Jurnal Kesehatan
Volume 16, Number 2, August 2025
ISSN 2086-7751 (*Print*), ISSN 2548-5695 (*Online*)
http://ejurnal.poltekkes-tjk.ac.id/index.php/JK

Optimization of Whole Blood Specimen Handling for Complete Blood Count (CBC) Results and Blood Cell Morphology

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ARTICLE INFO

Article history

Submitted:
5 Jul 2025

Revise:
27 Aug 2025

Accepted:
22 Sept 2025

Keywords*:*

Anticoagulant;
Complete blood count;
Delay time;
Blood cell morphology;
Whole blood.

ABSTRACT

Hematology analyzers are used in almost all clinical laboratories for CBC testing. CBC test results are crucial, as they play a significant role in establishing diagnoses and determining the appropriate therapy for patients. This study aims to analyze the effect of anticoagulant type, storage temperature, and delay time on the results of erythrocyte, leukocyte, and thrombocyte counts in whole blood samples. The study design employed was a laboratory experiment with a total of 64 samples, which were subjected to a combination of treatments involving anticoagulant type (Vacutainer and conventional), storage temperature (room temperature and refrigerator), and delay time for testing (0, 6, 12, and 24 hours). The examination was conducted using a hematology analyzer, and data analysis was performed using the Kruskal–Wallis test and the post hoc Mann–Whitney U test. The results showed that there was no significant effect of the three factors on the number of erythrocytes and leukocytes. However, the delay time significantly affected the platelet count (p=0.004), with significant differences observed between 6 hours and 24 hours, and between 12 hours and 24 hours. The delay in blood examination with K_3EDTA and Na_2EDTA affects the morphology of erythrocytes and leukocytes. Abnormal changes occur after 6, 12, and 24 hours of storage. The gold standard is a fresh whole blood sample collected in a K3EDTA vacutainer tube with the correct volume and analyzed within a maximum of 2 hours at room temperature.

INTRODUCTION

Health laboratories are essential facilities that support health services, providing the necessary examination, analysis, interpretation, and identification of specimens to establish a diagnosis for patients (Kementerian Kesehatan Republik Indonesia, 2022). The laboratory quality control process consists of three essential stages: pre-analytical, analytical, and post-analytical stages. The pre-analytical stage encompasses all activities conducted before specimen examination, including patient preparation, specimen collection, and specimen handling. Improper specimen handling can result in invalid results because the specimen is unsuitable for examination (Hasan et al., 2017).

The pre-analytical stage plays an important role in specimen quality. Errors in the pre-analytical stage account for 50-75% of errors, including identification errors and specimen problems (Plebani et al., 2014). Errors in specimen collection and handling, such as clotting in whole blood, improper specimen volume, hemolysis, anticoagulants, and improper specimen storage temperature, can affect specimen stability (McPherson & Pincus, 2011).

The addition of anticoagulants to specimens is a pre-analytical step that affects the accuracy of testing (Naz et al., 2012). Ethylene Diamine Tetra Acetate (EDTA) is a commonly used anticoagulant in hematology laboratories for Complete Blood Count (CBC) testing. EDTA anticoagulants include conventional EDTA (10% Na_2EDTA solution) and EDTA vacutainers (K_2EDTA/K_3EDTA in vacuum tubes) (Kuman, 2019). Anticoagulants in vacutainers tend to be more expensive, so some laboratories still use conventional anticoagulants (Wahdaniah &

Tumpuk, 2018). In previous studies, significant differences were observed in platelet count results between EDTA vacutainers and conventional EDTA (Sigit & Aini, 2013). However, another study showed no difference (p=0.822) in red blood cell counts between conventional EDTA and vacutainers (Dewi et al., 2022)

Hematology analyzers are used in almost all clinical laboratories for CBC testing. These automated instruments have the disadvantage of being unable to identify cell morphology, so peripheral blood smear testing is required. This test is used to confirm warning signs (flagging) indicated by the instrument if there are suspected factors that may be affecting the results. Peripheral blood smear examinations can identify blood cell morphology and estimate their numbers, particularly for observing abnormal blood cell morphology not detected by automated instruments. CBC test results are crucial, as they play a significant role in establishing diagnoses and determining the appropriate therapy for patients. Delayed analysis of specimens can result in changes in the parameters being tested and complicate the interpretation of the results (Pintér et al., 2016).

