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THE EFFECT OF DELAY, STORING WHOLEBLOOD WITH K_3EDTA AND Na_2EDTA ANTICOAGULANTS ON EXAMINATION RESULTS OF ERYTHROCYTE COUNTS, HEMOGLOBIN LEVELS AND ERYTHROCYTE MORPHOLOGY

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Keywords:
K_3EDTA,
Na_2EDTA,
Erythrocyte Count,
Hemoglobin,
Erythrocyte Morphology.

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ABSTRACT

Background: Sample stability refers to the ability of blood to maintain its quantitative values under specific storage conditions at room temperature (18–25°C) and refrigerated temperature (2–8°C). **Objective:** To determine the differences in red blood cell count, hemoglobin levels, and erythrocyte morphology using K3EDTA vacutainer and conventional Na2EDTA blood samples under varying temperatures and delay times. **Method:** This experimental laboratory research used a cross-sectional design involving four male students from the D4 Medical Laboratory Technology Program at Universitas Muhammadiyah Sidoarjo. Samples were stored at room temperature (18–25°C) and cold temperature (2– 8°C) with delays of 0, 12, and 24 hours. Examinations were conducted using a hematology analyzer and blood smear preparations. Should include a concise description of the process by which you conducted your research. **Result:** Wilcoxon Signed-Rank test results showed no significant differences ($p>0.05$) in red blood cell count and hemoglobin levels. However, morphological changes were observed, including crenation and hypochromia in K3EDTA samples stored cold for 24 hours, and spherocytes and hyperchromia in Na2EDTA samples stored at room temperature for 12 hours should list the results or outcomes of the work you have done so far. **Conclusion:** The type of anticoagulant and storage conditions did not significantly affect red blood cell count or hemoglobin levels. However, erythrocyte morphology showed alterations influenced by the type of EDTA, temperature, and storage duration. K_3EDTA was more stable in maintaining erythrocyte morphology compared to Na_2EDTA, particularly at cold temperatures.

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INTRODUCTION

Laboratory examination, especially in the field of hematology, plays an important role in supporting the diagnosis, monitoring, and evaluation of patient therapy. To obtain accurate and reliable examination results, the application of Good Laboratory Practice (GLP) principles is needed. GLP emphasizes the importance of quality control at every stage of the examination process, which includes pre-analytical, analytical, and post-analytical stages. Among the three stages, the most errors occur in the pre-analytical stage, which includes errors in patient identification, patient preparation, specimen collection and handling procedures, type of anticoagulant used, and specimen distribution to the laboratory[1]. An inappropriate pre-analytical stage can reduce the quality of the specimen, which has a direct impact on the accuracy of the test results. Common errors include Whole Blood Clotting, inappropriate sample volume, inappropriate anticoagulant selection, and sample storage at inappropriate temperatures, which can lead to hemolysis and destabilize hematological parameters[1]. Blood sample stability is defined as the ability of blood to maintain quantitative values under specific storage conditions over a period of time. 0. Ideally, all blood samples should be examined within two hours of collection due to the risk of cell lysis and bacterial growth. Temperature and storage duration

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play an important role in this. In practice,EDTA blood samples can still be used for whole blood tests for up to 24 hours if stored at room temperature (18–25 °C)or in a refrigerator (4-8°C) [2].

However, some hematological parameters show shorter stability limits. For example, hemoglobin levels and erythrocyte counts tend to remain stable for the first 6 hours. However, storing blood for too long, especially at room temperature, can cause erythrocyte hemolysis and result in inaccurate data [3]. The most commonly used anticoagulant for hematological examinations is EDTA, which is available in the form of sodium salt (Na_2EDTA) and potassium salt (K_2EDTA/K_3EDTA) K_3EDTA (Potassium EDTA) is an anticoagulant in the form of a tripotassium salt of ethylendiamintetraacetic acid which is usually used in vacutainer tubes and has a pH close to the pH of blood, making it more stable in maintaining the integrity of blood cells. Meanwhile, Na_2EDTA (Sodium EDTA) is a disodium EDTA salt that is commonly used in liquid or powder form[4]. Several studies compared the effectiveness of these two types of anticoagulants. Based on the results of a 2019 study, it was found that although the examination values of several blood parameters with K_3EDTA were lower than K_2EDTA, but for the parameters of the number of erythrocytes and hemoglobin levels, there was no significant effect between the two in various storage times[1].

