# Erythrogram Profile of Blood Samples Anticoagulated with Tri-potassium Ethylene Diamine Tetraacetic Acid ($K_3$EDTA) Stored for 48 Hours at 4ºC

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## Abstract

Several pre-analytical variables influence hematological results, including anticoagulant use, storage temperature, and time between blood sample collection and analysis. Delayed sample analysis owing to prolonged storage could result in erythrogram profiles, which could complicate the interpretation of the resulting data. This study investigated the erythrogram profile of tripotassium ethylenediaminetetraacetic acid ($K_3$EDTA) in blood samples stored for 48 h at 4°C. Ten healthy blood samples of Ongole crossbred cattle were collected into $K_3$EDTA tubes from the jugular or coccygeal veins and analyzed for erythrogram profiles (erythrocyte counts, hemoglobin levels, hematocrit value, and erythrocyte morphology). Blood sample analysis for the control (0 h) was performed within ± 1.5 hours after collection, then the samples were refrigerated (4°C) and analyzed at 3, 6, 9, 12, 24, and 48 h. The results showed increased ($p < 0.05$) erythrocyte counts and hematocrit values after 9–24 and 6–48 h of storage, respectively. There was a significant difference in erythrocyte diameter between 0 h and other time observations ($p < 0.05$). Echinocytes were observed at 0 h of storage and continued to increase up to 48 h. Hypochromasia was also found at 6 to 48 hours of storage. Therefore, the analysis of blood samples for erythrogram parameters should be performed as soon as possible, preferably within three hours after collection, to ensure clinically reliable results.

Keywords: erythrogram, erythrocyte morphology, $K_3$EDTA anticoagulant, storage duration

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## INTRODUCTION

Blood is a body fluid that transfers oxygen from the lungs to body tissues and carbon dioxide from body tissues to the lungs; thus, physiological processes can occur (Bijanti *et al*., 2010). Blood comprises plasma and cell components, including erythrocytes, leukocytes, and platelets. These blood cells support body activities (Keohane *et al*., 2015).

One of the most frequently performed blood tests in the field is the hematological examination or complete blood count. Hematological examinations in the clinical field assist in establishing a diagnosis, determining prognosis, and monitoring during the treatment process (Keohane *et al*., 2015). A hematological examination examines the cellular components of the blood and usually uses whole blood samples. The hematological examination requires the addition of anticoagulants to the blood samples to prevent coagulation (Hutomo 2011; Riswanto 2013). Ethylenediamine tetraacetic acid (EDTA) is the most commonly used anticoagulant in routine hematological examinations (Voigt and Swist 2011). The types of EDTA often used include tri-potassium ethylenediamine tetraacetic acid ($K_3$EDTA) (Alan 2006).

Many factors influence the results of hematological examinations, such as disease and physiological factors including age, sex, breed, environmental temperature, and geographic conditions (Mulyadi *et al*., 2015). Other factors influencing hematological tests are sample collection and retrieval, sample volume, type of anticoagulant, preservation samples, and time

interval storage until the samples are analyzed (Gandasoebrata, 2013; Lindstrom *et al*., 2015).

Routine hematological parameters included hemoglobin level, hematocrit value, erythrocyte count, total leukocyte count, and platelet count. Hematological examinations should be performed in laboratories with adequate facilities. Not all clinics or farms are equipped with supporting laboratory facilities. Blood samples must be sent to a commercial or animal health laboratory unit over a certain distance, which requires time to arrive. Therefore, this study determined the effect of storage time on the erythrogram profile of $K_3$EDTA anticoagulant blood samples for 48 h at 4°C.

## MATERIALS AND METHODS

### Ethical Approval

This study was submitted for ethical approval to the Animal Ethic Committee, Institute for Research and Community Services, IPB (LPPM - IPB).

### Study Period and Location

Blood sampling was conducted in December 2020 at Cipelang Animal Embryo Center (BET), Cipelang Village, Cijeruk District, Bogor Regency. Blood samples were performed in the Clinical Pathology Laboratory, Division of Veterinary Internal Medicine, School of Veterinary Medicine and Biomedical Science, IPB University, Dramaga Campus, Bogor.