Preceding research demonstrated that storage times of 3, 6, and 24 hours at room temperature storage (18–25°C) and refrigerator temperature storage (2–8°C) significantly affect CBC parameters for hematocrit levels (p=0.000) and platelet count (p=0.000), but do not affect hemoglobin levels (p=0.226), red blood cell count (p=0.297), and white blood cell count (p=0.664) (Puspitasari & Aliviameita, 2022). Another study concluded that red blood cells, platelets, hemoglobin, and Mean Corpuscular Hemoglobin (MCH) remain stable at room temperature for 24 hours. A temperature of 4°C is the most suitable storage temperature for this product. Another study found that CBC results did not undergo significant changes when stored at room temperature or 4 °C for 2 hours, but a significant effect was observed when storage lasted for 8 hours (Palmer et al., 2015). Another study states that the optimal time for performing a peripheral blood smear examination is storage for 2–8 hours at room temperature (18–22°C) and 12–24 hours at refrigerator temperature (4–8°C) (Palmer et al., 2015). Another study showed changes in erythrocyte morphology during storage at room temperature for 8 hours and at refrigerator temperature for 24 hours (Rahmnitarini et al., 2019).

Based on the above description, it is evident that specimen handling can impact test results. Therefore, further research is needed to optimize whole blood specimen handling in relation to CBC results and blood cell morphology.

METHOD

This study has passed the research feasibility test conducted by the Nahdlatul Ulama University Surabaya Health Research Ethics Commission, as certified by certificate number 0136/EC/KEPK/UNUSA/2025. The research design in this study is a quantitative analysis using laboratory experimental methods. The study was conducted at the Clinical Pathology Laboratory of the D4 Medical Laboratory Technology Program at Universitas Muhammadiyah Sidoarjo in May 2025. The population used in this study was blood from healthy individuals. This study utilized 64 blood samples, specifically blood from male students in the Medical Laboratory Technology Program at the Faculty of Health Sciences, Muhammadiyah University of Sidoarjo. The researchers collected blood samples from four respondents, each 14 mL in volume, with 2 mL in a Vacutainer tube and 2 mL in a conventional tube. Each sample underwent the following treatment: immediate examination (0 hours), storage for 6 hours at room temperature and refrigerator temperature, storage for 12 hours at room temperature and refrigerator temperature, and storage for 24 hours at room temperature and refrigerator temperature. That is a total of 64 samples that have been checked. Next, a CBC test was done using a hematology analyzer (Medonic M-32). Examination of blood smears using a microscope. Data analysis was performed using the Kruskal-Wallis nonparametric test with statistical applications.

RESULTS

Table 1. Results of the Kruskal-Wallis test on the number of erythrocytes, leukocytes, and thrombocytes based on three factors

Variable	Factor	Signifikansi (p)
RBC	Anticoagulant	0,747
	Temperature	0,436
	Deferral time	0,886
WBC	Anticoagulant	0,485
	Temperature	0,624
	Deferral time	0,906
Platelet	Anticoagulant	0,077
	Temperature	0,379
	Deferral time	0,004

Table 1 shows of the Kruskal-Wallis test on the number of erythrocytes showed that there were no significant differences based on the anticoagulant factor (p=0.747), storage temperature (p=0.436), or delay time for examination (p=0.886). The results of the Kruskal-Wallis test on the number of leukocytes showed that there were no significant differences based on anticoagulant factors (p=0.485), storage temperature (p=0.624), or delay time (p=0.906). The results of the Kruskal-Wallis test on platelet count showed no significant differences based on anticoagulant factor (p=0.077) or storage temperature (p=0.379), but showed a significant effect based on delay time factor (p=0.004).

Table 2. Post-Hoc test results (Mann-Whitney U) on platelet counts between deferral times

Deferral Time	Significance (p Bonferroni)
0 hours with 6 hours	1,000
0 hours with 12 hours	1,000
0 hours with 24 hours	0,327
6 hours with 12 hours	1,000
6 hours with 24 hours	0,004
12 hours with 24 hours	0,011

