



Comparison of diffcount results using Giemsa and Wright staining in immediate and delayed samples

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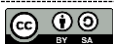
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Abstract

Background: Differential leukocyte counting is an important hematological examination that relies on accurate identification of leukocyte morphology in peripheral blood smears. Examination delays may affect cellular morphology and differential leukocyte count results, while staining methods may produce different microscopic characteristics. **Objectives:** This study aimed to compare differential leukocyte count results using Giemsa and Wright staining in immediate and delayed blood smear examinations. **Materials and Methods:** An experimental study with a *static group comparison* design was conducted using four EDTA-anticoagulated blood samples from students of Universitas Nahdlatul Ulama Surabaya. Each sample was prepared into eight peripheral blood smear slides, resulting in 32 preparations examined immediately and after 6, 12, and 24 hours using Wright and Giemsa staining. Differential leukocyte counts were performed microscopically at 1000× magnification. Data were analyzed using the Friedman test with $\alpha = 0.05$. **Results:** Descriptive variations were observed in basophils, eosinophils, band neutrophils, segmented neutrophils, lymphocytes, and monocytes across staining methods and examination times. However, the Friedman test showed no statistically significant differences for basophils ($p = 0.066$), eosinophils ($p = 0.864$), band neutrophils ($p = 0.763$), segmented neutrophils ($p = 0.066$), lymphocytes ($p = 0.954$), and monocytes ($p = 0.398$). Microscopic observations showed that leukocyte morphology remained identifiable despite differences in staining intensity and cellular contrast. **Conclusions:** Examination delays of up to 24 hours did not significantly affect differential leukocyte count results using Giemsa and Wright staining under the conditions of this study.

Keywords: *Differential Leukocyte Count, Giemsa Staining, Peripheral Blood Smear, Wright Staining*



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1. Introduction

Leukocytes are important components of the immune system that play a role in defending the body against various infections and diseases. The differential leukocyte count (*diffcount*) using peripheral blood smear examination provides information regarding the proportion and morphology of neutrophils, eosinophils, basophils, lymphocytes, and monocytes. The quality of a peripheral blood

smear is an important factor in determining the accuracy of leukocyte identification and morphological interpretation. Examination of eosinophil morphology on peripheral blood smears shows that differences in staining techniques can produce variations in cellular morphology, indicating that the selection of an appropriate staining method is an important aspect of hematological examination (Ardina & Rosalinda, 2020).

Delayed examination of blood samples is another factor that requires attention because it may affect the quality of hematological examination results. Blood storage at room temperature for a certain period can cause changes in hematological parameters, including leukocyte parameters. Nugraha et al. (2021) showed that hematological examination of blood samples left at room temperature resulted in changes in several examination parameters. These findings indicate that the time interval between blood collection and examination needs to be considered to obtain results that accurately represent the condition of the sample. Prolonged delays in examination may also affect cellular morphology and potentially influence the identification and interpretation of leukocytes during microscopic examination.

The staining method also plays an important role in producing clear visualization of leukocytes on peripheral blood smears. Giemsa and Wright stains are commonly used to enhance the visualization of blood cell structures, including nuclei, cytoplasm, and leukocyte granules. Fathun et al. (2025) compared the morphological characteristics of granulocytes using Wright, Giemsa, and May-Grünwald-Giemsa staining. The results showed that different staining methods could produce variations in granulocyte morphology that may support the identification of cells. Indrawan et al. (2025) also showed that variations in Giemsa concentration and incubation time need to be considered because they can affect the quality of peripheral blood smear staining.

Research on peripheral blood smear staining has also been conducted using various types of stains. Khasanah et al. (2023) showed that the use of different staining materials on peripheral blood smears could produce cellular appearances that can be observed microscopically. Hasibuan (2025) also compared the effectiveness of alternative stains on peripheral blood smears. Ardina and Rosalinda (2020) specifically compared eosinophil morphology using Giemsa, Wright, and combined Wright-Giemsa staining. These studies indicate that staining method and sample preparation conditions are important aspects in obtaining adequate leukocyte morphology. However, previous studies have not specifically compared differential leukocyte count (*diffcount*) results using Giemsa and Wright staining in samples examined immediately and after delays of 6, 12, and 24 hours.