### Animals

This study used blood samples from 10 Ongole crossbreed cattle from the BET, aged 4–5 years. The animals used in study were clinically healthy cows. Clinically healthy animals undergo a series of physical examinations, including checking their respiratory rate, heart rate, and rectal temperature.

### Hematological Examination

Blood samples were collected through the jugular vein for cows with codes P5, P6, and P8, then the coccygeal vein for cows with codes P1, P2, P3, P4, P7, P9, and P10. The blood samples obtained were immediately put into 0.5 mL $K_3$EDTA anticoagulant vacutainer tubes and immediately homogenized. Blood samples were stored in a cool box with a cooling gel to analyze the erythrogram profile. Blood samples were analyzed 0 h (±1.5 hours after blood sampling/before stored in the refrigerator), 3, 6, 9, 12, 24, and 48 h after storage at 4$^o$C. The number of erythrocytes, hemoglobin levels, and hematocrit values were examined using a hematology analyzer (VetScan HM5 Abaxis, Inc.).

### Preparation and Evaluation of Blood Smear

Blood smear preparations were performed to evaluate erythrocyte morphology. Blood smears were prepared according to the method described by Coles *et al*. (1986). Blood smear preparation was stained using diff quick dye (MDT Indoreagen®), which consisted of three stages: fixation using methanol solution for 40–60 seconds, staining using eosin solution for 20–30 seconds, and then staining using methylene blue solution for 15–30 seconds. The preparations were rinsed using distilled water, allowed to dry, and stored in an object glass box until readings were taken.

Erythrocyte morphology was evaluated using a Dino Eye-Lite® camera at 1000× magnification to observe erythrocyte diameter, shape, and color. The erythrocyte diameter was measured as described by Megarani *et al*. (2020). Diameter measurements were carried out in several visual fields using the battlement method, with 100 erythrocytes observed.

Observing blood smear preparations starts at the end of the preparation, moves to the next side, and then moves to two to three fields of view using the battlement method. The score for assessing erythrocyte morphology was based on Harvey (2012). Assessment score for the presence of poikilocytes: +1 if 5–10 are present; +2 if there are 10–100; +3 if there are 101–250; and +4 if >250 poikilocytes are present. The assessment score for hypochromasia was +1 if there were 1–10, +2 if there were 11–50, +3 if there were 51–200, and +4 if >200 hypochromic erythrocytes were present.

**Data Analysis**

The data were analyzed using the Statistical Product and Service Solution (SPSS). The quantitative data were analyzed by one-way analysis of variance (ANOVA), while the qualitative data were descriptively analyzed.

## RESULTS AND DISCUSSION

**Hematology Profiles**

Erithrogram profiles were observed using a hematology analyzer machine, such as erythrocyte total count (RBC), hemoglobin concentration (HGB), and hematocrit value (HCT). The result of the erythrogram profiles of Ongole crossbreed cattle at various times of storage is shown in Table 1 and Figure 1.

**Total Erythrocyte Count**

Erythrocytes are one of the blood cell components with the most significant number in circulation (Harvey, 2012). The number of bovine erythrocytes has a standard range of 5.8–10.4 × $10^6$/µL (Smith and Mangkoewidjojo, 1988). The number of erythrocytes obtained in this study was within the range of average values, with the lowest value found in blood samples stored for 3 hours and the highest value found 12 hours after storage, respectively 8.41 ± 0.92 and 8.88 ± 0.73 × $10^6$/µL (Table 1).

One of the factors that can influence the erythrocyte count is nutrition. Essential components influencing the erythrocyte count are amino acids, iron, vitamins, and Cu (Frandsond, 1993). Dewi *et al*. (2018) stated that several minerals and vitamins are essential in erythropoiesis. Iron is required for heme synthesis. Copper in ceruloplasmin is essential for releasing iron from tissue into plasma. Vitamin B6, or pyridoxine, is required as a cofactor in the first stage of enzymatic heme synthesis. Moreover, the essential component in ruminants is cobalt. Ruminants need cobalt for vitamin B12 synthesis, which is essential to the erythropoiesis process. Vitamin B12 and folic acid deficiency can cause maturation failure in the erythropoiesis process and a decline in the number of erythrocytes in the blood (Guyton and Hall, 2008).

