

# The Effect of Delayed K<sub>3</sub>EDTA Blood Samples on Hemoglobin Levels, Erythrocyte, Leukocyte and Platelet Counts with Correction of Peripheral Blood Smears in Referral Samples at Janapria Public Health Center

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## ABSTRACT

The results of laboratory examinations greatly influence the diagnosis of the patient's disease. Delay in examining blood samples for hemoglobin levels, leukocyte and platelet counts can allow changes in examination levels and cell morphology. In order for laboratory examination results to be accurate and reliable, there must be control over the pre-analytical, analytical and post-analytical. To determine the effect of delayed K<sub>3</sub>EDTA blood samples on hemoglobin levels, leukocyte and platelet counts with correction of peripheral blood smears in referral samples at the Janapria Health Center. This study used True Experiment method, 27 experimental units, with pre and post test control design. With data analysis used Anova test. 0 hour, 6 hour, 24 hour delayed experiment on hemoglobin level was 13.9 g/dl; 13.7 g/dl; 14.2 g/dl. Erythrocyte count was 4,87 million cells/ $\mu$ l; 5,84 million cells/ $\mu$ l; dan 5,92 million cells/ $\mu$ l. Leukocytes count was 5,733 cells/ $\mu$ l; 5,789 cells/ $\mu$ l; 5822 cells/ $\mu$ l. Platelet count was 158 thousand/ $\mu$ l; 140.2 thousand/ $\mu$ l; 140.8 thousand/ $\mu$ l. Obtained the probability value was (p) >0.05 on Hb value, leukocyte and platelet count, whereas in erythrocyte count was p < 0,05. There was no significant effect of K<sub>3</sub>EDTA blood delay on hemoglobin levels, leukocyte and platelet counts with correction of peripheral blood smears, whereas there is significant effect in erythrocyte count with correction of peripheral blood smears in referral samples at Janapria Public Health Center.

## INTRODUCTION

The Community Health Center is a health service facility that carries out public health efforts and first-level individual health efforts, by prioritizing promotive and preventive efforts in its work area to achieve the highest level of public health (Permenkes RI No 43, 2019). Health center as a center for primary individual health services functions as a gate keeper or first contact for formal health services and a referral provider according to medical service standards (Ratnasari, 2018).

The implementation of a system will not run well if its implementation does not comply with the provisions of the policy or guidelines. One of the problems in implementing a referral system is the limited resources and essential infrastructure in health institutions to provide minimal health services. The tiered referral system is one of the efforts made to strengthen primary services, as an effort to implement quality control. The tiered referral

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system is an effort to increase collaboration between health facilities. The tiered referral system is one of the efforts made to strengthen primary services, as an effort to implement quality and cost control in the National Health Insurance (Ratnasari, 2018) .

Laboratory examination is very important to help confirm the diagnosis of the disease. In order for laboratory examination results to be accurate and reliable, pre-analytical, analytical and post-analytical controls must be carried out. The pre-analytical stage includes patient preparation, blood sampling, handling, sample preparation, equipment and material preparation. The analytical stage includes sample processing and interpretation of results. The post-analytic stage includes recording results and reporting (Abdurrahman et al., 2021) . The pre-analytical stage is a stage that greatly influences the quality of the examination sample and the results that will be obtained.

Sample stability, part of the pre-analytical phase, is an important component that can influence clinical laboratory results. A study of factors in the pre-analytical phase including specimen collection, handling and storage showed that 93% of errors were not related to the analytical process (Puspitasari et al., 2022) . Anticoagulants are substances used to prevent blood clotting outside the body. One of the anticoagulants that is often used in routine blood tests is EDTA (Ethylen Diamine Tetra Acetate Acid) (Saputra & Aristotle, 2022) . EDTA as sodium (Na) or potassium (K) acts as an anticoagulant by changing calcium ions from the blood into non-ionic forms so that it can inhibit the blood clotting process (Gandasoebrata, 2010) .

Immediately after a blood specimen is taken from a patient, it is mixed with an anticoagulant to prevent clotting. A complete blood count (CBC) is one of the most common and routine laboratory tests, and is one of the first steps in diagnosing a disease. This test is quick, easy and can provide valuable information for doctors. CBC results can be influenced by various factors such as temperature and incubation period. Research by Peng et al., 2001; Tsuruda et al., 1999 found that blood specimens stored at room temperature or at 4-8°C (refrigerated) for up to 24 hours, provided results that could be used for CBC testing and automatic differential leukocyte counts (Obeidi et al., 2020) .

The results of laboratory examinations greatly influence the diagnosis of the patient's disease. As a result, laboratory examinations are a source of medical errors that can affect patient safety. Several factors that delay sample examination, which sometimes cannot be carried out immediately, can occur due to various things, including a larger number of samples being examined, problems with equipment damage, and limited number of laboratory personnel (Abdurrahman et al., 2021) .

