected from microscopic examination of thin blood smears stained with 10% Giemsa. The quality of staining was assessed using a scoring system: score 1 for optimal staining (clear background, blue cytoplasm, and red nucleus) and score 0 for suboptimal staining. Data normality was tested using the Shapiro-Wilk test, and group differences were analyzed using One-Way ANOVA if the data were normally distributed and homogeneous, or Kruskal-Wallis test if not. This study aimed to determine the optimal staining time using 10% Giemsa dilution for microscopic examination of malaria at Natar Medika Hospital. The study was conducted from December 2024 to February 2025, with 15 positive malaria blood samples collected. Staining was performed with durations of 10, 20, and 30 minutes.

RESULT

Table 1.
Giemsa 10% Staining Results

Sample Code	Staining Time		
	10 minutes	20 minutes	30 minutes
1	0	1	1
2	1	1	1
3	0	1	1
4	1	1	1
5	1	1	0
6	1	1	1
7	0	1	1
8	1	1	1
9	1	1	1
10	1	1	1
11	0	1	0
12	0	1	0
13	0	1	1
14	1	1	1
15	1	1	1

Table 1 shows the staining scores according to staining duration. At 20 minutes, all samples (15/15) showed good staining results (score 1). Staining for 10 minutes produced 9 samples with good quality and 6 with poor quality, while 30 minutes produced 12 good quality samples and 3 poor quality samples.

Staining evaluation was based on background clarity, cytoplasm color, and nuclear staining. A score of 1 indicated optimal staining (clear background, blue cytoplasm, and red nucleus), while a score of 0 indicated suboptimal staining.
The staining results are visually represented in Figure 1, illustrating the appearance at each staining time: 10 minutes, 20 minutes, and 30 minutes.

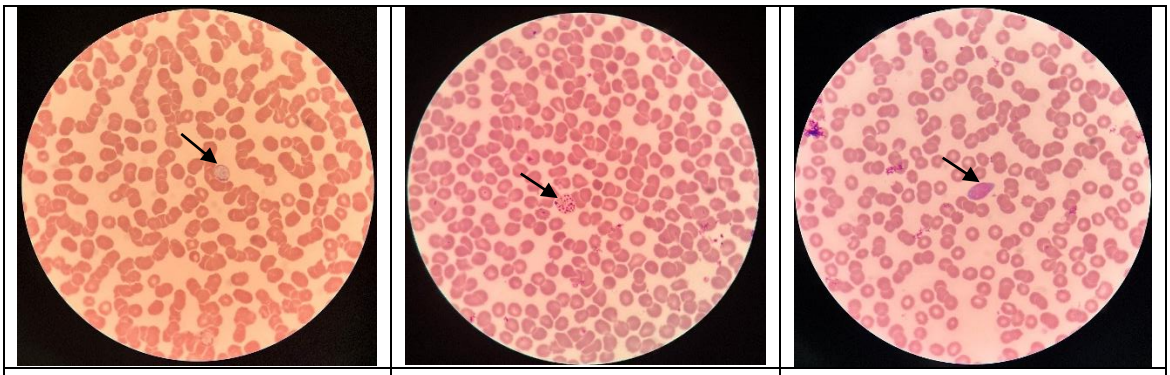

Figure 1. Giemsa 10% Staining on Thin Blood Smear (100x Magnification)

Normality testing using the Shapiro-Wilk test showed that all staining time groups had a significance value (p) of 0.000, indicating that the data were not normally distributed (Table 2).

Table 2.
Shapiro-Wilk Normality Test Results

Staining Time	Sig. (*p-value*)	Interpretation
10 minutes	0,000	Not normally distributed
20 minutes	0,000	Not normally distributed
30 minutes	0,000	Not normally distributed

As the data were not normally distributed, further analysis was conducted using the Kruskal-Wallis test. The Kruskal-Wallis test revealed a significant difference between groups (p = 0.026) (Table 3).

Table 3.
Kruskal-Wallis Test Results

Statistic	Value
Kruskal-Wallis H	7,333
df	2
Asymp. Sig.	0,026

A p-value < 0.05 indicated a significant difference between the 10-minute, 20-minute, and 30-minute staining groups. Based on these findings, a staining time of 20 minutes provided the best quality for microscopic identification of malaria using 10% Giemsa.

DISCUSSION

Microscopic examination of thin blood smears remains the gold standard for malaria diagnosis and identification of Plasmodium species and stages (Potutu, 2022; Pootschi, 2017). According to Misnarliah (2023), the results of the examination of malaria parasites on thin blood smears, the color of the preparation is clear, and easy to identify. To obtain effective microscopic examination results, it is necessary to determine the optimal time and concentration of Giemsa (Syaifudin et al., 2018). In this study, 10 minutes, 20 minutes, and

30 minutes Giemsa 10% staining times were tested to determine the optimal staining duration for blood smears. The results showed that staining for 20 minutes yielded the best quality, with all samples (100%) scoring in the good category. Staining for 20 minutes provided clear backgrounds, blue cytoplasm, and red nuclei, aligning with the criteria for high-quality thin blood smears according to Kemenkes RI (2017) and Andayani (2016). These findings are consistent with Hassor (2023), who also reported that 20 minutes produced the best staining results for malaria blood smears. At this time, the parasite mass after being stained with Giemsa solution, appears clear and compact (solid). Giemsa solution stains chromatin (parasite nucleus) to carmine red (fire red) and stains cytoplasm (parasite cell fluid) to purplish blue (Gestiawan, 2020).