The statistical analysis showed a significant increase in erythrocytes ($p < 0.05$) at 9 to 24 hours after storage at 4°C. However, the value is still within the standard range according to Smith and Mangkoewidjojo (1988), which ranges from 5.8–10.4 × $10^6$/µl. The same study by Daves *et al*. (2015) showed significant differences in the number of erythrocytes in human blood samples stored for 6 hours in $K_2$EDTA at 4°C. Different study results were reported by Ali (2017); the number of erythrocytes in human blood samples stored for 72 hours in $K_3$EDTA at 4°C did not show significant differences. The results of another study reported that storing human blood samples for 8 hours in $K_3$EDTA at room temperature did not show a significant difference in the number of erythrocytes (Putri *et al*., 2019). Schapkaitz and Pillay (2015) reported that the number of erythrocytes was stable during 168 hours of storage in $K_2$EDTA at a temperature of 4–8°C. Also reported by Mingoas *et al*. (2020), the number of erythrocytes remained stable during 48 hours of storage in EDTA anticoagulant at 4°C.

The statistical analysis results in this study showed that storing blood samples in $K_3$EDTA anticoagulant for 48 hours at 4°C affected ($p < 0.05$) the number of erythrocytes at 9, 12, and 24 hours after storage. Observations at 48 hours after storage, although not significantly different ($p > 0.05$), showed a tendency to increase the number of erythrocytes. Observations made on individual blood samples revealed an increase in the number of erythrocytes that exceeded the normal reference range (10.89 × $10^6$/µL), which occurred in one of the blood samples (P5) with a storage period of 48 hours.

A similar study's results were reported by Brigden and Dalal (1999) that human blood samples stored in $K_3$EDTA anticoagulant for 48 hours at 4°C could cause pseudoincreases. This apparent increase is due to the presence of giant platelets and leukocytes, which are counted by the hematology analyzer as erythrocytes and cause the number of erythrocytes to increase artificially.

**Table 1.** Erythrocyte count (RBC), hemoglobin concentration (HGB), and hematocrit value of blood samples in $K_3$EDTA anticoagulant were stored at 4°C for 48 hours

| Parameters | Time (Hours) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **3** | **6** | **9** | **12** | **24** | **48** |
| RBC ($10^6$/µL) | 8.43 ± 0.89$^{ab}$ | 8.41 ± 0.92$^{a}$ | 8.62 ± 0.81$^{abc}$ | 8.76 ± 0.74$^{cd}$ | 8.88 ± 0.73$^{d}$ | 8.79 ± 1.11$^{cd}$ | 8.74 ± 1.08$^{bcd}$ |
| HGB (g/dL) | 12.57 ± 1.75$^{a}$ | 12.83 ± 1.69$^{a}$ | 13.00 ± 1.51$^{a}$ | 13.17 ± 1.47$^{a}$ | 13.73 ± 1.99$^{a}$ | 13.10 ± 1.74$^{a}$ | 13.02 ± 1.86$^{a}$ |
| HCT (%) | 36.11 ± 4.17$^{a}$ | 36.41 ± 4.14$^{a}$ | 37.46 ± 4.21$^{c}$ | 38.00 ± 4.04$^{c}$ | 38.47 ± 4.30$^{c}$ | 37.82 ± 4.75$^{c}$ | 38.30 ± 4.91$^{c}$ |

RBC= erythrocyte count; HGB= hemoglobin concentration; HCT= hematocrit value.
The different superscripts showed significant differences ($p < 0.05$) between storage times.

**Table 2.** Erythrocyte diameter of blood sample in $K_3$EDTA anticoagulated blood samples stored for 48 hours at 4°C

| Time (Hours) | Diameter (µm) | Interval (µm) |
|---|---|---|
| 0 | 5.88 ± 0.53$^{a}$ | 5.3–6.4 |
| 3 | 6.20 ± 0.76$^{b}$ | 5.4–7.0 |
| 6 | 6.64 ± 0.50$^{e}$ | 6.1–7.1 |
| 9 | 6.37 ± 0.39$^{d}$ | 6.0–6.8 |
| 12 | 6.17 ± 0.39$^{b}$ | 5.8–6.6 |
| 24 | 6.19 ± 0.48$^{b}$ | 5.7–6.7 |
| 48 | 6.39 ± 0.44$^{d}$ | 6.0–6.8 |

Numbers in the same column followed by different superscript letters indicate significant difference ($p < 0.05$) in erythrocyte diameter between storage times.