At the Janapria Community Health Center, referral samples from the network of auxiliary community health centers (pustu) and village birthing centers (polindes), as well as networks (private clinics, independent practicing doctors, independent practicing midwives, independent practicing nurses) were sent using zip without a cool box. The distance traveled and the length of delay in examining samples will affect the results of the examination. The length of delay and storage time has a significant effect on platelet counts. Research by Nugraha et al (2021) found that delaying EDTA blood samples for 2 hours had a significant effect on platelet parameters. Another study by Puspitasari and Aliviameita (2022) also found a decrease of 2.32% between the average number of EDTA blood platelets examined immediately and a delay of 1 hour.

Hemoglobin examination using EDTA blood samples should be carried out immediately or less than 1 hour after collection, if necessary it can be stored in a refrigerator at 4°C for 3 hours. EDTA blood stored at 4°C for 24 hours in the refrigerator did not cause significant deviations, except for platelet counts and hematocrit values. If examinations with EDTA blood must be postponed, you must pay attention to the storage time limits for each

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examination, one of which is the stability of blood hemoglobin levels in anticoagulants, namely a temperature of 18-25oC for <4 hours and a temperature of 8oC for 24 hours. Storage of EDTA blood at room temperature for a long time causes a series of changes in erythrocytes such as rupture of the erythrocyte membrane (hemolysis) so that hemoglobin is free to enter the surrounding medium (plasma) (Muslim, 2017) .

Previous research on the description of hemoglobin levels before and after being stored for 2 hours at room temperature in a total of 22 blood samples using the cyanmethemoglobin method, obtained results by delaying the examination time for 2 hours at room temperature resulting in a decrease in hemoglobin levels in EDTA blood (Obeidi et al. , 2020) . For leukocyte parameters that are checked immediately and delayed for 1, 2 and 3 hours at room temperature using the automatic method, the longer the delay in the examination, the lower the number of leukocytes (Wahyuningsih, 2017) .

The novelty of this research is that it can see the results of examination of hemoglobin, erythrocytes, leukocytes and platelets which influence the delay time in reference samples with correction of peripheral blood smears. The sample reference at the Janapria Community Health Center does not use adequate sample reference facilities, the sample sender does not write down the sampling time, the distance traveled varies and the temperature when the sample is taken to the Community Health Center is not known for certain, so the author is interested in conducting research on the effect of delays in K3EDTA blood samples in reference samples for hemoglobin examination parameters, erythrocyte, leukocyte and platelet counts.

#### **MATERIALS/METHOD**

The type of research used in this research is *True Experiment* , which aims to determine a symptom or influence that arises as a result of treatment. The research design used a pre and post test control design to determine the initial and post-treatment conditions and measure the effect of treatment on the experimental group by comparing the group with the control group. The research sample used EDTA blood which is a reference sample at the Janapria Community Health Center, Central Lombok Regency, West Nusa Tenggara. The sampling technique was carried out by Non-Random Purposive Sampling, namely purposive sampling based on certain considerations made by the researchers themselves, where the inclusion criteria were blood samples with EDTA anticoagulant, while the exclusion criteria were hemolysis, icteric and lipemic blood samples.

#### **RESULTS AND DISCUSSION**

Based on research carried out on K3EDTA blood samples on hemoglobin levels, number of erythrocytes, leukocytes and platelets, obtained data on the characteristics of respondents based on gender in Figure 1 below:

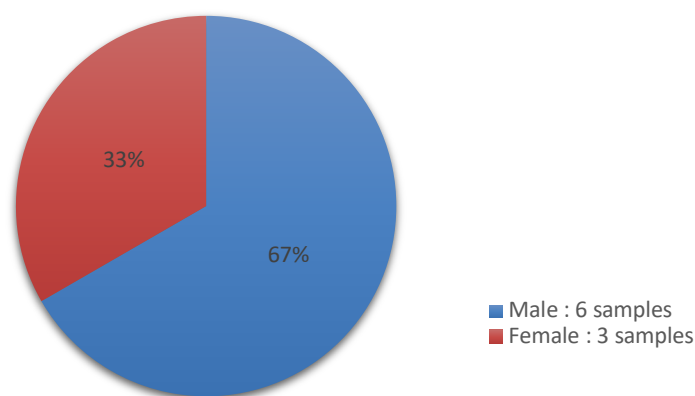


Figure 1 Number of samples by gender

Based on Figure 1, there were more male respondents than female, which the samples of this research was 6 male (66.67%) and 3 female (33.33%).

The complete results of examination of hemoglobin levels, number of erythrocytes, leukocytes and platelets in the EDTA blood samples of research respondents are in table 1 below:

Table 1 Results of examination of hemoglobin levels, number of erythrocytes, leukocytes and platelets in research samples