The limited evidence regarding the combined comparison of staining methods and examination delays represents a gap in previous research. Previous studies have generally focused on morphological characteristics, staining quality, or specific leukocyte types. Research specifically evaluating the results of *diffcount* for neutrophils, eosinophils, basophils, lymphocytes, and monocytes using Giemsa and Wright staining at different examination times remains limited. The novelty of this study lies in combining the staining method and sample examination delay to evaluate differential leukocyte count results. This approach is important because differences in staining characteristics and changes in blood cell morphology during sample storage may influence leukocyte identification and *diffcount* interpretation.

This study aimed to determine and compare differential leukocyte count (*diffcount*) results using Giemsa and Wright staining in samples examined immediately and after delays of 6, 12, and 24 hours. The study also aimed to determine whether there were differences in *diffcount* results between Giemsa and Wright staining at each examination time. The findings are expected to provide information regarding the influence of staining method and sample examination delay on differential leukocyte count results and to serve as a consideration for laboratory personnel in selecting an appropriate staining method when blood samples cannot be processed immediately.

2. Materials and Methods

2.1 Research Design

This study used an experimental method with a *static group comparison* design. The study was conducted to compare differential leukocyte count (*diffcount*) results on peripheral blood smears using Giemsa and Wright staining at four examination times: immediately, 6 hours, 12 hours, and 24 hours. The timing was based on previous research showing changes in the scattergram when using the CELL-DYN RUBY hematology analyzer at time intervals of 6, 12, and 24 hours (Nugraha, 2021). The study was conducted at the Department of Health Analyst, Faculty of Health, Universitas Nahdlatul Ulama Surabaya.

2.2 Samples and Specimens

The specimens used in this study were EDTA-anticoagulated blood samples obtained from four students of Universitas Nahdlatul Ulama Surabaya. Each blood sample was prepared into eight peripheral blood smear preparations, resulting in a total of 32 preparations. The preparations consisted of samples examined immediately and samples examined after delays of 6, 12, and 24 hours using Giemsa and Wright staining.

2.3 Examination Procedure

Peripheral blood smear preparations were examined microscopically to determine the differential leukocyte count, including neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Leukocyte identification was performed using a microscope at 1000× magnification. The results obtained from each preparation were recorded and subsequently compared according to the staining method and examination time.

2.4 Data Analysis

The differential leukocyte count data obtained from the microscopic examination were recorded and analyzed statistically. Statistical analysis was performed using a significance level of $\alpha = 0.05$. The statistical test was selected according to the distribution and characteristics of the data to determine differences in differential leukocyte count results based on staining method and examination time.

3. Results and Discussion

3.1 Characteristics of Research Subjects

This study was conducted to determine differences in differential leukocyte count (*diffcount*) results using Giemsa and Wright staining based on examination delay. The study involved four female subjects aged 21–22 years. Examinations were performed immediately and after delays of 6, 12, and 24 hours. The parameters examined included basophils, eosinophils, band neutrophils, segmented neutrophils, lymphocytes, and monocytes.

Table 1. Characteristics of research subjects based on age and sex

Characteristics	Frequency (n)	Percentage (%)
Age		
21 years	2	50.0
22 years	2	50.0
Sex		
Male	0	0.0
Female	4	100.0
Total	4	100.0

Table 1 shows that two subjects (50.0%) were 21 years old and two subjects (50.0%) were 22 years old. All subjects were female, comprising four subjects (100.0%), while no male subjects were included. The study received ethical approval from the Health Research Ethics Committee of Universitas Nahdlatul Ulama Surabaya under approval number 0046/EC/KEPK/UNUSA/2026.

3.2 Differential Leukocyte Count Results Based on Staining Method and Examination Delay

Differential leukocyte count examinations were performed using Wright and Giemsa staining under immediate examination conditions and after delays of 6, 12, and 24 hours. The mean and standard deviation values of the six leukocyte types are presented in Table 2.