Temperature factors and length of delay also affect erythrocyte morphology. A 2019 study reported that erythrocyte crenation began to appear after 8 hours of storage, both at room temperature and in the refrigerator. However, morphological changes were more pronounced at room temperature. Spherocytes began to form after 16 hours of storage at room temperature and after 24 hours at refrigerated temperatures[5]. Meanwhile, a study in 2022 showed that there was no statistically significant effect between the length of delay and storage temperature on leukocyte counts, hemoglobin levels, and erythrocyte counts[2]. Another study in 2019 also showed that there was no significant effect in morphological changes between blood samples using Na_2EDTA and K_3EDTA anticoagulants. However, the length of storage was shown to affect the shape of the erythrocytes although it did not affect the size and color[6].

Based on this description, it is important to conduct further research to evaluate the effect of length of delay and type of anticoagulant on the results of erythrocyte, hemoglobin, and erythrocyte morphology examinations.

Corrections:

- Ensure all reference numbers are placed immediately after the relevant claim.
- Clarify abbreviations upon first use (e.g., RBC, Hb).
- Improve language for conciseness; e.g., “Samples that are not examined immediately… may cause results to be invalidated” could be simplified to “Delayed analysis may compromise validity.”

METHODS/ CASE PRESENTATION

This study has received an ethics certificate number 0527/HRECC.FODM/IV/2025 on April 29, 2025, from the Health Research Ethics Committee of the Faculty of Dentistry, Airlangga University, Surabaya. This study design is a quantitative study with a cross-sectional approach using experimental methods. The study was conducted in the clinical pathology laboratory of the D4 Medical Laboratory Technology Program, Muhammadiyah University of Sidoarjo in May 2025. The sampling technique used was purposive sampling. This study involved 4 respondents who were students of the Medical Laboratory Technology Study Program, Faculty of Health Sciences, Muhammadiyah University of Sidoarjo. The selection criteria for respondents were males aged 17 to 25 years and willing to sign a consent form based on information provided by the researcher.

The tools used in this study were holder, tourniquet, tube rack, 5-50 µL micropipette, 10 mL maat pipette, dropper pipette, staining bridge, object glass, bulb, binocular microscope. Materials used: blood, vacutainer Na2EDTA, K3EDTA. tube, 96% alcohol, giemsa mother solution, phosphate buffer solution pH 7 years alcohol cotton, immersion oil. Researchers used venous blood sampling of 16 mL with macrosampling method. The number of samples used was 4 respondents. The blood obtained was put into a K3EDTA vacuum tube and a conventional tube. tube with the addition of Na2EDTA. Samples were categorized stored at room temperature (18-25 °C) and cold temperature (2-8 °C), then delayed for 0, 12 hours, and 24 hours. Examination of the number of erythrocytes and hemoglobin levels used an automatic method with a Hematology Analyzer (Medonic M32). Microscopic examination of blood smears using a binocular microscope (Olympus CX33). Data

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normality was tested using the Shapiro-Wilk test, and nonparametric statistical analysis used the Wilcoxon Signed-Rank test.

Corrections:

- Reorganized into **shorter, clearer paragraphs**.
- Adjusted grammar (e.g., "ethical certificate with number" → "ethical clearance certificate (No. …)").
- Clarified research design and location.
- Standardized units and terms (µL, mL, °C, etc.).
- Replaced redundant phrasing (e.g., "agreement to fill in and sign informed consent" → "who provided informed consent").
- Smoothed methods description (tools → materials → procedure → analysis).

RESULTS

Tables 1 and 2 show that the average erythrocyte count and hemoglobin levels in samples with K3EDTA and Na2EDTA anticoagulants remained stable (within normal limits) after 24 hours of storage at room temperature (18-25 °C) and cold temperatures (2-8 °C).