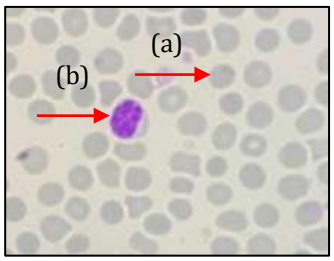
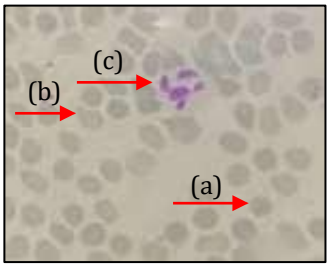
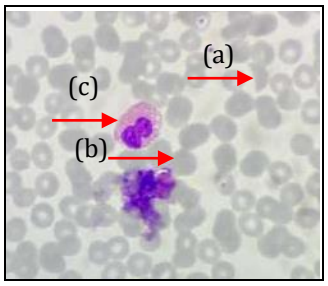
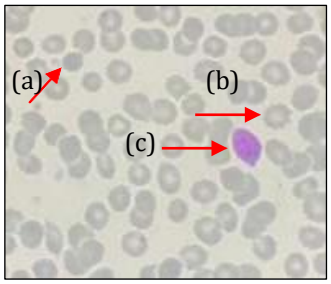
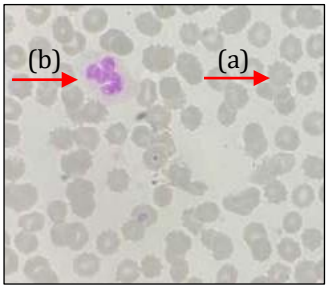
Table 2 shows no significant differences between the 0-hour delay group and the 6-hour delay group (p=1.000), the 0-hour delay group and the 12-hour delay group (p=1.000), the 0-hour delay group and the 24-hour delay group (p=0.327), and between the 6-hour delay group and the 12-hour delay group (1.000). However, there is a significant difference between the 6-hour delay group and the 24-hour delay group (p=0.004), as well as between the 12-hour delay group and the 24-hour delay group (p=0.011).

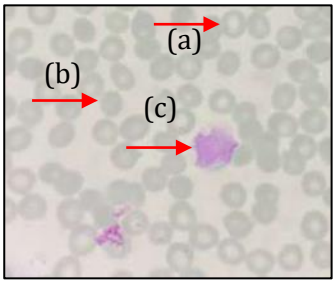
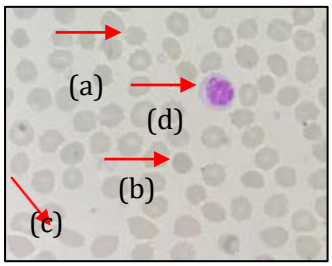
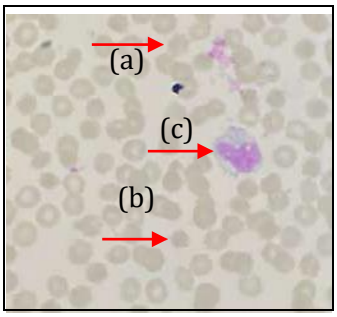
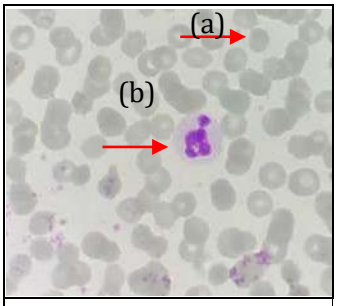
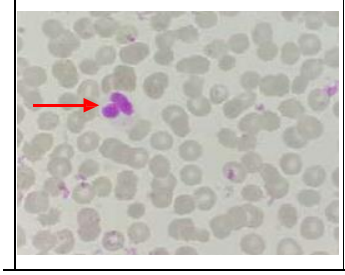
Table 3 shows that whole blood samples with K3EDTA anticoagulant stored at room temperature for 6 hours, 12 hours, and 24 hours caused abnormal changes in the morphology of erythrocytes and leukocytes. Abnormal erythrocytes found included spherocytes, burr cells, elliptocytes, and teardrop cells. The abnormal leukocytes identified were: abnormal homogeneous chromatin, hypersegmented neutrophils, vacuolated lymphocyte nuclei, and vacuolated monocytes. In whole blood samples with K3EDTA anticoagulant stored at cold temperatures for 6 hours, 12 hours, and 24 hours, abnormal erythrocytes were found, namely: helmet cells, spherocytes, and hypocromic cells. Abnormal leukocytes included: vacuolated neutrophils, hypogranulated neutrophils, smudge cells, hypersegmented neutrophils, and degranulation or Pelger-Huet forms.