Staining for 10 minutes resulted in 6 of 15 samples with poor quality, characterized by unclear backgrounds and indistinct Plasmodium morphology. Short staining times may result in inadequate absorption of the stain, as noted by Sathpathi et al. (2014) and Worung et al. (2020). However, according to Meiliza (2024), within 10 minutes, the quality of the results of coloring malaria preparations at a temperature of 20-25C 10% had good color intensity. Although WHO (2010) permits rapid Giemsa staining within 8–10 minutes, this study indicates that, using the stock Giemsa at Natar Medika Hospital, 10 minutes was not sufficient for optimal staining.Meanwhile, 30-minute staining produced overly dark slides, making parasite morphology difficult to interpret. This finding is consistent with observations by Hassor (2023), where 30-minute staining resulted in unclear backgrounds, with excessive blue or red tones causing overlapping colors that obscured parasite visibility. Also, Pandey et al. (2023) and Yagmur et al. (2015) reported that prolonged Giemsa staining results in overly dark coloration of blood cells and parasites, which hampers accurate identification and differentiation of Plasmodium species.After tabulating the staining results scores for each staining time of 10 minutes, 20 minutes, and 30 minutes, a normality tes was carried out using SPSS. If the p-value is greater than 0.05, the data is considered normally distributed. Conversely, if the p-value is less than or equal to 0.05, the data is considered not normally distributed (Ahadi and Zain, 2023; Lantz et al., 2016). Normality testing using the Shapiro-Wilk test showed that all staining time groups had a significance value (p) of 0.000, indicating that the data were not normally distributed (Table 2).

As the data were not normally distributed, further analysis was conducted using the Kruskal-Wallis test (Ostertagová dkk., 2014; Victor dkk., 2022). Statistical analysis using the Kruskal-Wallis test showed a significant difference among staining time groups ($p = 0.026$), confirming that staining time affects the quality of malaria blood smear preparation. Therefore, the null hypothesis was rejected. The alternative hypothesis, suggesting that 10 minutes is the optimal staining time, was also rejected. Instead, 20 minutes was established as the optimal staining time for Giemsa 10% at Natar Medika Hospital.Operational challenges such as the limited number of medical laboratory personnel at Natar Medika Hospital were identified as factors that must be addressed to maintain diagnostic quality. Hospital management should consider adjusting human resource allocation to ensure rapid and accurate malaria diagnosis. Overall, this study reinforces the importance of local validation of Giemsa staining methods and supports the adoption of a 20-minute staining standard for accurate microscopic malaria diagnosis.

CONCLUSION

Based on the results and discussion, it can be concluded that there is variation in the microscopic quality of malaria blood smears using 10% Giemsa staining at different times (10, 20, and 30 minutes) at Natar Medika Hospital. Staining for 20 minutes yielded the best quality, with all samples showing good staining results characterized by a clear background, blue cytoplasm, and red nuclei. Therefore, 20 minutes is recommended as the standard staining time for 10% Giemsa in microscopic examination of malaria at Natar Medika

Hospital.

REFERENCES

Ahadi, G. D. dan Zain, N. N. L. E. (2023). Pemeriksaan Uji Kenormalan Dengan Kolmogorov-

Smirnov, Anderson-Darling Dan Shapiro-Wilk. Eigen Mathematics Journal, 11–19. hlm. https://doi.org/10.29303/emj.v6i1.131

Andayani, G.A.S. & Sriasih, N.M. (2016). The Quality Of Colouring And Time Efectivity Of Staining

Using Rapid St Reagensia Compared To Giemsa Dye On The Identification Of Malaria Slide. Karya Ilmiah PLP pada SENASLAB Universitas Udayana, 255-231.

Gestiawan, Y. dkk. (2020). Hubungan di Temukannya Stadium Tropozoit Parasit Malaria (Plasmodium sp) Dengan Gejala Klinik Awal Penderita Yang Ada di Beberapa desa Kecamatan Simpenan Kabupaten Sukabumi. Technology in Medical Laboratory for Environmental Disease, Vol. 1, No. 1.

Hassor, R.S., Mulia, Y.S., Solihat, M.F., & Sulaeman. (2023). Analisis Perbandingan Waktu Pewarnaan Menggunakan Giemsa 10% Terhadap Hasil Sediaan Darah Malaria. Jurnal Kesehatan Siliwangi, Vol. 4, No. 1.

Kemenkes RI. (2017). Pedoman Teknis Parasit Malaria. Direktorat Pencegahan Dan Pengendalian

Penyakit Tular Vektor Dan Zoonotik Kementerian Kesehatan Republik Indonesia.