**Table 3.** Erythrocyte morphology scoring* in $K_3$EDTA anticoagulated blood samples stored for 48 hours at 4°C

| Time (Hours) | Echinocytes | Hypochromasia |
|---|---|---|
| 0 | 2+ | - |
| 3 | 2+ | - |
| 6 | 2+ | 4+ |
| 9 | 3+ | 4+ |
| 12 | 3+ | 4+ |
| 24 | 3+ | 4+ |
| 48 | 4+ | 4+ |

*) Harvey (2012); (-) did not find poikilocyte.

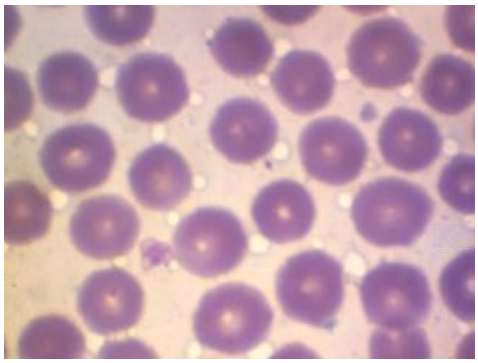

**Figure 1.** Morphology of erythrocyte Ongole Crossbreed Cattle.

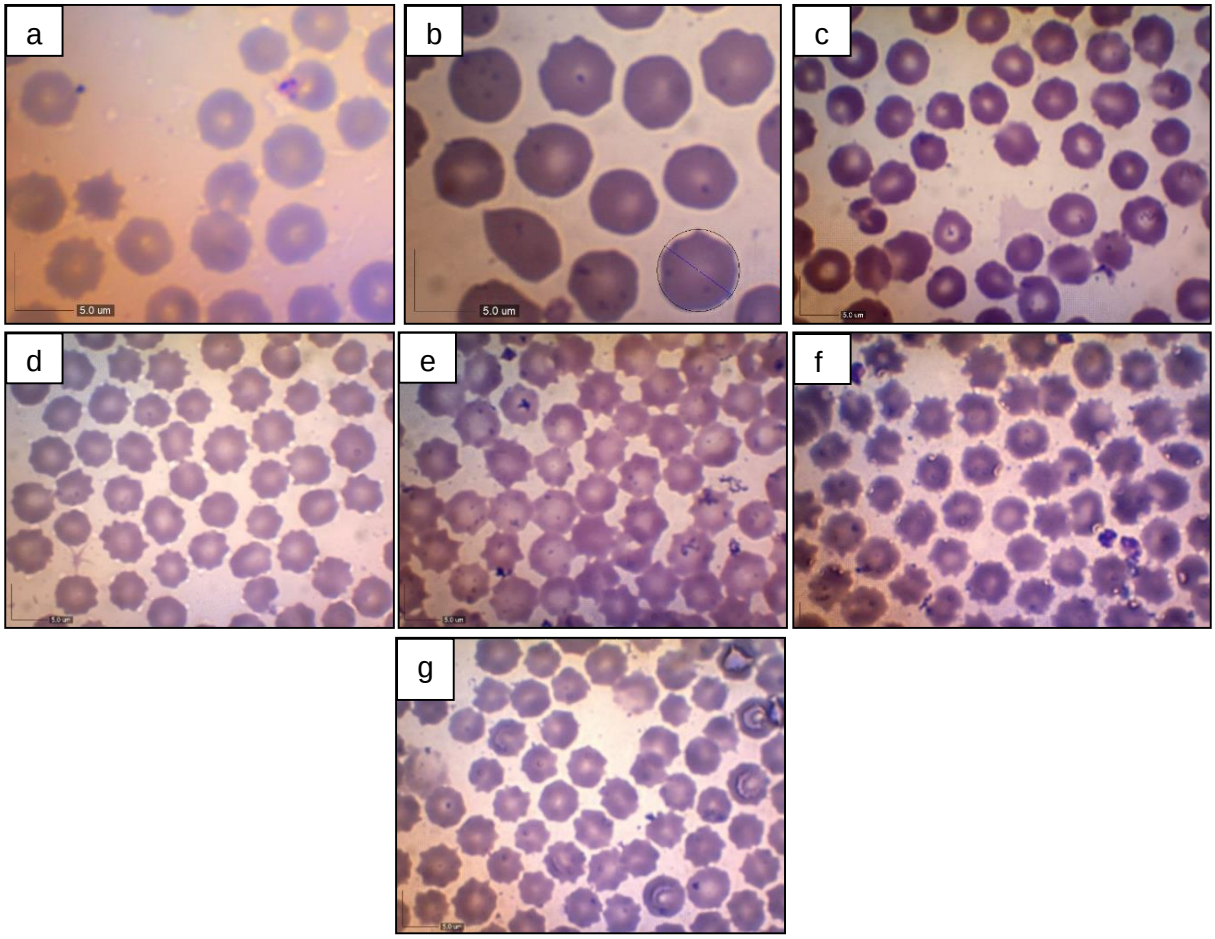


**Figure 2.** Morphological pattern of erythrocyte (echinocyte) at (a) 0, (b) 3, (c) 6, (d) 9, (e) 12, (f) 24, and (g) 48 hours stored at 4°C.

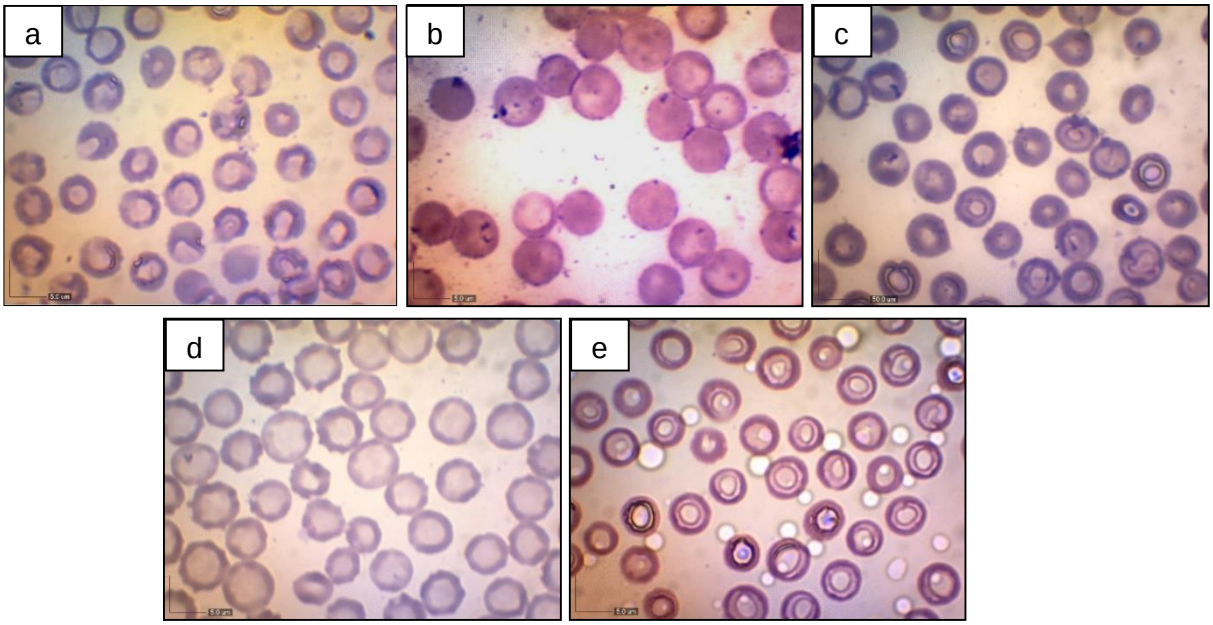


**Figure 3.** Morphological pattern of erythrocytes (hypochromasia) at (a) 6, (b) 9, (c) 12, (d) 24, and (e) 48 hours stored at 4°C.

