

## THE EFFECT OF K2EDTA BLOOD STORAGE TIME TOWARD TEMPERATURE SPACE, HEMOGLOBIN LEVELS AND ERYTHROCYTE MORPHOLOGY

Tedy Febriyanto<sup>2</sup>; Putri Widelia Welkriana<sup>1</sup>

<sup>1,2</sup> Department of Medical Laboratory Technology, Politeknik Kesehatan Kementerian Kesehatan Bengkulu, Indonesia  
Corresponding email: [tedy@poltekkesbengkulu.ac.id](mailto:tedy@poltekkesbengkulu.ac.id)

### Abstract

Venous blood mixed with anticoagulants is usually used for hematological examination, in order to blood material does not clot. Checking hemoglobin levels and erythrocyte morphology uses an anticoagulant, one of which is EDTA (ethylenediaminetetraacetate), as sodium or potassium salt. Most of the hospitals and clinical laboratories in Kota Bengkulu still have many checks that are not carried out immediately. Then, there are delays caused by the accumulation of samples, especially when there is a Medical Check-Up program for company or factory workers with a large number of workers. This research was an experimental study in a laboratory. The sample in this study were 39 Students level 2 and 3 Department of Medical Laboratory Technology of Poltekkes Kemenkes Bengkulu 2020. The treatments were in the form of K2EDTA blood count storage time of less than 1 hour and more than 3 hours on hemoglobin levels and erythrocyte morphology at room temperature. The results showed the mean hemoglobin level of less than 1 hour was 16.15 g / dL and the mean hemoglobin level for more than 3 hours was 14.87 g / dL. Erythrocyte morphological mean results of less than 1 hour and more than 3 hours are normal. The results of this study showed there is an effect of storage time of less than 1 hour and more than 3 hours of K2EDTA blood at room temperature on hemoglobin levels and erythrocyte morphology. It is recommended to conduct further research on the risk factors for the effect of K2EDTA blood storage at room temperature on erythrocyte morphology.

**Key Words:** *Blood Saving Time, K2EDTA, Hemoglobin, erythrocyte morphology*

### INTRODUCTION

The results of laboratory examinations, especially hematology, are requested by doctors to establish the diagnosis, support the diagnosis, make a differential diagnosis, monitor the course of the disease, assess the severity of the pain and determine the prognosis. Laboratory examinations generally pass through 3 stages, namely pre-analytic, analytical and post-analytic. The pre-analytic stage includes patient preparation, retrieval, shelter, storage and delivery of materials. Laboratory examinations that have gone through all three stages of examination must be carried out according to existing procedures, so that results are obtained that are thorough, precise, fast and reliable. Hematology examination includes hemoglobin level parameters, leukocyte count, erythrocytes, platelets, hematocrit, erythrocyte index, reticulocyte count, blood sediment rate and other special examinations (Muslim, 2015).

Blood tests in general can be divided into two, namely routine blood tests and complete blood. One example of a complete blood test is the examination of hemoglobin levels. For the hematology examination, venous blood is usually used mixed with anticoagulants, so that the blood material does not clot. Anticoagulants that can be used include K<sub>2</sub>EDTA, Na<sub>2</sub>EDTA, heparin, sodium citrate in a solution of 3.8%, a mixture of omoniumoxalat and potassiumoxalate (Muslim, 2015). Determination of hemoglobin levels using anticoagulants one of which is EDTA (ethylene diamine tetra acetate), as sodium or potassium salt. The addition of anticoagulants to the blood hemoglobin examination to avoid clotting. In addition, EDTA has no effect on the magnitude and shape of erythrocytes and also on the form of lectocytes. The amount of EDTA used is 1 mg / mL of blood (Parwati, et al 2016).