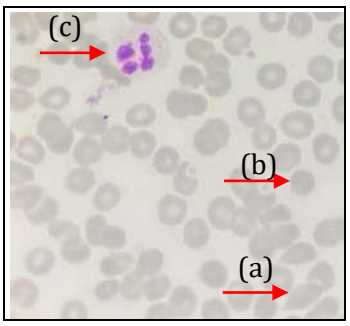
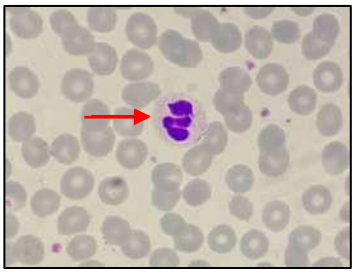
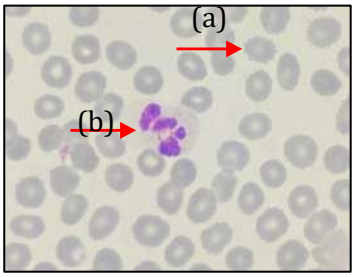
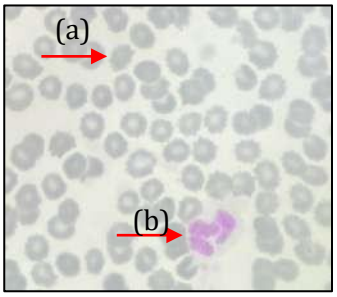
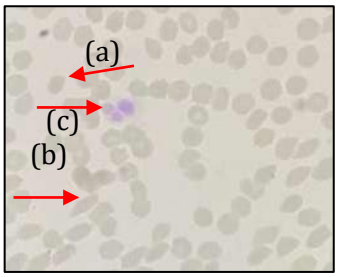
Delaying examination for 6 hours, 12 hours, and 24 hours at room temperature on whole blood samples with Na2EDTA anticoagulant caused changes in the morphology of erythrocytes and leukocytes. Abnormal erythrocytes observed include teardrop cells, spherocytes, burr cells, ovalocytes, and hypochromic cells. Abnormal leukocytes observed include: hypersegmented neutrophils, hypogranulated neutrophils, Pelger-Huet forms with degranulation, and abnormal homogeneous chromatin. Delaying examination for 6 hours, 12 hours, and 24 hours at cold temperatures on whole-blood samples with Na2EDTA anticoagulant caused changes in the morphology of erythrocytes and leukocytes. Abnormal

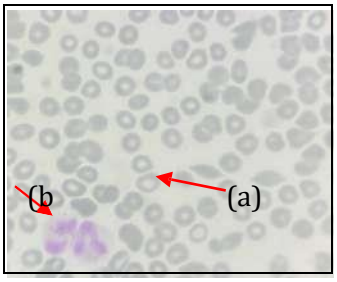
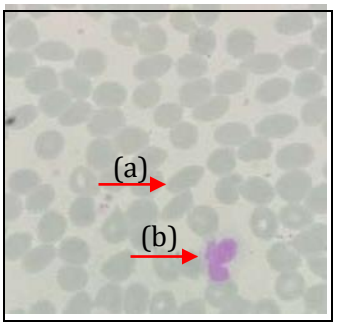
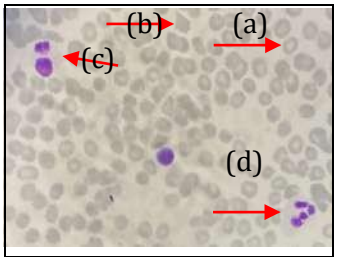
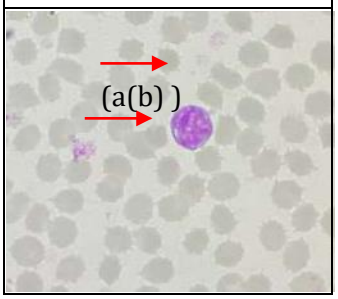
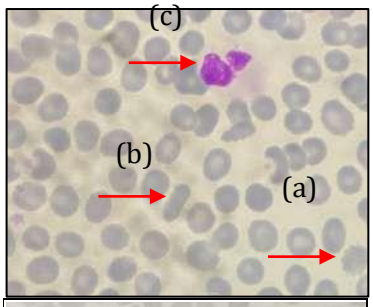
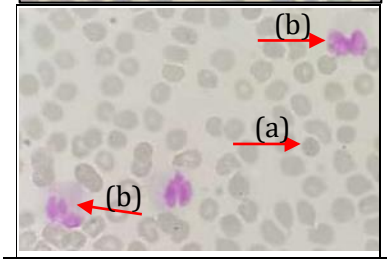
erythrocytes found included: burr cells, elliptocytes, hypochromic cells, and spherocytes. Abnormal leukocytes found included: elongated nucleoplasm neutrophils, hypersegmented neutrophils, hypogranulated neutrophils, and unidentified cells.

