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THE EFFECT OF EDTA BLOOD STORAGE TIME AND ROOM TEMPERATURE ON THE EXAMINATION OF ERYTHROCYTE SEDIMENTATION RATE

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Abstract

Background*: Erythrocyte Sedimentation Rate (ESR) is an examination that aims to determine the speed of erythrocytes settling in the blood measured for 1 hour. The examination of ESR performed within 2 hours at most. However, the examination is often not performed immediately due to several reasons that cause delays for several hours. The room temperature during the examination affects the examination. The temperature in West Java has the highest average reaching 32°C.* ***Aims:*** *To determine an effect of EDTA blood storage time and room temperature on the examination of ESR.* ***Method:*** *This research is included in the type of quasi eksperimental research by involving variations in blood storage time immediately, 4 hours, and 5 hours and room temperature during examination is room temperature 20-25^{o}C and temperature 29-32^{o}C.* ***Result:*** *The result of this study showed that there was a significant effect of the variable EDTA blood storage time and room temperature on the examination of ESR which is indicated by the value of Sig. 0,00 (Sig. <0,05). This is because EDTA blood stored more than 2 hours after blood collection will affect the shape of the cells and the temperature can affect the settling speed.* ***Conclusion:*** *Based on the results of statistical tests, it can be concluded that there is an effect of EDTA blood storage time and room temperature on the examination of ESR.*

Keywords*: Erythrocyte Sedimentation Rate, EDTA Blood Storage Time, Room Temperature*

1. Introduction

Erythrocyte sedimentation rate (ESR) is an examination that aims to determine the speed at which erythrocyte settle in blood examined after 1 hour and containing anticoagulants. ESR is one of the examination used to detect and monitor the presence of damage in tissues, inflammation, and shows both chronic and acute illness (but not the severity) (1).

The Westergren method is the gold standard for ESR. This mesthod is recommended by the International Council for Standardization in Haemotology (ICSH) 1973 because it has high sensitivity, is reliable, and has a long tube that makes reading easer (2);(3).

The International Council for Standardization in Haemotology (ICSH) 1973 recommended an anticoagulant for ESR using 3,8% Sodium citrate (2).

However, the examination of ESR is always accompanied by a complete blood count which causes blood to be drawn simultaneously using EDTA anticoagulant because it is more economical (4);(5). This because EDTA anticoagulant can also be used for ESR (6).

The examination of ESR with EDTA blood needs to pay more attention to the limit of blood collection to avoid in vitro changes during the storage procces and preferably no later than 2 hours after the blood collection (7). If the examination is carried out for more than 2 hours, the blood cells will change their shape to become more spherical and it is difficult to form roulex which causes a decrease in the ESR (8). The examination of ESR is often not performed immediately due to several reasons, including blood collected for examination at the same time, repeated examination using stored blood samples, large number of spesimens examined at a time, limited equioment, distance of the examination site, length of transportation when referred to other laboratories, and limited health analyst personnel causing delays in examination up to several hours (9);(10).

The examination of ESR also needs to pay attention to the room temperature during the examination. The temperature used for ESR is room temperature 20-25ºC (1). Temperature can affect the settling speed. If the temperature used is high, the viscosity of blood will decrease and make the ESR increase (11);(12). Meteorogical, Climatological, and Geophsical Agency stase that the temperature in West Java has the highest average temperature reaching 32ºC. When the examination of ESR requires room temperature for examination, can be overcome by the use of AC. However, in previous research it as found that some laboratories in West Java do not use AC, making the room temperature for ESR not optimal (9).

The results of research concuted by Riana et al., (2023) found that there was no effect of the blood stored for 3 hours at 28ºC on the examination of ESR. Another result of research concuted by Candrakirana, (2018) found that there were differences in the value of ESR examined directly and stored for 6 hours at room temperature, so researchers will conduct research with immediate storage time, 4 hours, and 5 hours with room temperature 20-25ºC and temperature 29-32ºC.

2. Research Methods

This research is included in the type of quasi eksperimental research by involving variations in blood storage time immediately, 4 hours, and 5 hours and room temperature during examination is room temperature 20-25ºC and temperature 29-32ºC. The research was conducted in May 2024 which was carried out at the Hematology Laboratory, Medical

Laboratory Technology Departemen of Bandung Health Polytechnic.

Subject were students of Medical Laboratory Technology Departemen of Bandung Health Polytechnic who were examined of ESR and met the inclusion criteria is willing to become respondents, filling out informed consent, aged 18-24 years, in state of fasting 8-12 hours and exclusion criteria is in a state of illness, hemolysis samples, and lipemic samples.

Subject were taken from the population and the determination number of subject was carried out using Federer's formula (t-1) (n-1) ≥ 20, where (t) is a treatment group of 6 resulting from 2 room temperature treatments (20-25ºC and 29-32ºC) and 3 treatment from storage time (immediately, 4 hours, and 5 hours) at each room temperature, and (n) is the subject. Based on the calculation, a minimum of 5 subject was obtained, and in this research using 6 normal blood subject.

The sample selection in this study was using purposive sampling technique by selecting samples among the population in accordance with the inclusion and exclusion criteria of the study.