Spl No	P/L	Treatment											
		Hemoglobin (g/dL)			Erythrocytes (million cells/ $\mu$ l)			Leukocytes (thousand cells/ $\mu$ l)			Platelets (thousand cells/ $\mu$ l)		
		T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
1	P	12.0	12.0	12.4	4.15	4.17	4.25	2.8	2.9	3.0	174	143	137
2	L	11.9	11.6	12.0	3.99	3.91	4.05	3.7	3.6	3.5	123	116	118
3	L	12.6	12.5	12.8	4.00	3.98	4.08	6.2	6.1	6.1	103	102	104
4	L	17.8	17.4	18.2	6.08	6.04	6.18	3.0	3.0	3.0	24	22	23
5	L	14.0	13.4	14.1	5.79	5.48	5.82	10.2	9.9	9.8	291	280	267
6	P	13.7	13.2	14.0	4.81	4.63	4.78	6.5	6.3	6.7	219	204	201
7	P	13.5	13.5	14.0	4.88	5.00	5.00	5.9	6.3	6.1	173	134	144
8	L	15.2	15.2	15.5	5.35	5.45	5.31	6.0	6.2	6.2	214	200	202
9	L	14.2	14.3	14.8	4.83	4.93	4.82	7.3	7.8	8.0	102	61	72
Lowest value		11.9	11.6	12.0	3.99	3.91	4.05	2.8	2.9	3.0	24	22	23
The highest score		17.8	17.4	18.2	6.08	6.04	6.18	10.2	9.9	9.8	291	280	267
Average		13.9	13.7	14.2	4.87	4.84	4.92	5.73	5.78	5.82	158.0	140.2	140.8

Normal Values:

Hemoglobin: female 12 – 16 g/dL, male 13 – 17 g/dl.

Erythrocytes: female 4.0 – 5.0 million/  $\mu$ l, male 4.5 - 5.5 million/  $\mu$ l

Leukocytes: 5,000 – 10,000 cells /  $\mu$ l

Platelets: 150,000 – 400,000 cells/  $\mu$ l

Table 1 shows the results of examining hemoglobin levels, leukocyte and platelet counts in EDTA blood samples from research respondents in a total of 9 samples, 3 replications, and a total of 27 experimental units. The mean hemoglobin levels T0, T1, T2 respectively were 13.9 g/dl; 13.7 g/dl; 14.2 g/dl. The mean of erythrocytes T0, T1, T2 respectively was 4.87 million cells/ $\mu$ l; 4,84 million cells/ $\mu$ l , 4,92 million cells/ $\mu$ l. The mean number of leukocytes T0, T1, T2 respectively was 5,730 cells/ $\mu$ l; 5,780 cells/ $\mu$ l; 5,820 cells/ $\mu$ l. The mean number of platelets T0, T1, T2 respectively was 158,000 cells/ $\mu$ l; 140,200 cells/ $\mu$ l; 140,800 cells/ $\mu$ l.

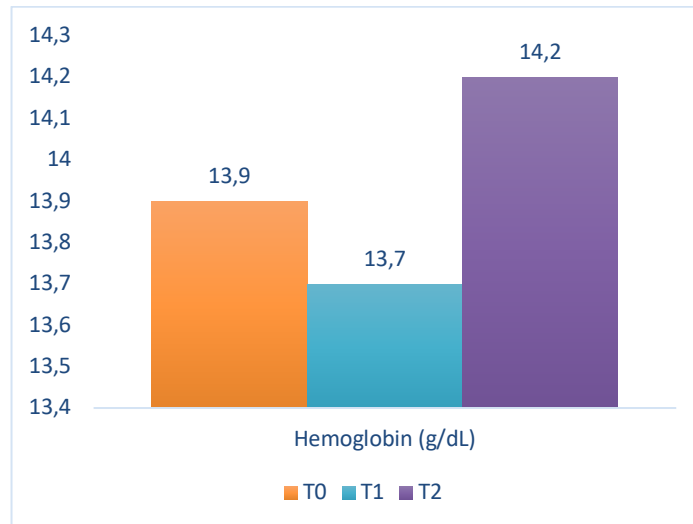


Figure 2. Mean hemoglobin levels in research samples treated T0, T1, and T2

Based on Figure 2 on the hemoglobin examination parameters, there was a decrease in the average hemoglobin level from T0 to T1 by 0.2, and an increase in the average value from T1 to T2 by 0.5.

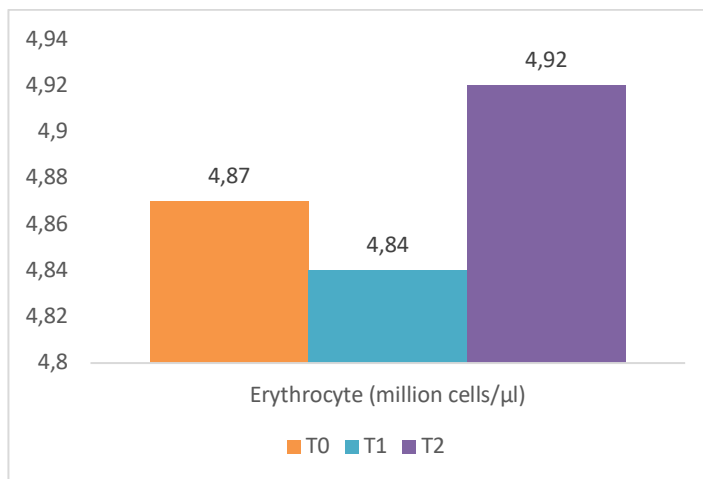


Figure 3. Average erythrocyte levels in research samples treated with T0, T1 and T2

Based on Figure 3, in the erythrocyte examination parameters, there was a decrease in the average number of erythrocytes from T0 to T1 by 30,000 cells/ $\mu$ l and from T1 to T2 by 80,000 cells/ $\mu$ l.