Hemoglobin examination using a blood sample of Ethylene Diamine Tetra Acetate (EDTA) should be done immediately or less than 1 hour after taking, if needed can be stored in the refrigerator 4OC for 3 hours. EDTA blood stored at 4OC for 24 hours in the refrigerator does not cause significant irregularities, except for platelet count and hematocrit value. Examination with EDTA blood if forced to be delayed should pay attention to the storage deadline of each examination (Stability of blood hemoglobin levels in anticoagulants 18-25OC <4 hours and 8OC 24 hours). EDTA blood storage at prolonged room temperature causes a series of erythrocyte changes such as rupture of membranerocytes (hemolysis) so that hemoglobin is free to enter the surrounding medium (plasma) (Muslim, 2015).

According to Rahayu (2018) an overview of hemoglobin levels in students / i Ill level of medan health analyst department before and after being stored for 2 hours at room temperature that has been done in the Hematology Laboratory of the Department of Health Analysts Medan, against 22 blood samples using cyanmethemoglobin method, obtained results with delay of examination time for 2 hours at room temperature resulted in hemoglobin levels in the blood EDTA decreased.

Based on the survey conducted by researchers that most hospitals and clinical laboratories in bengkulu city are still many examinations that are not done immediately and there are delays caused by the accumulation of samples that come, Medical Check-Up Program on company workers or factories with a large number of workers. Where the laboratory officer must come to take a blood sample to be examined. The distance of sampling and examination of patient samples with each other already takes time, so patient samples have been delayed in examination of samples.

## **METHOD**

### **Research Design and Subject**

This type of research was experimental research in the laboratory. Treatment in the form of K2EDTA blood examination time less than 1 hour and more than 3 hours against hemoglobin levels and erythrocyte morphology at room temperature. The samples in this study were 39 Students level 2 and 3 Department of Medical Laboratory Technology of Poltekkes Kemenkes Bengkulu 2020.

### **Instruments and Data Analysis Techniques**

Tools and materials used include: test tubes, spectrophotometer, micropipette (20 L and 1000 L), yellow tip, blue tip, tissue, cotton, tourniquet, EDTA tube, label paper, 3 cc syringe, alcohol cotton, plaster, venous blood samples, drabkin's solution, Aquadest. Test tube with 5000 L of drabkin solution, and added 20 L of EDTA blood using a micropipette. Mixed until homogeneous so that there will be a change in hemoglobin to cyanmethemoglobin. Then it was incubated for 5 minutes, then read with a spectrophotometer at a wavelength of 546 nm, program C/F, factor 36.77, and drankin's solution as a blank. Hemoglobin level is determined from the ratio of its absorbance to the standard absorbance of cyanmethemoglobin.

The data was obtained through blood samples and will be examined at the Hematology Laboratory of the Health Analyst Department of the Health Polytechnic of the Ministry of Health Bengkulu. Analysis of the data used was univariate analysis aimed at knowing the description of each research variable and bivariate analysis to determine the relationship between the effect of K2EDTA blood storage time at room temperature on hemoglobin levels and erythrocyte morphology, which will then be tested by Chi-square test.

## **RESULTS**

The result of examination of hemoglobin levels and erythrocyte morphology with a shelf time of less than 1 hour and more than 3 hours on 39 students presented in Table belows:

**Tabel 5.1 Results of Examination of Hemoglobin Levels and Erythrocyte Morphology with a Shelf Time of Less than 1 Hour and More Than 3 Hours**

<b>Examination</b>	<b>Save Time for Inspection</b>	<b>n</b>	<b>Mean ± SD</b>	<b>P Value</b>
Rate of hemoglobin	< 1 hour	39	16.15 ± 1.857	0.003
	> 3 hours	39	14.87 ± 2.355	
Erythrocyte Morphology	< 1 hour	39	1.05 ± 0.223	0.006
	> 3 hours	39	1.28 ± 0.456	

**Average results of hemoglobin K2EDTA levels with a shelf life of less than 1 hour and more than 3 hours at room temperature**

Based on table 4.1 above, the results obtained that the average hemoglobin levels of students level 2 and 3 department of health analysts of the Bengkulu Ministry of Health in less than 1 hour is 16.15 gr / dL and the average hemoglobin levels of students level 2 and 3 department of health analysts of Poltekkes Kemenkes Bengkulu more than 3 hours is 14.87 gr/ dL.