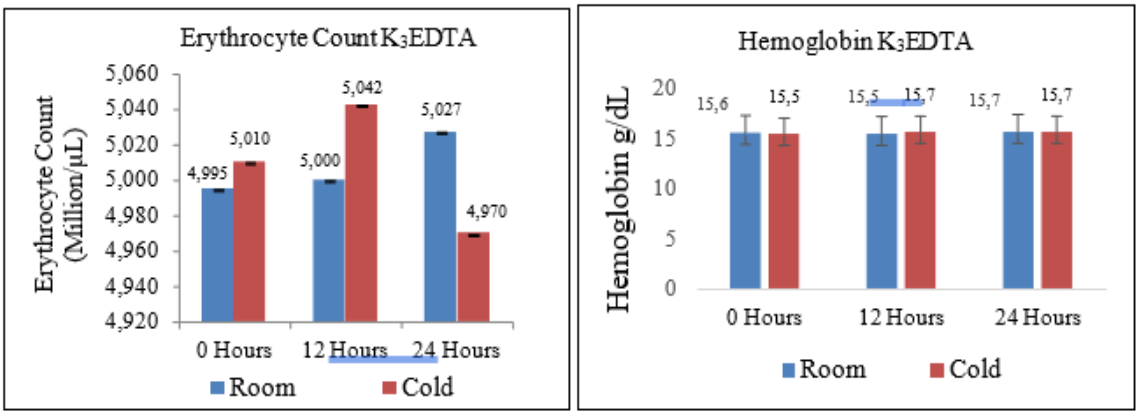

Figure 1.Erythrocyte Count and Hemoglobin Level in K3EDTA vacutainer Treatment, All Parameters Not Significantly

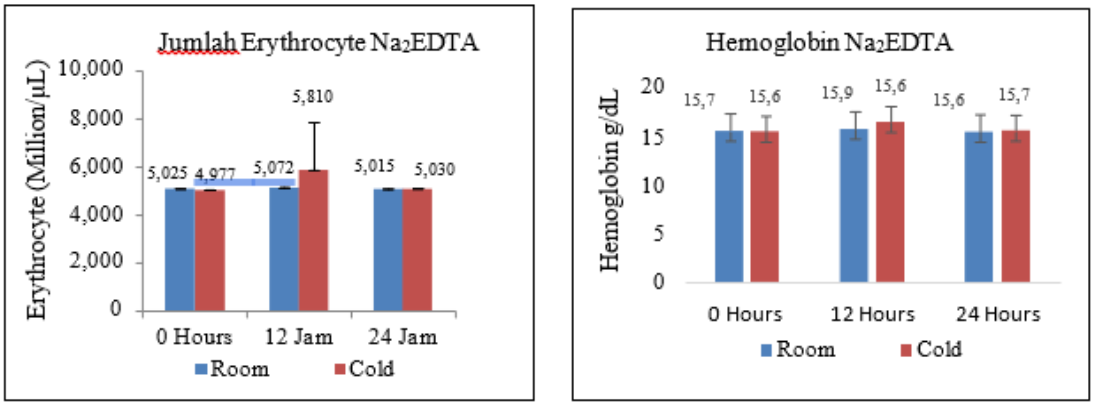

Figure 2. Erythrocyte Count and Hemoglobin Level in Na2EDTA Konvensional Treatment, All Parameters Not Significantly

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The observation results in Figure 3 show the morphology of erythrocytes in cold temperature K3EDTA tubes, found damage to the shape of erythrocytes starting from 24 hours of delay in the form of crenated cells such as burr cells, elliptocytes, and tear drop cells and abnormal erythrocyte color (hypochrome). Based on the observation of Figure 5 at room temperature, new damage is more clearly visible after 12 hours of Na2EDTA storage in the form of spherocytes and erythrocyte color (hyperchrome).