Gandasoebrata (2013) reported that low results in the number of erythrocytes could be caused by inaccurate ratios between the volume of anticoagulant and blood samples. A

disproportionate ratio of blood samples with anticoagulants will cause hypertonic conditions in the blood sample (Novel *et al*., 2012). Hidayatulloh (2021) reports that hypertonicity causes the fluid in the erythrocytes to move out to maintain osmotic pressure. The fluid that comes out of the erythrocytes will cause the erythrocytes to shrink, and hemodilution occurs, which results in the number of erythrocytes decreasing (Purnama *et al*., 2021). Another factor that can influence the number of erythrocytes is a sample that needs to be appropriately homogenized (Hartina *et al*., 2018). Blood samples that need to be appropriately homogenized can cause inaccurate examination results (Mindray, 2006).

**Hemoglobin Concentration**

Hemoglobin is the main component of erythrocytes, which transport oxygen and carbon dioxide (Price and Wilson, 2006). According to Cunningham (2002), hemoglobin carries oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. Hemoglobin also plays a role in maintaining normal blood pH. The red color of blood is caused by hemoglobin contained in erythrocytes, as reported by Nair and Peate (2013). The ability of the blood to carry oxygen is influenced by hemoglobin levels in the blood and the chemical characteristics of hemoglobin (Cunningham, 2002).

The average value for hemoglobin levels in cattle blood is 8.6–14.4 g/dL (Smith and Mangkoewidjojo, 1988). The hemoglobin levels of blood samples obtained in this study were in the expected range, with the lowest value at 0 hours of storage and the highest at 12 hours after storage, namely 12.57 ± 1.75 g/dL and 13.73 ± 1.99 g/dL, respectively (Table 1). Meanwhile, normal hemoglobin levels in cows, according to Wood and Quiroz-Rocha (2010), are in the range of 8.4–12.0 g/dL.

Hemoglobin levels are influenced by many factors, including season, body activity, whether there is damage to erythrocytes, handling of blood samples, and nutrient content in feed (Andriyanto *et al*., 2010). Hemoglobin levels can also be influenced by environmental temperature. The number of erythrocytes and hemoglobin levels will increase in environments with low temperatures and decrease in environments with high temperatures (Guyton, 1991). Hemoglobin levels are also influenced by the adequacy of protein in feed, age, sex, and species (Schalm *et al*., 1975).

Septiarini *et al*., (2020) study reported a decrease in hemoglobin levels in Bali cattle fed organic forage. It proves that nutrients in feed can also influence hematological levels. Hemoglobin levels are also influenced by the maintenance system and the condition of the cage, which is open throughout the day and allows for sufficient oxygen. Bain (2014) reported that iron (Fe) can influence hemoglobin levels. Hemoglobin is a protein that contains iron, consisting of four polypeptide chains called globin chains. Each globin chain has a deep pocket, a storage place for the heme group that contains iron (Kristanto and Septiyani, 2023).

The statistical analysis results on hemoglobin levels in this study did not show significant differences ($p > 0.05$) during 48 hours of storage at 4°C. Putri *et al*. (2019) reported a similar study's results that the hemoglobin levels of human blood samples stored for 8 hours in $K_3$EDTA anticoagulant did not show significant differences. The results of the study by Ali (2017) reported that hemoglobin levels in fresh blood samples with $K_3$EDTA anticoagulant at 24, 48, and 72 hours of storage at 4°C did not show significant differences.

Although hemoglobin levels were not significantly different ($p > 0.05$), there was a tendency for the average hemoglobin level to increase at 3 hours to 12 hours after storage and a tendency to decrease again at 24 hours to 48 hours of storage at 4ºC. However, despite dynamics in hemoglobin levels during 48 hours of storage, the values were still within the normal range, according to Smith and Mangkoewidjojo (1988), which ranged from 8.6–14.4 g/dL.