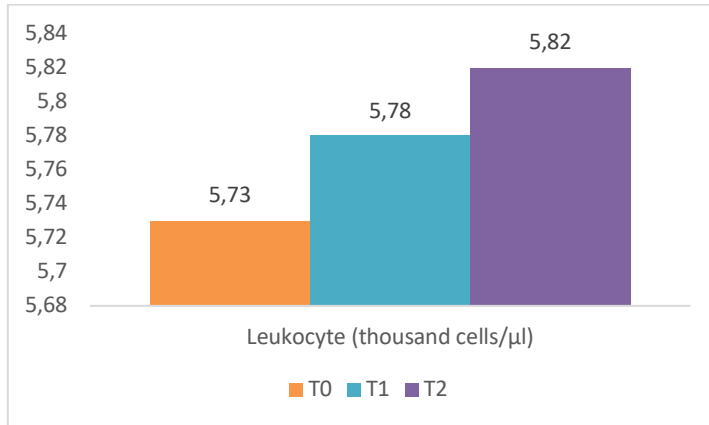


Figure 4 Mean leukocyte levels in research samples treated with T0, T1 and T2

Based on Figure 4, in the leukocyte examination parameters, there was an increase in the average number of leukocytes from T0 to T1 by 50 cells/μl and from T1 to T2 by 40 cells/μl.

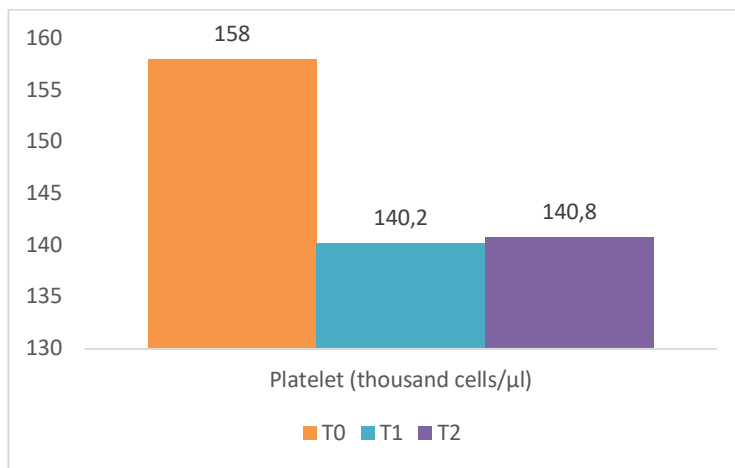


Figure 5 Average platelet levels in research samples treated with T0, T1 and T2

Based on Figure 5 on the platelet count examination parameters, there was a decrease in the mean platelet count from T0 to T1 of 17.8 thousand cells/μl (17,800 cells/μl), and an increase in the mean value from T1 to T2 of 0.6 thousand cells/μl (600 cells/μl).

The research samples were corrected using peripheral blood smears to read cell morphology including erythrocytes, leukocytes and platelets after and before treatment with T0, T1 and T2. The results of the peripheral blood smear correction are obtained in table 2 as follows:

Table 2 Results of peripheral blood smear

Spl	P/L	0 Hours (T0)			6 Hours (T1)			24 Hours (T2)		
		Eri	Leu	Plt	Eri	Leu	Plt	Eri	Leu	Plt
1	P	Crenation	Normal	Normal	Crenation	Normal	Fragment	Crenation	Neg sitop The core is	scanty/ LP
2	L	Normal	Normal	Normal	Normal	Normal	Normal	Crenation	The core is	scanty/ LP

3	L	Normal	Normal	Normal	Normal	Normal	Faded	Crenation	damaged Neg sitop	scanty/ LP
4	L	Normal	Normal	Normal	Crenation	The core is damaged	Lysis	Crenation	Neg sitop	scanty/ LP
5	L	Normal	Normal	Normal	Crenation/hypochromy	Normal	Thrombocytosis	Crenation	Neg sitop	scanty / LP 1 GT
6	P	Normal	Normal	Normal	Crenation	Normal	Very rarely	Crenation	Neg sitop	scanty/ LP
7	P	Rouleaux / hypochrome	Normal	Normal	Rouleaux / hypochrome	Normal	Normal	Crenation	Neg sitop	scanty/ LP
8	L	Normal	Normal	Normal	Crenation/hypochromy	The core is damaged	Fragment	Crenation	Neg sitop	scanty/ LP
9	L	Normal	Normal	Normal	Crenation/hypochromy	Normal	Fragment	Crenation	Neg sitop	scanty/ LP

Based on table 4.2, it shows changes in erythrocytes, leukocytes and platelets at a delay in examination of 0 hours, 6 hours and 24 hours. Erythrocytes in treatment without delay (0 hours) tend to show results with a normal erythrocyte shape, except in a few patient clinical conditions. However, after a delay, the erythrocytes become crenate. Meanwhile, in the leukocyte image, a delay will cause the cytoplasm to be absent, and in the platelet image it causes the number of platelets to be almost non-existent (scanty) in each field of view that is counted.