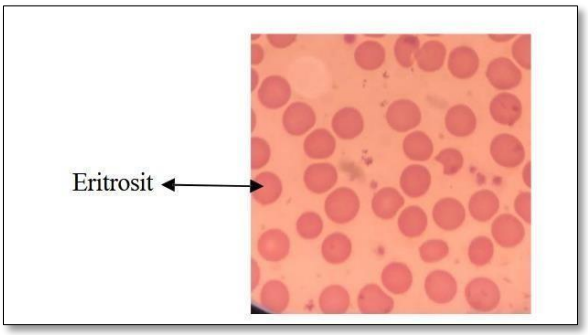

Figure 3.Normal Erythrocyte Morphology

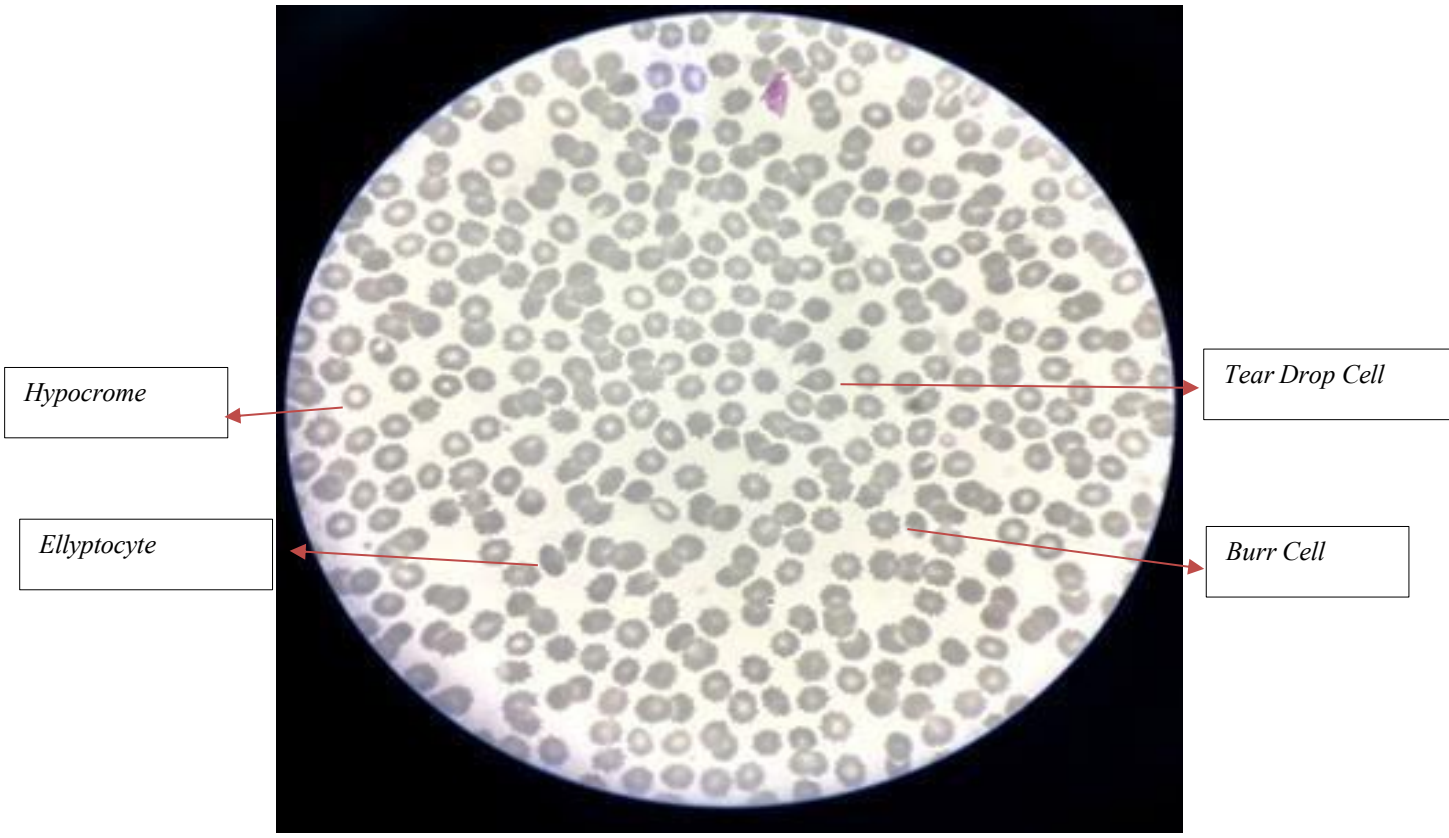

Figure 4. Abnormal Erythrocyte Morphology on K3EDTA Anticoagulant, 24 Hours, Cold Temperature at 100x10 Magnification