Based on observations of individual blood samples, there was an increase in hemoglobin levels that exceeded the normal reference value range. The increase in hemoglobin levels was found in blood samples code P5 at 24 and 48 hours of storage (14.6 g/dL and 16 g/dL) and code

P6 at 12, 24, and 48 hours of storage (15.9 g/dL, 15.9 g/dL, and 16.2 g/dL), and code P10 at 12 hours of storage (18.3 g/dL).

The dynamics of hemoglobin levels during the storage period could be influenced by erythrocyte damage. Saputro and Lestari (2021) reported that stored blood samples could increase hemoglobin levels. The longer the stored blood sample, the more erythrocytes can swell due to stiffness and loss of lipids in the cell membrane. These conditions trap the plasma and are read as hemoglobin by the hematology analyzer.

**Hematocrit Value**

Hematocrit is the volume of erythrocytes in 100 mL of blood, and the value is expressed in decimal fractions or percentages. The hematocrit value can be used as a simple screening test for anemia (Dewi *et al*., 2018; Kiswari, 2014). Hematocrit is also called packed cell volume (PVC). Hematocrit is one of the tests to calculate the volume of erythrocytes that is practically carried out in clinical practice (Harvey, 2012).

The average percentage of hematocrit in cattle blood is in the range of 24–46% (Schalm *et al*., 1975), while the hematocrit value in cattle, according to Wood and Quiroz-Rocha (2010), is in the range of 21–30%. The lowest hematocrit value in this study was 36.11 ± 4.17 %, and the highest was 38.47 ± 4.30% (Table 1). This value is still within the standard value range, according to Schalm *et al*. (1975).

A lack of amino acids in the feed can cause a decrease in the hematocrit value (Purnama *et al*., 2019). In contrast, an increase in the hematocrit value can be caused by dehydration so that the ratio of erythrocytes to blood plasma is above the average value range (Frandsond, 1993). Pathological conditions that can affect the hematocrit value are increased hematocrit values (polycythemia) and decreased hematocrit values (anemia) (Corwin, 2009).

The average hematocrit value in $K_3$EDTA anticoagulant blood samples stored for 48 hours at 4°C can be seen in Table 1. The statistical analysis results show that the hematocrit value increased ($p < 0.05$) at this temperature for 6 to 48 hours of storage at 4°C. However, the hematocrit value at each storage time point was still within the normal range, according to Schalm *et al*. (1975), which ranged from 24–46%. Similar results in a human study were reported by Ozmen and Ozarda (2021), who found that storing blood samples with $K_2$EDTA anticoagulant for 48 hours at a temperature of 4°C affected the hematocrit value. Putri *et al*. (2019) reported that the hematocrit value of human blood samples stored for 8 hours in a room-temperature $K_3$EDTA anticoagulant did not significantly differ.

The results of observations of hematocrit values in individual blood samples showed an increase that exceeded the normal value range that occurred in blood samples code P6 at 6 to 48 hours of storage, with hematocrit values of 47.14%, 47.02%, 49.49%, 47.95%, and 47.69%, respectively.

An increase in hematocrit values in blood samples stored for 12 hours in EDTA tubes at 4°C was reported by Mingoas *et al*. (2020). Britton (1963) and Mingoas *et al*. (2020) reported that hematocrit is a combination of measuring the number and size of erythrocytes. This study showed an increase in erythrocyte size (swelling). This condition is caused by the progressive loss of the ability of fluid to enter and leave the erythrocytes. The erythrocyte membrane becomes weak due to hypoxia during the storage process (Majid *et al*., 2023).

**Morphology of Erythrocyte**

The erythrocyte morphology evaluation comprises cell diameter, the shape of the cell, and cell color intensity. Cell morphometry in a cytological examination is one method for detecting cellular abnormalities (Adili *et al*., 2016; Simeonov, 2012). Diameter measurements were based on Megarani *et al*. (2020), who carried them out in several fields of view using the battlement method, with 100 erythrocytes observed.