Table 2. Mean (mean $\pm$ SD) differential leukocyte count results based on staining method and examination delay (%)

Leukocyte type	Wright immediate	Wright 6 h	Wright 12 h	Wright 24 h	Giemsa immediate	Giemsa 6 h	Giemsa 12 h	Giemsa 24 h
Basophils	0.75 $\pm$ 0.96	0.75 $\pm$ 0.96	1.00 $\pm$ 0.82	0.25 $\pm$ 0.50	0.75 $\pm$ 0.96	0.75 $\pm$ 0.50	0.75 $\pm$ 0.50	0.25 $\pm$ 0.50
Eosinophils	1.25 $\pm$ 0.96	1.25 $\pm$ 0.50	1.00 $\pm$ 0.82	1.00 $\pm$ 0.82	1.25 $\pm$ 0.96	1.25 $\pm$ 0.50	0.75 $\pm$ 0.50	1.00 $\pm$ 0.82
Band neutrophils	5.25 $\pm$ 2.50	5.50 $\pm$ 3.11	4.75 $\pm$ 0.96	5.75 $\pm$ 1.26	4.50 $\pm$ 1.00	4.75 $\pm$ 2.36	4.75 $\pm$ 0.96	6.00 $\pm$ 0.82
Segmented neutrophils	52.75 $\pm$ 1.50	52.50 $\pm$ 2.08	50.75 $\pm$ 1.50	50.75 $\pm$ 3.30	54.75 $\pm$ 3.30	53.00 $\pm$ 3.56	51.25 $\pm$ 1.26	49.00 $\pm$ 1.41
Lymphocytes	35.25 $\pm$ 2.50	37.25 $\pm$ 2.87	36.25 $\pm$ 1.26	37.25 $\pm$ 3.20	35.75 $\pm$ 4.27	36.25 $\pm$ 3.30	36.25 $\pm$ 1.26	38.25 $\pm$ 2.87
Monocytes	4.75 $\pm$ 1.50	4.25 $\pm$ 2.87	5.75 $\pm$ 0.96	5.25 $\pm$ 0.96	4.25 $\pm$ 1.26	4.00 $\pm$ 2.83	6.00 $\pm$ 0.82	5.00 $\pm$ 0.82

Table 2 shows variations in differential leukocyte count values across staining methods and examination times. The highest basophil value was found in Wright staining after 12 hours (1.00 $\pm$ 0.82%), while the lowest was found in Wright and Giemsa staining after 24 hours (0.25 $\pm$ 0.50%). Eosinophils reached 1.25% in several groups, band neutrophils were highest in Giemsa staining after 24 hours (6.00 $\pm$ 0.82%), segmented neutrophils in immediate Giemsa staining (54.75 $\pm$ 3.30%), lymphocytes in Giemsa staining after 24 hours (38.25 $\pm$ 2.87%), and monocytes in Giemsa staining after 12 hours (6.00 $\pm$ 0.82%). Although descriptive variations occurred, no consistent increase or decrease was observed. Both Wright and Giemsa staining maintained identifiable leukocyte morphology. Anwar (2023) explained that Giemsa, Wright, and Wright-Giemsa staining can be used for differential leukocyte counting, with each providing different visual characteristics.

Basophil and eosinophil values showed relatively small variations. Basophils ranged from 0.25 $\pm$ 0.50% to 1.00 $\pm$ 0.82%, with a Friedman *p*-value of 0.066. Tigner et al. (2026) explained that basophil identification depends on nuclear and cytoplasmic granule morphology, while their low proportion may increase counting variation. Eosinophils ranged from 0.75 $\pm$ 0.50% to 1.25 $\pm$ 0.96%. Anggraeni (2025) showed that Wright staining can demonstrate granulocyte morphology through nuclear shape and cytoplasmic granules, while Anwar (2023) explained that staining selection affects cellular structure visibility. Despite these variations, eosinophil values did not show a consistent pattern or statistically significant difference.

Band neutrophils ranged from 4.50 $\pm$ 1.00% to 6.00 $\pm$ 0.82%, with a Friedman *p*-value of 0.763. Fathun et al. (2025) showed that staining methods can produce different granulocyte appearances, while Anwar (2023) described differences in Giemsa and Wright visualization. Segmented neutrophils

ranged from $49.00 \pm 1.41\%$ to $54.75 \pm 3.30\%$, with a p -value of 0.066. Elhassan et al. (2023) showed that nuclear morphology is important for leukocyte classification. Thus, although descriptive variations were observed, neither band nor segmented neutrophils showed statistically significant differences.

Lymphocyte values ranged from $35.25 \pm 2.50\%$ to $38.25 \pm 2.87\%$, with a Friedman p -value of 0.954, while monocytes ranged from $4.00 \pm 2.83\%$ to $6.00 \pm 0.82\%$, with a p -value of 0.398. Nursalsabila (2025) explained that differential leukocyte examination describes the distribution of individual leukocyte types, while Puspitasari (2025) emphasized its importance as a hematological parameter. Elhassan et al. (2023) explained that monocyte identification is based on cell size, nuclear shape, and cytoplasmic characteristics. Overall, the observed variations in lymphocytes and monocytes were not statistically significant, indicating that examination delays of up to 24 hours did not substantially affect differential leukocyte counts under the conditions of this study.