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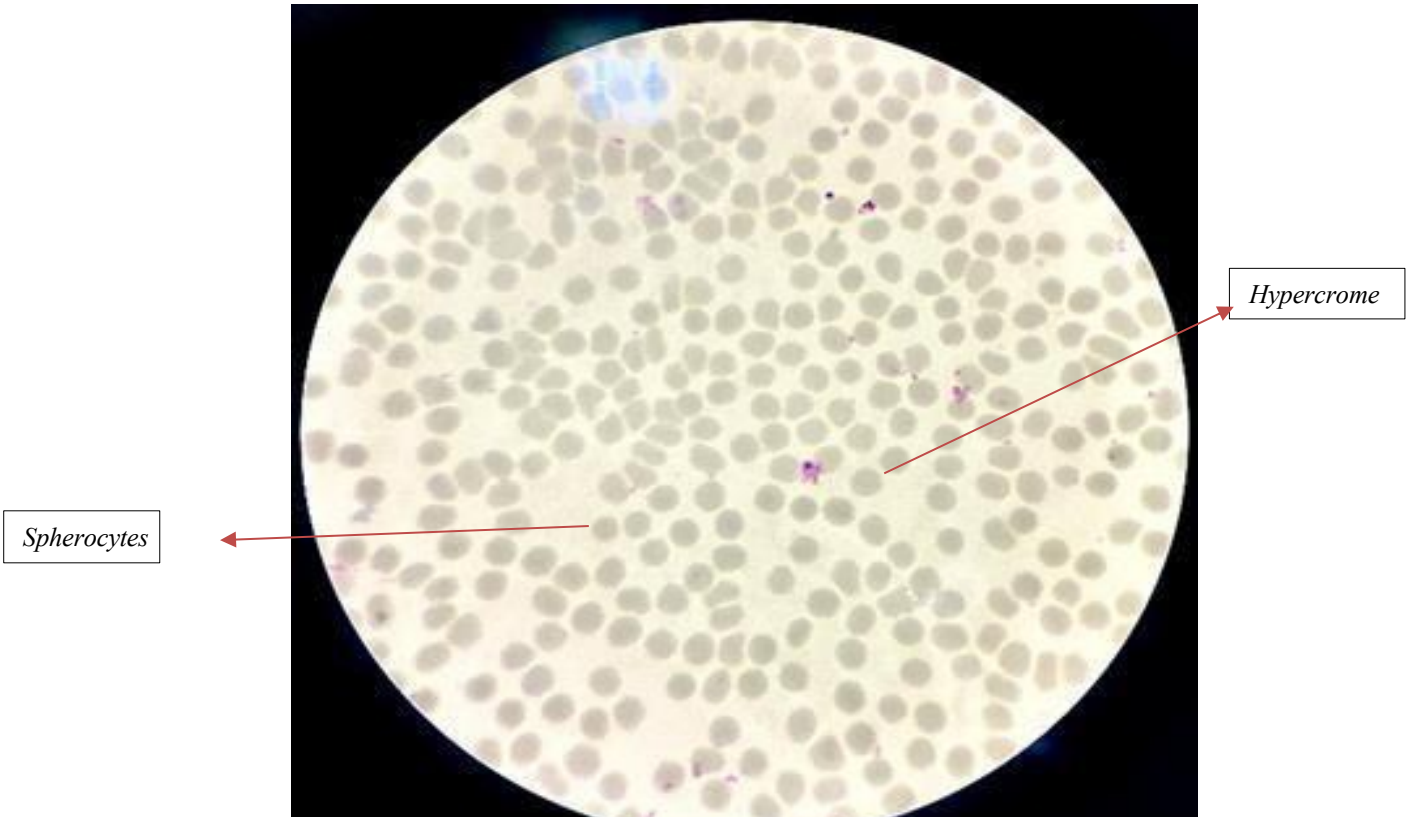

Figure 4. Abnormal Erythrocyte Morphology on Na2EDTA Anticoagulant, 12 Hours, Room Temperature at 100x10 Magnification

- Corrections:
- Minor Concerns
- Units should be standardized: "g/dL" → "g/dL."