The results of statistical analysis of erythrocyte diameter showed that there was a significant difference between the average erythrocyte diameter of blood samples at each storage time point (3, 6, 9, 12, 24, and 48 hours) versus 0 hours of storage at 4°C ($p < 0.05$) (Table

2). The erythrocyte diameter increased with an increasing storage period. However, the erythrocyte diameter is still within the normal range. Brockus (2011) states that the bovine erythrocyte diameter ranges from 5–6 μm. Similar study results were reported by Antwi-Baffour *et al*. (2013), who found that an increase in erythrocyte diameter occurred in blood samples stored in $K_2$EDTA for 72 hours at a temperature of 4–8°C. It is caused by degenerative changes that allow water to enter the erythrocytes and increase their diameter.

The morphology of erythrocytes is round, biconcave discs, and the center of the erythrocytes is flat (Alagbe *et al*., 2013). The morphological changes found in this study were changes in the shape and color of erythrocytes. The results of observing erythrocytes in blood smears are presented in Table 3. The erythrocyte morphology assessment score was based on Harvey (2012). Poikilocytosis is a term for abnormal variations in the shape of erythrocytes in peripheral blood, including artifacts (Christopher *et al*., 2014). The poikilocytes found in this study were echinocytes. Echinocytes are a form of erythrocyte with spicules or protrusions evenly distributed on the surface of the erythrocyte membrane. The echinocyte form can be related to hemolytic anemia or dehydration of erythrocytes (Harvey, 2012).

Echinocyte-shaped erythrocytes in this study were found in all blood samples at all storage time points (Table 3 and Figure 2) and reached the highest score at 48 hours of storage, namely 4+, or there were >250 echinocytes per field of view. Similar study results were reported by Fitria *et al*. (2016), who investigated mouse blood samples stored in EDTA for 48 hours at 4°C.

Another erythrocyte morphology change in this study was hypochromasia (Table 3 and Figure 3). Hypochromasia is an erythrocyte with a pale color in the central pallor, or the overall cell appearance looks pale (Septiyani *et al*., 2023). Hypochrormasia occurs in erythrocytes with low hemoglobin levels (Harvey, 2012; Kosasih and Kosasih, 2008; Li *et al*., 2018). This study found hypochromasia in blood samples stored for 6 to 48 hours, with a score of 4+. Saputro and Lestari (2021) reported that the longer blood samples are stored, the more hemoglobin in the erythrocytes will be released into the blood plasma, so that the hemoglobin level in the erythrocytes decreases and can cause hypochromasia in the erythrocytes.

Many factors influence the erythrocyte morphology, including the $K_3$EDTA anticoagulant, length of storage period, and temperature during storage (Fitria *et al*., 2016; Rahmanitarini *et al*., 2019; Saputro and Lestari, 2021). During storage, erythrocytes undergo several changes that affect their ability to carry oxygen. These changes include biomechanical changes that cause structural/morphological damage. The EDTA anticoagulant is hypertonic to blood cells. Inappropriate proportions of EDTA anticoagulant in blood samples can cause tonicity disorders, cell swelling, hemolysis, or crenation (Ekanem *et al*., 2012; Stokol *et al*., 2014; Wismaya, 2019). Erythrocyte morphology changes can occur due to intrinsic factors such as a decrease in adenosine triphosphate (ATP). Decreased ATP, which acts as an energy source for erythrocytes, can cause the loss of lipids and stiffen the cell membrane, resulting in changes in erythrocyte morphology (Cora *et al*., 2012; Andriyani *et al*., 2019). The temperature storage also influenced the erythrocyte morphology. Rahmanitarini *et al*. (2019) reported that the blood samples stored at a refrigerator temperature of 2–8°C can help maintain erythrocyte lifespan.

## CONCLUSION

Storing blood samples in $K_3$EDTA anticoagulant for 48 hours at 4°C caused an increase in erythrocytes at 9 to 24 hours of storage and increased hematocrit values at 6 to 48 hours. The diameter of erythrocytes increases with increasing storage time. The erythrocyte morphology changes that were found were echinocytes and hypochromasia.

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## AUTHORS' CONTRIBUTIONS

PA and APM: Conceptualization and drafted the manuscript. AE, APM, SDW, and RW: Validation, supervision, and formal analysis. PA, APM, and PIN: Performed sample evaluation. PA: Performed the statistical analysis and the preparation of tables and figures. All authors have read, reviewed, and approved the final manuscript.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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