The research results were processed in a statistical test, the ANOVA test or analysis of variance to see the comparison of the means of two or more groups, as well as assessing the differences between groups of data. The results obtained are in table 4 below:

Table 3 One Way Anova Tests

Anova test	F	Sig
Hemoglobin	0.232	0.795
Erythrocytes	4,320	0,000
Leukocytes	0.003	0.997
Platelets	0.155	0.858

Based on table 3 of the anova test, sig > 0.05 results were obtained for the hemoglobin, leukocyte and platelet parameters, while the erythrocyte parameters had a sig value < 0.05. In hemoglobin samples between groups, a significance of 0.795 > 0.05 was obtained so that the mean of the three experiments (T0, T1, and T2) was the same. In the samples, the number of leukocytes between groups obtained a significance of 0.997 > 0.05 so that the mean of the three experiments (T0, T1, and T2) was the same. In the platelet count samples between groups, a significance of 0.858 > 0.05 was obtained so that the mean of the three experiments (T0, T1, and T2) was the same. Meanwhile, the experiment on erythrocyte parameters

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between groups obtained a significance of  $0.000 < 0.05$  so that the average of the three experiments (T0, T1, and T2) .

This research was conducted to determine the effect of delaying K3 EDTA blood on hemoglobin levels, leukocyte and platelet counts with correction of peripheral blood smears in reference samples at the Janapria Community Health Center. Delaying the examination can cause changes in the results of blood samples, an increase or decrease in hemoglobin levels, the number of erythrocytes, leukocytes and platelets in 9 samples with 3 treatments, namely without delay, 6 hour delay and 24 hour delay.

*Complete blood count* (CBC) or complete blood examination is one of the most common blood tests requested by doctors to evaluate the number and characteristics of cell components in the blood (Seo & Lee, 2022) . The inspection process is divided into pre-analytic, analytical, and post-analytic. Pre-analytic errors account for up to 70% of all errors made in laboratory diagnostics, most of which arise from problems in patient preparation, sample collection, transport, and analytical preparation and storage, but are not related to highly standardized analytical processes. Sample stability, part of the pre-analytical phase, is an important component that can influence clinical laboratory results (Unalli & Ozarda, 2021) .

CBC results can be influenced by various pre-analytic factors such as temperature and incubation period. Although clinical hematology laboratories are equipped with modern *automatic analyzers* that are capable of processing large quantities of hematology tests in a short time, delayed sample analysis often occurs in the clinical laboratory workflow when samples are transported from other laboratories or medical centers to the laboratory, when analysis cannot be done immediately. carried out for technical reasons, or when samples are required for retesting. As a result, testing is often delayed 12 to 24 hours or more after sampling. However, excessive delays in processing can result in less accurate results (Unalli & Ozarda, 2021) .

Hemoglobin is a protein in red blood cells whose function is to transport oxygen from the lungs to the rest of the body. Hemoglobin can increase or decrease due to abnormalities or disease (Tutik & Ningsih, 2019) . Hemoglobin levels in the body will increase because the process of transporting oxygen by erythrocytes is affected by changes in the shape of red blood cells. Delaying the examination can affect hemoglobin levels because delaying the examination can affect the shape of the erythrocytes moving in the blood, which can cause hemoglobin levels to change. Delaying the examination can result in changes in hemoglobin which causes hemoglobin levels to also change (Puspitasari et al., 2022) .

The results of research on hemoglobin parameters showed that there was a not much increase, where statistically there was no significant difference between treatments T0, T1 and T2. The mean hemoglobin increased only by  $\leq 0.5$  with the mean sequentially namely 13.9 g/dl, 13.7 g/dl, 14.2 g/dl.

Several studies also obtained results that the delay time did not have a significant effect, with drastic changes to hemoglobin levels, but hemoglobin levels could still increase after the delay. In line with the research results of Puspitasari et al (2022), it shows that storage temperature does not have a significant effect on hemoglobin levels, but hemoglobin levels can increase after a delay. Hemoglobin levels will increase after a delay in examination, but the rate of increase in hemoglobin levels can vary depending on the delay time, storage temperature, and other conditions .

In research by Dameuli et al (2019) , hemoglobin levels from EDTA blood that was delayed 20 hours at a temperature of 2-8°C changed with the delay time. EDTA blood hemoglobin levels stored for 20 hours at 2-8°C on a hematology analyzer and HB meter



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increased. A false increase in hemoglobin levels is usually caused by a very high leukocyte count, hyperlipidemia and turbidity due to incomplete lysis.