3.3 Microscopic Characteristics of Peripheral Blood Smears

Microscopic examination was performed to observe leukocyte morphology on peripheral blood smears stained using Wright and Giemsa at different examination times. Representative microscopic images are presented in Figure 1.

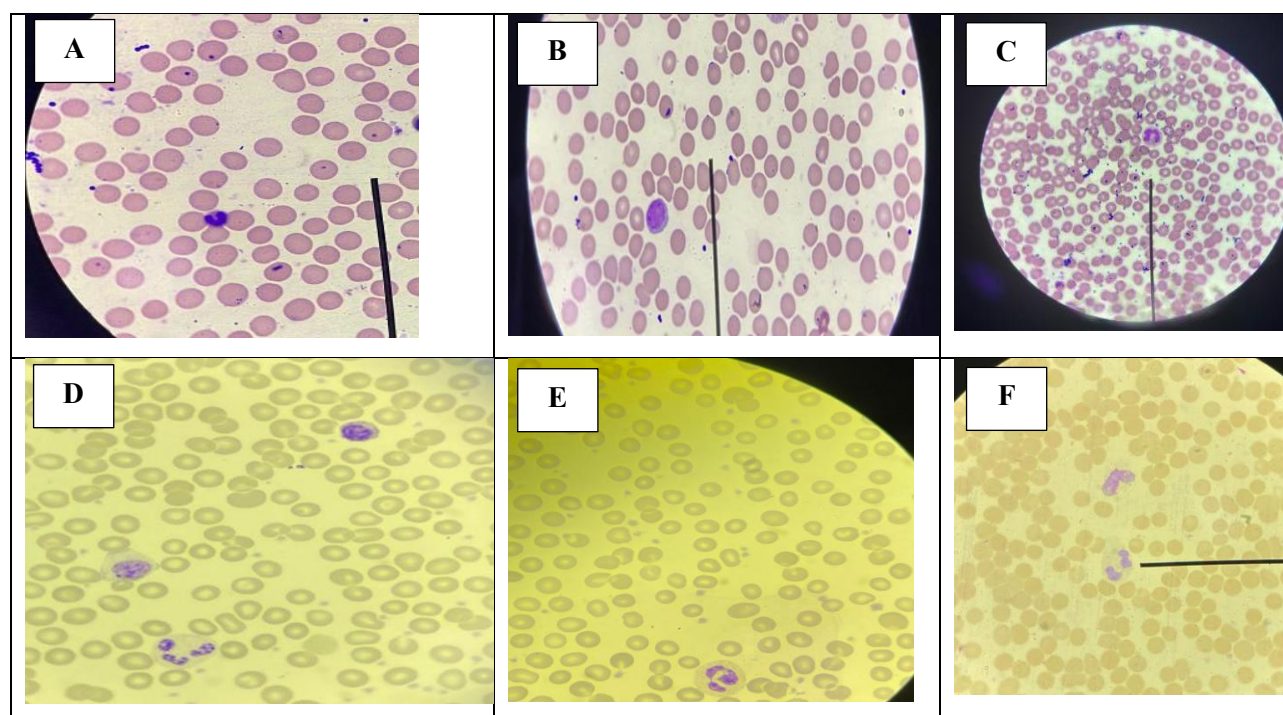


Figure 1. Microscopic appearance of peripheral blood smears stained using Wright and Giemsa at different examination times. (A) Immediate Wright staining, band neutrophil; (B) Wright staining after a 6-hour delay, lymphocyte; (C) Wright staining after a 24-hour delay, band neutrophil; (D) Immediate Giemsa staining, band neutrophil and lymphocyte; (E) Giemsa staining after a 6-hour delay, segmented neutrophil; (F) Giemsa staining after a 24-hour delay, segmented neutrophil.

The microscopic observations in Figure 1 show that leukocyte morphology remained observable and identifiable in both Wright- and Giemsa-stained preparations after different examination delays. Differences were mainly observed in staining intensity, contrast between the nucleus and cytoplasm, and granule clarity. Although changes in visual appearance were observed following examination delays, the main characteristics of leukocytes based on nuclear shape, cytoplasm, and granules remained recognizable.