Table 3. Blood cell morphology in treatment variations (anticoagulants, temperature, and storage duration)

Type of treatment	
1. K3EDTA 6-hour deferral at room temperature	
	Spherocyte (a), Abnormal homogeneous chromatin (b)
	Spherocyte (a), Tear drop cell (b), Hypersegmented neutrophil (c)
2. K3EDTA 6-hour deferral at room cold temperature	
	Helmet cell (a), Spherocyte (b), Vacuolated neutrophil (c)
3. K3EDTA 12-hour deferral at room temperature	
	Spherocyte (a), Burr cell (b), Abnormal homogeneous chromatin (c)
	Burr cell (a), Hypersegmented neutrophil (b)

Type of Treatment	
4. K3EDTA 12-hour deferral at room cold temperature	
	Hypochrome (a), Spherocyte (b), Smudge cell (c)
5. K3EDTA 24-hour deferral at room temperature	
	Burr cell (a), Spherocyte (b), Eliptocyte (c), Vacuolated lymphocyte nucleus (d)
	Burr cell (a), Spherocyte (b), Vacuolated monocyte (c)
6. K3EDTA 24-hour deferral at room cold temperature	
	Spherocyte (a), Hypersegmented neutrophil (b)
	Degranulation or Pelger-Huet forms

Type of Treatment	
7. Na2EDTA 6-hour deferral at room temperature	
	Tear drop cell (a), Spherocyte (b), Hypersegmented neutrophil (c)
8. Na2EDTA 6-hour deferral at room cold temperature	
	Elongated nucleoplasm neutropil
	Burr cell (a), Hypersegmented neutrophil (b)
9. Na2EDTA 12-hour deferral at room temperature	
	Burr cell (a), Hypersegmented neutrophil (b)
	Spherocyte (a), ovalocyte (b), hypogranulation neutrophil (c)

Type of Treatment	
10. Na2EDTA 12-hour deferral at room cold temperature	
	Hypochrome (a), Hypogranulation neutrophil (b)
	Elliptocyte (a), Unidentified cell
11. Na2EDTA 24-hour deferral at room temperature	
	Hypochrome (a), Tear drop cell (b), degranulation (c), Pelger-Huet form (c), Hipersegmented neutrophil (d)
	Burr cell (a), Abnormal homogeneous chromatin (b)
12. Na2EDTA 24-hour deferral at room cold temperature	
	Burr cell (a), Elliptocyte (b), Unidentified cell (c)
	Spherocyte (a), Unidentified cell

DISCUSSION

Based on the research data, of the three factors (anticoagulant, temperature, and delay time), only the delay time significantly affected platelet count. Meanwhile, no factor showed a significant effect on erythrocyte and leukocyte counts. Significant differences in platelet count occurred between the 6-hour vs. 24-hour delay and the 12-hour vs. 24-hour delay. This indicates that delaying the test for up to 24 hours has a significant effect on the decrease in platelet count compared to immediate testing. This indicates that erythrocytes and leukocytes are relatively stable to variations in anticoagulants, temperature, and delay time. Based on the research data, it was found that the storage temperature of blood samples had no significant effect on the results of red blood cell count, white blood cell count, and platelet count tests. This was true for both room temperature and refrigerator storage for 24 hours. The duration of blood sample storage at room temperature and refrigerator temperature did not cause statistically significant changes in the tested parameters. This aligns with Ozmen's 2021 study, which stated that red blood cells, hemoglobin, platelets, and Mean Corpuscular Hemoglobin remained stable for 48 hours at storage temperatures of 4°C, 10°C, or 23°C. However, some studies suggest that storing samples at room temperature for more than 24 hours may alter test results for specific hematology parameters.