The increase in hemoglobin levels in EDTA samples that were delayed for 20 hours at a temperature of 2-8°C using a hematology analyzer probably occurred due to turbidity caused by red blood cells that did not undergo an incomplete lysis process. According to the principle of the first stage of hemoglobin examination on a hematology analyzer, red blood cells are lysed and absorption of SLS on the red blood cell membrane will produce changes in protein structure. Delaying EDTA blood for 20 hours can result in swelling of erythrocytes, so that when reading hemoglobin using the *reflectance principle*, the hemoglobin in the swollen erythrocytes causes the reading results to increase (Dameuli et al., 2019).

The increase in hemoglobin levels can be caused by changes in the shape of erythrocytes. In research by Rahmнитарini et al (2019) it was found that there was a change in the shape of erythrocytes into crenation and spherocytes when stored at room temperature (18-25 °C) for 8 hours. This is also in line with the results of the examination, which showed that the morphology of erythrocytes experienced crenation after a delay in the examination, 6 of the 9 examination samples experienced crenation after a 6 hour delay, and all samples experienced crenation after a delay of 24 hours.

In terms of erythrocyte parameters, the delay caused an increase in the mean erythrocytes at T0 to T2 significantly and had a statistical effect, with mean T0, T1 and T2 respectively 4.87 million cells/  $\mu\text{l}$ , 5.84 million cells/  $\mu\text{l}$ , and 5.92 million cells/  $\mu\text{l}$ . With probability  $0.997 > 0.05$ . This increase can be caused by changes in the shape of erythrocytes. In research (Rahmнитарini et al., 2019) it was found that there was a change in the shape of erythrocytes into crenates and spherocytes when stored at room temperature (18-25 °C) for 8 hours. This is also in line with the results of the examination, which showed that the morphology of erythrocytes experienced crenation after a delay in the examination, 6 of the 9 examination samples experienced crenation after a 6 hour delay, and all samples experienced crenation after a delay of 24 hours.

Leukocytes are a heterogeneous group of nucleated cells that can be found in the blood circulation. Leukocytes consist of 5 types, namely neutrophils, eosinophils, basophils, lymphocytes and monocytes. When examining leukocytes, delaying the examination at room temperature or refrigerator temperature  $\pm 4^\circ\text{C}$  can affect the results of the leukocyte count in the examination sample (Buoro et al., 2016).

In terms of leukocyte parameters, the delay caused an increase in mean leukocytes, although it was not significant and had no statistical effect, with mean T0, T1 and T2 respectively 5,730 cells/ $\mu\text{l}$ , 5,780 cells/ $\mu\text{l}$ , and 5,820 cells/ $\mu\text{l}$ . With probability  $0.997 > 0.05$ . In line with research, there was a slight but not significant increase in the number of leukocytes in EDTA blood samples that were delayed for 4 hours (Asiyah et al., 2018).

According to Zini (2014), delaying leukocyte examination can cause changes in cell morphology in peripheral blood smears (SADT). Some neutrophils show nuclear swelling and a more homogeneous color, loss of lobe structure which becomes separated, faded cytoplasm, and vacuolization and loss of granules. This is in line with the results of peripheral blood smear examination, where a delay of 6 hours resulted in the appearance of leukocytes in 2 samples with damaged nuclei, and at a delay of 24 hours all research samples showed results of damaged nuclei and absence or faded cytoplasm in leukocytes.

In the platelet examination, the average number of platelets decreased after the delay. The mean number of platelets T0, T1, T2 respectively was 158 thousand/ $\mu\text{l}$ ; 140.2 thousand/ $\mu\text{l}$ ; 140.8 thousand/ $\mu\text{l}$ . A decrease in the number of platelets after delaying the examination can be caused by several factors such as an increase in the number of platelets

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that form clots or the presence of underlying conditions such as blood clotting disorders (Hennink et al., 2018) . In line with the research results of Puspitasari et al (2022) which found that the variables of delay time and storage temperature had a significant effect on the number of platelets. Research by Sujud et al (2015) also stated that the difference in results could be caused by the temperature delay. There was a decrease of 2.32% between the average EDTA blood platelet count that was examined immediately and the examination was delayed for 1 hour. In line with the results of the confirmation of the examination that had been carried out on the SADT smear, at a delay of 24 hours the number of platelets decreased to almost nothing in each field of view.

Another study showed a 13.24% decrease in mean platelets with an immediate examination versus a 60 minute delay. This is because blood samples with anticoagulants that are not immediately examined will cause morphological changes in blood cells. Delaying the examination causes the platelets to cluster and swell and then form fragments of a smaller size so that they are not counted as platelets in the tool (Lasmilatu, 2019).

In the picture of platelet results using SADT, platelets were almost not found in any field of view. These results are different from the results on automatic analyzers, which can still be counted. Several factors that influence the difference in results are that automatic hematology analyzers can give wrong results if there are similar sized particles or scattered light such as fragmented red blood cells, microcytic red blood cells, nucleated cells causing the platelet count to be false positive in the automatic tool, and The presence of giant platelets and platelet clumps affects the results of the device.

*Human error* in manual techniques is also a crucial factor in peripheral blood smear examination. Accuracy in making smears, staining, quality of reagents, and the reading process can cause differences in results between the two methods (De La Salle et al., 2012).