**K2EDTA erythrocyte morphology results with a shelf life of less than 1 hour and more than 3 hours at room temperature**

To see the erythrocyte morphological results data are categorized with information 1 for normal morphology and 2 for abnormal morphology. Based on table 4.1 above, the average erythrocyte morphology in students of level 2 and 3 of the Department of Health Analysts of The Ministry of Health Bengkulu less than 1 hour and more than 3 hours is normal.

**Effect between shelf time of less than 1 hour and more than 3 hours of K2EDTA blood at room temperature against hemoglobin levels**

Based on table 4.1 above obtained the results of hemoglobin levels of students level 2 and 3 Department of Health Analysts Of The Ministry of Health Bengkulu less than 1 hour and more than 3 hours is the value of P 0.003 this means that the value of P is smaller than 0.05 so it can be concluded that there is an influence between the storage time of less than 1 hour and more than 3 hours of K2EDTA blood at room temperature against hemoglobin levels.

**Effect of examination time of less than 1 hour and more than 3 hours of K2EDTA blood at room temperature on Erythrocyte Morphology**

Based on table 4.1 above obtained the morphological results of erythrocytes students level 2 and 3 Department of Health Analysts Of The Ministry of Health Bengkulu less than 1 hour and more than 3 hours is the value of P 0.006 this means that the value of P is smaller than 0.05 so it can be concluded that there is an effect of examination time of less than 1 hour and more than 3 hours of K2EDTA blood at room temperature to erythrocyte morphology.

**DISCUSSION**

From the results of the study found that the average hemoglobin level of less than 1 hour is 16.15 gr / dL and the average result of hemoglobin levels more than 3 hours is 14.87 gr / dL, which means there has been an average decrease in hemoglobin levels and also in accordance with the results of the Mann-Whitney test analysis with the addition of average information and standard stores showed there is an influence.

Hemoglobin (Hb) is a protein substance found in red blood cells (HR), which gives red color to the blood. Hemoglobin consists of iron which is a carrier of oxygen. Abnormally high hemoglobin levels occur due to a hemoconcentration state resulting from dehydration (fluid loss). Low hemoglobin levels are associated with a variety of clinical problems (Kee, 2007). The main function of Hb is to transport O<sub>2</sub> from the lungs, where O<sub>2</sub> pressure is high, while in tissues, the pressure is low. Determining the correct hb levels is important and has become one of the routine tests performed on almost every patient (Kiswari, 2014).

According to Rahayu (2018) on the research about overview of hemoglobin levels in students level III of Medan Health Analyst Department before and after being stored for 2 hours at room temperature that has been done in the Hematology Laboratory of the Department of Health Analysts Medan to 22 blood samples using cyanmethemoglobin method, obtained results with delay of examination time for 2 hours at room temperature resulted in hemoglobin levels in the blood EDTA decreased. Examination using EDTA blood should be done immediately, only if necessary can be stored in the refrigerator (4 ° C). EDTA blood stored at 4°C for 24 hours provides a higher hematocrit value. To make a blood smear preparation edge can be used EDTA blood stored for a maximum of 2 hours. In general, EDTA blood can be stored 24 hours in the refrigerator without bringing significant irregularities, except for platelet count and hematocrit value (Gandosoebrata, 2010).

EDTA blood delayed by more than 2 hours at room temperature or more than 24 hours at 4°C erythrocytes will swell so that hematocrit values, Average Erythrocyte Volume (VER) increases and average erythrocyte hemoglobin (KHER) concentration decreases. The container that is not tightly closed results in evaporation from the examination material so that the examination results are higher than actually (Muslim, 2015). This is possible because erythrocyte morphological abnormalities that occur are forms of crenation / echinocyte (Munandar, 2016). Crenation is an erythrocyte form which shrinks and bulges rise on its surface. Crenation usually forms in blood that is left at room temperature for a long time which means it is also increasingly exposed to EDTA. This change in erythrocyte form can be caused by the influence of intrinsic factors such as reduced adenosine triphospat (ATP) or due to extrinsic factors such as an increase in anticoagulant pH.