Use consistent terminology: "room temperature" should always be paired with its defined range (18–25 °C).

- Remove unnecessary narrative repetition of values already shown in figures/tables. Instead, highlight trends (e.g., "RBC counts remained stable up to 24 h in both conditions").
- Major Revision: The results section should be rewritten with attention to clarity, avoidance of redundancy, correction of possible data inconsistencies, and improved figure/table integration. A concise synthesis emphasizing trends and interpretation rather than repeating numerical values is needed.

DISCUSSION

Sample stability is defined as the ability of a sample to maintain the initial value of the measured quantity for a specified period of time within specified limits when stored under specified conditions. In a laboratory context, the stability of erythrocyte and hemoglobin parameters is highly dependent on sample quality, including the type of anticoagulant used, storage temperature, and length of delay before examination[6].

Based on the results of this study, it was found that the number of erythrocytes in K_3EDTA tubes at room temperature (18–25 °C) increased significantly after 12 hours of delay, and then decreased again at 24 hours. In contrast, at cold temperature (2-8°C), the number of erythrocytes remained stable (12 hours), and only slightly decreased (24 hours). The increase at room temperature is most likely due to hemoconcentration

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effects due to plasma fluid evaporation or examination artifacts due to mild hemolysis and changes in erythrocyte morphology due to long-term interaction with EDTA[7].

In observations in Na_2EDTA tubes, the number of erythrocytes at room temperature was stable throughout the observation time (0, 12 and 24 hours), while at cold temperatures there were fluctuations, increasing at (0 hours) and (12 hours), then decreasing again at (24 hours). This indicates that cold temperatures in Na_2EDTA can cause osmotic changes or other cellular effects that affect the concentration of red blood cells in the blood, especially when there are changes in plasma viscosity or cell agglutination. Hemoglobin levels in both K_3EDTA and Na_2EDTA showed relatively good stability under both temperature conditions. In the K_3EDTA tube, the hemoglobin level in room temperature only changed slightly and in cold temperature increased after (24 hours). While in Na_2EDTA, the hemoglobin level at room temperature was stable at (0 hours), increasing at (12 hours). At cold temperatures, there was a more pronounced increase at 12 hours before returning to normal at 24 hours due to cell fluid redistribution and stability. However, at 24 hours, this compensatory effect is lost and cells begin to degrade or morphologically change[8].

Normality test using Shapiro-Wilk showed that all erythrocyte and hemoglobin count data were not normally distributed at each time delay and storage condition. There was no statistically significant effect between erythrocyte counts or hemoglobin levels at 0,12, and 24 hours delay. These results indicate that although there are small variations in the results of erythrocyte counts and hemoglobin levels, the changes are still within the limits of clinical tolerance. In anticoagulation, K_3EDTA showed better stability of erythrocyte and hemoglobin examination results than Na_2EDTA, especially in maintaining red blood cell morphology. This is because K_3EDTA has a pH that is close to physiological (~6.4) and is more hypo-osmolar, thus maintaining the stability of cell volume[4]. Meanwhile, Na_2EDTA is more acidic than K_3EDTA, which can affect the osmotic pressure of red blood cells. This condition causes an imbalance in fluid flow (influx or efflux) so that erythrocytes tend to enlarge or shrink abnormally[9]. In line with previous research, which states that K_3EDTA is more effective in maintaining the structure and integrity of blood cellular component[6]. Research in 2021 also supports this finding, where the results of the number of erythrocytes did not experience a significant effect up to 6 hours of storage, both at room temperature and cold temperature. In the context of this study which examined up to 24 hours, the results still show that erythrocyte and hemoglobin parameters can be maintained, especially when storage is carried out at cold temperatures[6].