This is in line with research by Balakrishnan et al (2018) which states that there is no significant variation in platelet counts between manual methods when compared with automatic analysis tools. The IRM ( *International Reference Method* ) values have a lower average than the median value of all automatic analysis methods. The majority (67%) of automated analyzer results overestimated platelet count compared with IRM, with significant differences in 16.5% of cases. There are differences in performance between these analyzer models, where the observed differences may depend in part on material properties and analysis technology (De La Salle et al., 2012) .

The results of this study show that the three parameters, namely hemoglobin, leukocytes and platelets, do not have a significant effect on increasing or decreasing the levels or number of cells. This is in line with research by Tendulkar et al (2015) that the number of erythrocytes and hemoglobin levels is almost not affected by storage at 4-8 °C or room temperature for 72 hours. Leukocytes, platelets and hemoglobin are stable at 4 °C for 72 hours. In line with research (Gunawardena et al., 2017) shows that most of the complete blood examination parameters are not affected by storage temperature for 48 hours, except for the platelet count.

The results of platelet examination are greatly influenced by time and temperature, so a standard is needed regarding storage conditions if blood samples are not immediately examined. Studies examining the comparison of platelet levels between delayed and immediate examinations have been carried out in various laboratory examination centers (Sujud et al., 2015) .

The storage time for EDTA blood is no more than one hour at room temperature. Where a delay in the examination of more than one hour can cause a reduction in the number and function of platelets. This happens because platelets very easily stick to each other or experience platelet aggregation or adhesion to foreign objects. Erythrocytes, platelets and

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hemoglobin were found to be stable for 48 hours at 4 °C, 10 °C and 23 °C storage, or without refrigeration for 24 hours (Unalli & Ozarda, 2021) .

## CONCLUSIONS

The probability value ( $p > 0.05$ ) can be concluded that there is no significant effect of K3EDTA blood delay on hemoglobin levels, leukocyte and platelet counts with peripheral blood smear correction, while the probability value ( $p < 0.05$ ) on erythrocyte parameters so there is a significant influence significant delay in K3EDTA blood on erythrocyte count levels with correction of peripheral blood smears in reference samples at the Janapria Community Health Center.

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## REFERENCE

- Abdurrahman, S., Yuyu, S. 2, & Rahayu, S. (2021). Pengaruh Waktu Penyimpanan Serum Pada Pemeriksaan Kolesterol Total. *Jurnal MediLab Mandala Waluya*, 5(2).
- Asiyah, N., Ariyadi, T., & Sukeksi, A. (2018). Perbedaan Jumlah Lekosit Sampel Segera Diperiksa dan Tunda 2 Jam Dan 4 Jam Pada Pasien Lekositosis. *Jurnal Universitas Muhammadiyah Semarang*, 15–16. <http://repository.unimus.ac.id/id/eprint/3182>
- Balakrishnan, A., Shetty, A., & Vijaya, C. (2018). Estimation of platelet counts: auto analyzer versus counts from peripheral blood smear based on traditional and platelet: red blood cell ratio method. *Tropical Journal of Pathology and Microbiology*, 4(5), 389–395. <https://doi.org/10.17511/jopm.2018.i05.04>
- Buoro, S., Mecca, T., Seghezzi, M., Manenti, B., Cerutti, L., Dominoni, P., Napolitano, G., Resmini, S., Crippa, A., Ottomano, C., & Lippi, G. (2016). Assessment of blood sample stability for complete blood count using the Sysmex XN-9000 and Mindray BC-6800 analyzers. *Revista Brasileira de Hematologia e Hemoterapia*, 38(3), 225–239. <https://doi.org/10.1016/j.bjhh.2016.05.010>
- Dameuli, S., Ariyadi, T., & Nuroini, F. (2019). Perbedaan Kadar Hemoglobin menggunakan Hb Meter, Spektrofotometer dan Hematology Analyser pada Sampel Segera dan Ditunda Periksa 20 Jam. *Ilmu Kesehatan*, 7–17. <http://repository.unimus.ac.id>
- De La Salle, B. J., McTaggart, P. N., Briggs, C., Harrison, P., Doré, C. J., Longair, I., Machin, S. J., & Hyde, K. (2012). The accuracy of platelet counting in thrombocytopenic blood samples distributed by the UK National External Quality Assessment Scheme for General Haematology. *American Journal of Clinical Pathology*, 137(1), 65–74. <https://doi.org/10.1309/AJCP86JMBFUCFCXA>
- Gandasoebrata, G. (2010). *Penuntun Laboratorium Klinik* (Cetakan ke). Jakarta: Penerbit Dian Rakyat.
- Gunawardena, D., Jayawera, S., Madhubhashini, G., Lokumarakkala, D. D., & Senanayake, S. J. (2017). Reliability of Parameters of Complete Blood Count With Different Storage Conditions. *Journal of Clinical Laboratory Analysis*, 31(2). <https://doi.org/10.1002/jcla.22042>
- Hennink, I., van Leeuwen, M. W., Penning, L. C., & Piek, C. J. (2018). Increased number of tissue factor protein expressing thrombocytes in canine idiopathic immune mediated hemolytic anemia. *Veterinary Immunology and Immunopathology*, 196(November 2017), 22–29. <https://doi.org/10.1016/j.vetimm.2017.12.009>
- Muslim, A. (2017). Pengaruh Waktu Simpan Darah K2EDTA dan Na2EDTA Pada Suhu Kamar Terhadap Kadar Hemoglobin Influence On Storetime of K 2 EDTA and Na 2 EDTA Blood In Room Temperature To Hemoglobin Concentration. *Jurnal Analis Kesehatan*, 4(2), 392–396.
- Nugraha, G., Ningsih, N. A., Sulifah, T., & Fitria, S. (2021). Stabilitas Pemeriksaan Hematologi Rutin Pada Sampel Darah Yang Didiamkan Pada Suhu Ruang Menggunakan Cell-Dyn Ruby. *The Journal of Muhammadiyah Medical Laboratory Technologist*, 4(1), 21. <https://doi.org/10.30651/jmlt.v4i1.8255>
- Obeidi, N., Safavi, E., & Emami, H. (2020). Evaluation of the effect of temperature and time of incubation on complete blood count ( CBC ) tests. *African Journal of Biotechnology*, 11(7), 1–4. <https://doi.org/10.5897/AJB11.2012>
- Permenkes RI No 43. (2019). Peraturan Menteri Kesehatan RI No 43 tahun 2019 tentang Pusat Kesehatan Masyarakat. In *Kementerian Kesehatan Republik Indonesia: Vol. No. 1335*.