The results of this study are also in accordance with previous studies, which stated that there were no significant differences in hemoglobin, hematocrit, and erythrocyte count between K2EDTA and K3EDTA at various storage times. However, there were significant differences in the values of MCH (4 hours storage), MCV (8 hours), and MCHC (6-8 hours) in both anticoagulants, indicating changes in erythrocyte morphology after storage for more than 4-6 hours, especially in K3EDTA which is more alkaline and hyperosmolar so that it can cause shrinkage and degenerative changes in erythrocytes(Utami et al., 2019). Research conducted by Kadam in 2023 found that storing blood samples at room temperature for 24 hours can lead to a significant increase in Mean Corpuscular Volume (MCV) and a decrease in Mean Corpuscular Hemoglobin Concentration (MCHC), as well as a decrease in the number of leukocytes. These changes did not occur in blood samples stored at refrigerator temperature (4°C), suggesting that cold storage of blood samples is more effective in maintaining the stability of hematological parameters (Kadam et al., 2023).

Delaying the examination for more than 6 hours can cause a decrease in the number of erythrocytes due to hemolysis and cell membrane damage. Storage for 6 hours at cold temperatures (4-8 °C) showed better erythrocyte stability without any significant changes (Afriansyah et al., 2021). At the same time, storage at room temperature (20-25 °C) for more than 2 hours causes erythrocyte swelling and increases hematocrit and MCV values (Jaya et al., 2022). Delaying the examination for more than 6 hours can cause a decrease in the number of leukocytes due to hemolysis and a decrease in ATP (Adhenosine Triphosphate) (Agistriany et al., 2024). Storage for 24 hours at cold temperature (4-8 °C) shows leukocyte stability with a decrease within normal limits. While storage at room temperature (20-25 °C) can be done for a maximum of 2 hours to get valid results (Darmadi & Permatasari, 2018).

A delay in examination of more than 2 hours causes aggregation and a decrease in platelet count (Puspitasari & Aliviameita, 2022). Storage at room temperature (20-25 °C) can accelerate the decrease in cell number due to cell adhesion. At the same time, storage at cold temperatures (4-8 °C) inhibits platelet metabolism, allowing platelet stability to be maintained for up to 6 hours (Handini & Dewi, 2022). In addition, research by Sree Ramya in 2020 stated that blood storage at room temperature can cause changes in blood cell morphology, such as the formation of echinocytes in erythrocytes and platelet aggregation, which can also affect the results of hematological examinations (Ramya et al., 2022). Thus, although this study found no significant effect of storage temperature on hematological parameters within 24 hours, it is important to consider that long-term storage at room temperature may affect examination results. Therefore, to maintain sample integrity and accuracy of results, it is recommended that blood samples be stored at a temperature of 4 °C if the examination cannot be performed immediately after sample collection.

Based on the Kruskal-Wallis statistical test, it was found that the length of time delay had a significant effect on the results of the platelet count examination (p=0.004), but did not

significantly affect the number of erythrocytes or the number of leukocytes. The Mann-Whitney U post-hoc test with Bonferroni correction identified that significant differences in results occurred between the 6-hour delay and 24-hour delay (p=0.004), and the 12-hour delay and 24-hour delay (p=0.011). This finding aligns with research conducted by Handini & Dewi (2022), which demonstrated that delaying the examination for up to 24 hours can significantly impact platelet count. Platelets tend to remain stable initially, as the decrease in platelet count is progressive, and statistically significant changes are only observed after the delay reaches or exceeds 24 hours (Kadam et al., 2023). Physiologically, platelets are cytoplasmic fragments of megakaryocytes that have no nucleus, but are sensitive to environmental conditions and storage time. After blood collection, the first few hours of platelet function and number will be relatively stable, especially if the storage temperature is maintained at an appropriate level. However, as the time delay for examination increases, platelets undergo degradation processes, including cell membrane damage, spontaneous activity, aggregation, and lysis, especially when storage is carried out at room temperature (Ozmen & Ozarda, 2021). Additionally, the use of EDTA anticoagulant in blood sample storage tubes can accelerate changes in platelet morphology, including the formation of microaggregates and swelling, which occur more rapidly upon delayed examination. A delay of more than 24 hours can increase the likelihood of platelet agglutination or aggregation, which may prevent them from being accurately detected as platelets on the hematology analyzer (Ramya et al., 2022).