Anwar (2023) showed that Wright, Giemsa, and Wright-Giemsa staining have different staining characteristics for various blood cell components. Dewi and Aryandi (2022) showed that staining quality in peripheral blood smears contributes to the ability to observe cellular morphology. Wagut et al. (2025) also showed that granulocyte characteristics can be observed through blood cell morphological examination. These findings support the present observations because leukocyte types remained identifiable across the different treatment groups. Differences in staining intensity observed microscopically did not directly result in statistically significant changes in differential leukocyte counts.

3.4 Friedman Test Results and Statistical Interpretation

Statistical analysis was performed using the Friedman test to determine differences in differential leukocyte count results based on examination delay. The Friedman test was selected because the data were not entirely normally distributed based on the Shapiro–Wilk test. The results are presented in Table 3.

Table 3. Friedman test results for differential leukocyte count based on examination delay

Leukocyte type	<i>p</i> -value	Interpretation
Basophils	0.066	Not significant
Eosinophils	0.864	Not significant
Band neutrophils	0.763	Not significant
Segmented neutrophils	0.066	Not significant
Lymphocytes	0.954	Not significant
Monocytes	0.398	Not significant

Table 3 shows that all leukocyte types had *p*-values >0.05, namely 0.066 for basophils, 0.864 for eosinophils, 0.763 for band neutrophils, 0.066 for segmented neutrophils, 0.954 for lymphocytes, and 0.398 for monocytes. These results indicate that there were no statistically significant differences in differential leukocyte counts based on examination delay. The lowest *p*-values were observed for basophils and segmented neutrophils at 0.066, but these values remained higher than 0.05. Therefore, examination delays of up to 24 hours under the conditions of this study did not produce statistically significant differences in differential leukocyte counts using Giemsa and Wright

staining.

The absence of statistically significant differences indicates that the descriptive variations observed in Table 2 were not sufficiently large to demonstrate meaningful differences between examination times. The findings also correspond with the microscopic observations in Figure 1, in which the main morphological characteristics of leukocytes remained identifiable despite differences in staining appearance. This suggests that the examination delay under the conditions used in this study did not substantially affect the ability to perform differential leukocyte counting.

Technical conditions during smear preparation and staining should also be considered when interpreting these findings. Putri and Solikhati (2026) showed that variations in phosphate buffer pH can affect the quality of Giemsa staining in differential leukocyte examination. Research concerning drying and staining conditions also indicates that changes in processing conditions can affect cell size and leukocyte classification, although some changes in staining conditions do not result in statistically significant differences in differential leukocyte counts. Standardization of smear preparation and staining procedures is therefore necessary to minimize technical variation. Fardani and Ningty (2025) and Hanafiah et al. (2025) demonstrated the importance of morphological and cytological examination in evaluating cellular characteristics in biological research. Relatively standardized preparation conditions in this study may have contributed to the continued identification of leukocytes across all examination times.

The findings of this study should also be interpreted in relation to its limitations. The study involved only four subjects, which limits the generalizability of the findings and may reduce the ability to detect small differences between examination conditions. The results therefore describe the experimental conditions of this study and should not be generalized to a broader population without further investigation. Future research should involve a larger number of subjects and more strictly control blood storage conditions, smear preparation, staining concentration, buffer pH, and staining duration. These approaches would provide stronger evidence regarding the effects of examination delay and staining method on differential leukocyte count results.

4. Conclusions

The study concludes that examination delays of up to 24 hours did not produce statistically significant differences in differential leukocyte count results using Giemsa and Wright staining. Although descriptive variations were observed in basophils, eosinophils, band neutrophils, segmented neutrophils, lymphocytes, and monocytes, no consistent pattern of increase or decrease was found. The Friedman test showed p -values >0.05 for all leukocyte types, indicating that the

observed variations were not statistically significant. Microscopic observations also showed that the main morphological characteristics of leukocytes remained identifiable in both staining methods, despite differences in staining intensity, nuclear and cytoplasmic contrast, and granule clarity. Therefore, Giemsa and Wright staining remained suitable for differential leukocyte identification under examination delays of up to 24 hours in the conditions of this study. Further research with larger samples and better control of storage and staining conditions is recommended to strengthen these findings.

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