- 
- Puspitasari, P., Aliviameita, A., Wahyudhi, S. D. Y., & Purnawati, F. P. (2022). Stabilitas Sampel Darah Terhadap Profil Hematologi Dengan Metode Otomatis. *The Journal of Muhammadiyah Medical Laboratory Technologist*, 5(1), 1. <https://doi.org/10.30651/jmlt.v5i1.12667>
- Rahmнитарini, A., Hernaningsih, Y., & Indrasari, Y. N. (2019). The Stability of Sample Storage for Complete Blood Count (CBC) Toward the Blood Cell Morphology. *Bali Medical Journal*, 8(2), 391–395. <https://doi.org/https://doi.org/10.15562/bmj.v8i2.1369>
- Ratnasari, D. (2018). Analisis Pelaksanaan Sistem Rujukan Berjenjang Bagi Peserta JKN di Puskesmas X Kota Surabaya. *Jurnal Administrasi Kesehatan Indonesia*, 5(2), 145. <https://doi.org/10.20473/jaki.v5i2.2017.145-154>
- Saputra, O. D., & Aristoteles, A. (2022). Perbedaan Pemeriksaan Darah Segera Dan Ditunda Selama 6 Jam Pada Suhu 4-8Oc Terhadap Kadar Hemoglobin Dengan Hematology Analyzer. *Jurnal 'Aisyiyah Medika*, 7(2), 49–56. <https://doi.org/10.36729/jam.v7i2.852>
- Seo, I.-H., & Lee, Y.-J. (2022). Usefulness of Complete Blood Count (CBC) to Assess Cardiovascular and Metabolic Diseases in Clinical Settings: A Comprehensive Literature Review. *Biomedicines*, 10(11). <https://doi.org/10.3390/biomedicines10112697>
- Sujud, S., Hardiasari, R., & Nuryati, A. (2015). Perbedaan Jumlah Trombosit Pada Darah EDTA Yang Segera Diperiksa dan Penundaan Selama 1 Jam di Laboratorium RSJ Grhasia Yogyakarta. *Medical Laboratory Technology Journal*, 1(2), 91. <https://doi.org/10.31964/mltj.v1i2.21>
- Tendulkar, A., Jain, P., Gujral, S., Tambe, M., Kenjale, R., & Ganesh, B. (2015). Stability of Selected Hematological Parameters in Stored Blood Samples. *Journal of Cell Science & Therapy*, 06(05). <https://doi.org/10.4172/2157-7013.1000220>
- Tutik, & Ningsih, S. (2019). Pemeriksaan Kesehatan Hemoglobin di Posyandu Lanjut Usia (Lansia) Pekon Tulung Agung Puskesmas Gadingrejo Pringsewu. *Jurnal Pengabdian Farmasi Malahayati*, 2(1), 22–26.
- Unalli, O. S., & Ozarda, Y. (2021). Stability of hematological analytes during 48 hours storage at three temperatures using Cell-Dyn hematology analyzer. *Journal of Medical Biochemistry*, 40(3), 252–260. <https://doi.org/10.5937/jomb0-27945>
- Wahyuningsih, F. D. (2017). *Perbedaan Jumlah Leukosit Berdasarkan Waktu Pemeriksaan Pada Suhu Kamar Metode Automatic*. Poltekkes Kemenkes Semarang.
- Zini, G. (2014). Stability of complete blood count parameters with storage: toward defined specifications for different diagnostic applications. In *International journal of laboratory hematology* (Vol. 36, Issue 2, pp. 111–113). <https://doi.org/10.1111/ijlh.12181>