In addition, EDTA will cause a decrease in the surface tension of the erythrocyte membrane so that the erythrocyte membrane becomes weak and unstable, erythrocytes will swell and bulges are formed on its surface causing a change in shape from discoid to echinocyte. Some results of the study above means that the delay in the time of checking K2EDTA blood hemoglobin levels that are too long at room temperature can affect the results of hemoglobin levels. K2EDTA is the best type of anticoagulant and is advocated by International Council for Standardization in Hematology and National Committee for Clinical Laboratory Standard. It because the ratio between anticoagulant doses with blood volume can be accounted for (Garini, 2011).

K2EDTA serves to prevent coagulation, but has certain stability standards in examinations, especially hematology examinations. K2EDTA blood only serves to inhibit the occurrence of coagulation, not to prevent or withstand changes in the shape of erythrocyte morphology. However, when the K2EDTA blood test passes the specified stability standard, there will be erythrocyte morphological deformities into a form of crenation or echinocyte in which the erythrocyte form shrivels and bulges arise on its surface. Then the erythrocyte membrane will break (hemolysis) and hemoglobin is free into other mediums (plasma). So hemoglobin is difficult to detect (read) by the tool and the level of hemoglobin obtained is lower than it should be.

## **CONCLUSIONS AND SUGGESTIONS**

The average hemoglobin level of K2EDTA with a shelf time of less than 1 hour and more than 3 hours at room temperature are 16.15 gr/dL. and 14.67 gr /dL. Meanwhile, the morphology of erythrocytes K2EDTA with a storage time of less than 1 hour and more than 3 hours at room temperature is normal. It can be concluded there is an effect between the shelf time of less than 1 hour and more than 3 hours of blood K2EDTA on hemoglobin and there is an effect of examination time of less than 1 hour and more than 3 hours of K2EDTA blood at room temperature against erythrocyte morphology. It is recommended to conduct further research on the risk factors for the effect of K2EDTA blood storage at room temperature on erythrocyte morphology.

## REFERENCE

- Adhawiah, R. (2016). *Pengaruh Senam Hamil Terhadap Perubahan Kadar Hemoglobin (Hb) pada Ibu Dengan Usia Kehamilan Lebih dari 5 Bulan Di Puskesmas Samata.*
- Damuel, S. (2018) Perbedaan Kadar Hemoglobin Menggunakan Hb Meter, Spektrofotometer dan *Hematology Analyzer* pada Sampel Segera Diperiksa dan Ditunda 20 jam. 1-6
- Elsy, P.P., sayekti, S., & isro'aini. (2016). Gambaran Pemeriksaan Kadar Hemoglobin (Hb) Cyanmethemoglobin yang Diperiksa Segera dan Ditunda 4 Jam. (5), 1–7.
- Gandasoebrata, R. (2007). *Penuntun Laboratorium Klinik.* Dian Rakyat: Jakarta.
- Gandosoebrata. (2010). *Penuntun Laboratorium Klinik.* Jakarta: Dian Rakyat.
- Hasanan, F. (2018). Hubungan Kadar Hemoglobin dengan Daya Tahan Kardiovaskular pada Atlet Atletik FIK Universitas Negeri Makassar. *Jurnal Olahraga Dan Kesehatan*, (16), 1–16.
- Hasri, S. I. P. (2016). Perbandingan Pemeriksaan Kadar Hemoglobin Metode Cyanmeth Secara Langsung dan Tidak Langsung. *Jurnal Medika*, (8), 1–8.
- Kiswari, d. R. (2014). *Hematologi & Transfusi.* Jakarta: Penerbit Erlangga.
- Muslim, A. (2015). Pengaruh Waktu Simpan Darah K2EDTA, Na2EDTA Pada Suhu Kamar Terhadap Kadar Hemoglobin. *Jurnal Analis Kesehatan*, 392-396.
- Rahayu, C. (2018). Gambaran Kadar Hemoglobin Pada Mahasiswa/i Tingkat III Analis Kesehatana Medan Sebelum Dan Sesudah Disimpan Selama 2 Jam Pada Suhu Kamar. *Jurnal Analis Kesehatan*, 2(2): 55-67.