In addition to conducting CBC examinations with a hematology analyzer, this study also observed blood smears on whole blood samples, with variations in the type of anticoagulant (K3EDTA and Na2EDTA), length of time delay (6 hours, 12 hours, and 24 hours), and storage temperature (room temperature and cold temperature). Observation of blood smear preparations with a microscope showed that there were changes in the morphology of erythrocytes and leukocytes at 6 hours, 12 hours, and 24 hours of storage at room temperature and cold temperature. Abnormal erythrocyte morphology found, namely: *Spherocyte, burr cell, tear drop cell, Elliptocyte,* and *hypochrome*. In leukocytes, abnormal morphology was also found, including: *Hypersegmented neutrophil, Abnormal homogeneous chromatin, Vacuolated neutrophil, Vacuolated lymphocyte, Smudge cell, Degranulation or Pelger-Huet forms, Elongated nucleoplasm neutropil, Hypogranulation neutrophil,* and *Unidentified cell*.

The discovery of abnormal erythrocyte and leukocyte morphology is in accordance with previous research showing that prolonged blood storage with EDTA anticoagulant for 2 hours causes changes in erythrocyte morphology in peripheral blood smears. Changes that occur include the size, shape, and color of erythrocyte cells becoming abnormal. This suggests that the delay in examination has a significant impact on the morphology of blood cells (Yunus et al., 2022). Another study reported that delaying the EDTA blood test causes swelling of neutrophil nuclei, loss of lobes and granules, and vacuolization of leukocytes. This can affect the interpretation of blood cell morphology results. Whole blood samples stored at room temperature for 8-16 hours can cause erythrocytes to change into crenated and Spherocyte forms (Nugraha et al., 2021).

Previous studies have found that a 2-hour delay in EDTA blood test results in a decrease in leukocyte counts due to cell disintegration and swelling of leukocyte nucleic acids, which can cause misreading by the hematology analyzer (Rosyidah Rudina Azimata, Aulia Tata Ningrum, 2024). Another study found no significant difference in the number of erythrocytes between EDTA blood stored at room temperature (20-25°C) and cold temperature (4-8°C) for 6 hours. However, the longer the delay, the more the number of erythrocytes tends to decrease due to hemolysis and morphological changes such as swelling or membrane shrinkage (Afriansyah et al., 2021). Research in 2023 indicated that delayed hematological examination of EDTA blood can lead to changes in leukocyte morphology, including nuclear lobe separation and cell membrane damage, as well as platelet aggregation if storage is prolonged. However, in a study with the Neutrophil Lymphocyte Ratio (NLR) parameter up to 8 hours, there was no significant effect between K2EDTA and K3EDTA or storage time on the NLR value, although morphological changes should still be aware if the examination is delayed for more than 6 hours (Afriansyah et al., 2021).

This study has limitations due to its small sample size. For future studies, it is

recommended to increase the number of patients so that the data produced is more representative and the statistical test results are more valid.

CONCLUSION

The type of anticoagulant, storage temperature, and examination delay time do not significantly affect the number of erythrocytes and leukocytes. While the platelet count showed a significant effect of the time delay factor, the type of anticoagulant and storage temperature had no significant effect. The results of the post hoc test showed significant differences in platelet counts between the 6-hour and 24-hour time groups and between the 12-hour and 24-hour time groups, while the other time groups did not exhibit significant differences.

The delay in blood examination with K3EDTA and Na2EDTA affects the morphology of erythrocytes and leukocytes. Abnormal changes occur after 6, 12, and 24 hours of storage. A delay of more than 6 hours can lead to a decrease in the number of erythrocytes due to hemolysis, separation of nuclear lobes, and damage to leukocyte cell membranes, as well as platelet aggregation. Therefore, the timing and conditions of blood sample storage are crucial for the accuracy of examination results. The examination temperature is delayed.

AUTHOR'S DECLARATION

Authors' contributions and responsibilities

AA: Writing of the draft article and review. **PP, WIA, IES:** Data collection and editing.

Funding

We express our gratitude to Majelis Pendidikan Tinggi Penelitian dan Pengembangan (Diktilitbang) Pimpinan Pusat Muhammadiyah through RisetMu Batch VIII program in 2025, which has funded this research in accordance with 0258.388/I.3/D/2025

Availability of data and materials

All data are available from the authors.

Competing interests

The authors declare no competing interests.

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