In addition to quantitative examination, this study also evaluated erythrocyte morphology through microscopic observation of each sample. The results showed that in the cold temperature K3EDTA tube, damage to the shape of erythrocytes was found starting from 24 hours of delay in the form of crenation cells such as Burr cell (Serrated Shape), elliptocyte (Shape like an ellipse), and tear drop cell (Shape like a teardrop) and the color of erythrocytes in the pale area is more extensive (hypocrom). Meanwhile, at room temperature, significant changes were observed previously after 12 hours in Na2EDTA anticoagulant. Spherocytes are perfectly round red blood cells without central pallor, smaller in size, and appear dense and dark under a microscope. This is due to the partial loss of the cell membrane, which increases the surface volume-area ratio[10]. As well as the color of erythrocytes there are no pale areas (hyperchrome). This is consistent with previous research in 2019 which reported that erythrocyte crenation began to form after 8 hours of storage, and spherocytes formed after 16 hours at room temperature. At cold temperatures, morphological changes tend to be slower and minimal. Morphological damage to erythrocytes occurs due to osmotic stress, pH changes, enzyme activity, and interaction with anticoagulants over a long period of time. Crenation, spherocytosis and other shape distortions can lead to inaccurate analysis results if the examination is delayed for too long. These changes are important to note, especially when the sample will be used for manual or microscopic morphological examination[7]. The limitations of this study are the relatively limited number of samples and the absence of further examination with special staining methods or digital analysis, so that the interpretation of erythrocyte morphology still depends on manual microscopic observation.

Corrections:

- Smoothed grammar and syntax (e.g., “the drastic

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increase" → "the increase").

- Consolidated repeated phrases and numerical data into **trends** rather than restating raw values.
- Standardized units and ranges (e.g., "18–25 °C" instead of "18-25°C").
- Improved academic tone by avoiding colloquial phrasing (e.g., "more clearly visible" → "notable changes were observed earlier").
- Structured into logical flow: **definition → quantitative results → statistical findings → anticoagulant comparison → morphology → limitations**.

CONCLUSION

Based on the results of the study, it can be concluded that there was no significant effect ($p>0.05$) on erythrocyte counts or hemoglobin levels in blood samples anticoagulant with K3EDTA vacutainer tubes dan conventional Na2EDTA tubes across different storage durations in the length of delay of 0 hours, 12 hours and 24 hours and storage conditions (18-25 °C) and cold temperature (2-8 °C).

The results of erythrocyte morphology showed that in cold temperature K3EDTA tubes, erythrocyte shape alterations of erythrocytes was found starting from 24 hours of delay in the form of crenated cells including as burr cell, elliptocyte, and tear drop cell and abnormal erythrocyte color (hypochromic changes). While at room temperature, noticeable morphological alterations appeared earlier after 12 hours of Na2EDTA storage in the form of spherocytes and (hyperchromic features).

Changed **"there is" → "there was"** for consistency with past tense.

- Rephrased **"between the number of erythrocytes and hemoglobin" → "on erythrocyte counts or hemoglobin levels"** for clarity.
- Simplified anticoagulant phrasing: **"using K3EDTA vacutainer anticoagulant and conventional Na2EDTA anticoagulant" → "samples anticoagulated with K_3EDTA vacutainer tubes or conventional Na_2EDTA tubes."**
- StreamLined time/temperature description: replaced **"with variations... with variations" → "across different** storage **durations... and storage conditions."**
- Improved morphology description: **"damage to the shape... in the form of..." → "erythrocyte shape** alterations**... including..."**
- Corrected terminology: **"hypochrome" → "hypochromic changes"**; **"hyperchrome" → "hyperchromic features."**
- Made comparison explicit: added **"In contrast"** for Na_2EDTA results at room temperature.
- Replaced **"the new damage is more clearly visible" → "noticeable morphological alterations appeared earlier."**
- Adjusted overall tone to be **concise, academic, and parallel in structure.**

ETHICAL APPROVAL

His study has obtained an ethical certificate with number 0527/HRECC.FODM/IV/2025 on April 29, 2025 from the Health Research Ethics Committee of the Faculty of Dentistry, Airlangga University Surabaya.

CONFLICTS OF INTEREST

The author declare no conflict of interest

FUNDING

No Specific funding was provided for this article

AUTHOR CONTRIBUTIONS

This research is the result of a collaboration between the student and his/her supervisor. The student played an active role in all stages, from problem formulation and data collection and analysis to article preparation. Meanwhile, the supervisor provided direction, methodological guidance, and in-depth scientific evaluation to ensure the quality and validity of the research results. The contributions of both parties complemented each other in realizing this scientific work.

ACKNOWLEDGMENTS

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