Kemenkes RI. (2023). Buku Saku Tatalaksana Kasus Malaria. Jakarta: Kementerian Kesehatan Republik Indonesia.

Kemenkes RI. (2024). Kasus Malaria di Indonesia. https://malaria.kemkes.go.id/case. Diakses pada tanggal 29 Oktober 2024.

Lantz, B., Andersson, R. dan Manfredsson, P. (2016). Preliminary Tests of Normality When

Comparing Three Independent Samples. Journal of Modern Applied Statistical Methods, 15(2), 135–148. hlm. https://doi.org/10.22237/jmasm/1478002140

Meiliza, L. (2024). Kualitas Hasil Pewarnaan Malaria Dengan Giemsa Pada Suhu Dan Konsentrasi 20

- 25°C 10% Dan 36 - 37°C 5% Selama 10 Menit. Skripsi. Poltekkes Kemenkes Yogyakarta.

Misnarliah, Wulandhani, S., Mudrika. (2023). Gambaran Hasil Pemeriksaan Mikroskopik Apusan

Darah Parasit Malaria Dengan Teknik Apusan Darah Yang Berbeda. JOA: Journal Omicron Adpertisi, Vol. 2, No. 2, 13-18.

Open Data Lampung. (2023). Kesakitan Dan Kematian Akibat Malaria Menurut Jenis Kelamin

Kabupaten/Kota Se-Provinsi Lampung Tahun 2022. https://opendata.lampungprov.go.id/dataset/kesakitan-dan-kematian-akibat-malaria-menurut-jenis-kelamin-kabupatenkota-seprovinsi-lampung-tahun-2022. Diakses pada tanggal 29 Oktober 2024.

Ostertagová, E., Ostertag, O. dan Kovác, J. (2014). Methodology and Application Of the Kruskal-

Wallis Test. Applied Mechanics and Materials, 611, 115–120. hlm. https://doi.org/10.4028/www.scientific.net/amm.611.115

Pandey, O., Hona, E., Shrestha, E., Khadka, V. dan Ghising, T. (2023). Unusual Presentation and

Difficult to Diagnose: A Case of Malaria With Negative Thick and Thin Giemsa Stain Smear Tests. Cureus. https://doi.org/10.7759/cureus.39675

Pramudiyatika, R.A. (2022). Pengaruh Waktu Pewarnaan Giemsa Dengan Variasi Konsentrasi Pada

Pemeriksaan Mikroskopik Malaria di Puskesmas Remu Kota Sorong. Naskah Publikasi. Fakultas Ilmu Kesehatan Universitas Aisyiyah Yogyakarta.
Pootschi, M., et al. (2017). Image Analysis And Machine Learning For Detecting Malaria.
Translational Research, Vol. 194, 36-55.
Potutu, N.W.R. (2022) Optimalisasi Waktu Pewarnaan Giemsa 10% Pada Pemeriksaan Mikroskkopik
Malaria. Naskah Publikasi. Fakultas Ilmu Kesehatan Universitas Aisyiyah Yogyakarta.
Sathpathi, S., et al. (2014). Comparing Leishman and Giemsa Staining for the Assessment of Peripheral
Blood Smear Preparations in a Malaria-Endemic Region in India. Malaria Journal, 13(1). https://doi.org/10.1186/1475-287513-512
Syaifudin, M., Irma, I., & Ramadhani, D. (2018). Optimalisasi Pewarnaan Giemsa Pada Apusan Darah
Tipis Terinfeksi Plasmodium Berghei Untuk Mendukung Vaksin Malaria Iradiasi. Jurnal Biotek Medisiana Indonesia, Vol. 7.1, 77-84.
Victor, E. U., Ugoh, C. I., Eze Francis Chukwuemeka, Omeje, I. L. dan Besiru, M. (2022). Analytical
Methods in Proximate Composition of Ten Commonly Used Seasonings in Nigeria. European Journal of Food Science and Technology, 10(2), 24–34. hlm. https://doi.org/10.37745/ejfst.2013/vol10n2pp2434
WHO. (2010). Basic Malaria Microscopy Part I. Learner's Guide. Geneva: World Health
Organization.
WHO. (2016). Giemsa Staining of Malaria Blood Films. Malaria Microscopy Standard Operating
Procedure – MM-SOP-07A. Geneva: World Health Organization
WHO. (2023). World Malaria Report 2023. Geneva: World Health Organization.
Worung, I. M., Mahartini, N. N. dan Herawati, S. (2020). Hitung Trombosit Metode Otomatis
Dikonfirmasi Dengan Hapusan Darah Tepi (HDT) Tanpa Pewarnaan Dan Dengan Pewarnaan Giemsa Di RSUP Sanglah, Bali, Indonesia. Intisari Sains Medis, 11(3), 1387–1391. hlm. https://doi.org/10.15562/ism.v11i3.799
Yagmur, G., et al. (2015). Two Cases of Malaria Due to Plasmodium Falciparum Resulting in Death:
Postmortem Identification. Romanian Journal of Legal Medicine, 23(1), 33–36. hlm. https://doi.org/10.4323/rjlm.2015.33