The procedure carried out in this study began with the distribution of informed consent and if the subject was willing to become a respondent, venous blood was taken from the subject. The examination of ESR was carried out by the Westergren method using EDTA anticoagulant which was stored immediately, 4 hous, and 5 hours and then given a 0,85% sodium chlorida for dilution (0,4 mL 0f 0,85% sodium chlorida + 1,6 mL of blood) and sucked into the Westergren tube until the 00 mm limit mark. After that, the treatment was carried out in 2 treatment is at a room temperature 20-25ºC and temperature 29-32ºC and storaged in an upright position in the Westergren tube rack and the result of ESR were reported by measuring the plasma height in the Westergren tube that had been stored for 1 hour.

The data in this study are primary data obtained from the result of ESR using Westergren method. Data processing of the research results is played in tabular form and continued with statistical tests using General Linear Model (GLM) test to see the effect of EDTA blood storage time and room temperature on the examination of ESR.

3. Result and Discussion

A. Result

The result data of the examination of ESR using Westergren method with EDTA blood storage time immediately, 4 hours, and 5 hours and room temperature during examination is room temperature 20-25ºC and temperature 29-32ºC are shown in Table 1

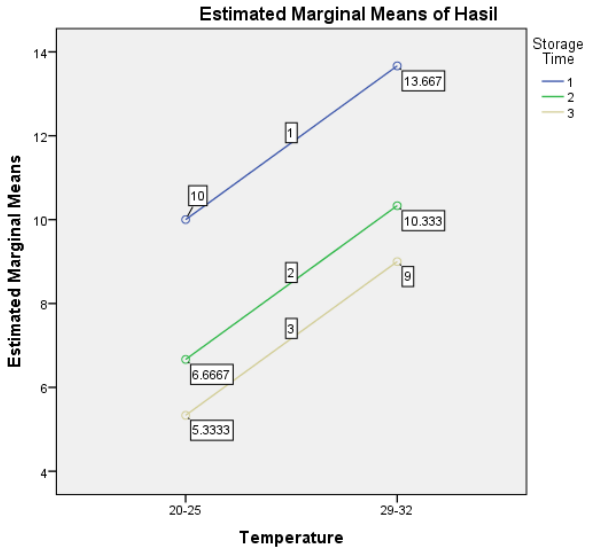
Table 1. Result Data of ESR (mm/h)

Sample	Room Temperature 20-25ºC			Room Temperature 29-32ºC		
	Immediately	4 hours	5 hours	Immediately	4 hours	5 hours
1	11	8	6	15	12	11
2	9	5	4	13	9	8
3	10	7	5	13	9	7
4	10	7	6	14	11	10
5	9	6	5	13	10	9
6	11	7	6	14	11	9

Source: Primary Data (2024)

In Table 1, it can be seen that the examination of ESR value with immediate, 4 hours, and 5 hours decreased. Meanwhile, when viewed from the room temperature at during examination, the temperature of 29-32ºC has higer ESR value than the room temperature of 20-25ºC.

Figure 1. Profil Plot of ESR values



The mark (1) indicates an immediately, (2) indicates a storage time of 4 hours, and (3) indicates a storage time of 5 hours. In Figure 1. It can be seen that the longer the blood is stored, the lower of ESR value and the higer the temperature used, the higer of ESR value produced.

The General Linear Model (GLM)-Repeated Measure test was conducted to determine whether there was an effect of EDTA blood storage time and room temperature on the examination of ESR using Westergren method. The result of the *General Linear Model* (GLM)-*Repeated Measure* test are shown in Table 2.

Table 2. Test General Linear Model (GLM)-Repeated Measure

	Data Group	Sig.	Result
Storage Time	Immediately vs 4 hours	0,000	Sig. <0,05
	Immediately vs 5 hours	0,000	Sig. <0,05
Room Temperature	Room temperature 20-25ºC vs temperature 29-32ºC	0,000	Sig. <0,05
	Temperature 29-32ºC vs room temperature 20-25ºC	0,000	Sig. <0,05

Source: Primary Data (2024)

The result of General Linear Model (GLM)-Repeated Measures test showed that there was a significant effect between immediate storage time and 4 hours, and immediate storage time and 5 hours on the examination of ESR using Westergren method as indicated by Sig. <0,05. Futhermore, there is a significant effect between room temperature at the time of examination at room temperature 20-25^{0}C and room temperature 29-32^{0}C on the examination of ESR using Westergren method as indicated by Sig. <0,05. It can be concluded that there is a statistically significant effect on both variables.

B. Discussion

In statistical data processing tha has been carried out using the General Linear Model (GLM)-Repeated Measure test regarding the effect of EDTA blood storage time on the examination of ESR using Westergren method contained in Table 2. obtained Sig. 0,000 (Sig. value <0,05), which can be interpreted that there is a significant effect of EDTA blood storage time variable on the examination of ESR using Westergren method. This is because EDTA blood storage time more than 2 hours after blood collection will affect the shape of the cells. The longer the blood stored, the smaller of ESR value produced.

The formation of roulex can occur due to the shape of red blood cells in the form of biconcave discs that allow contact with other red blood cells and stick together resulting in roulex formation (12). In the blood that stored, blood cells will change shape to become more spherical (more rounded) because the amount of adenosine triphosphate produced from glucose deconstruction is reduced, causing the pump function of sodium and potassium ions in maintaining cell volume to be distruped. Sodium ions and calcium ions entering the cell and potassium ions leaving the cell cause water osmosis to occur in the cell, resulting in the swelling of erythrocytes. Erythrocytes that are deformed into abnormal shapes will cause difficulty in roulex formation and result in decreased of ESR (8);(13).

The examination of ESR has 3 phases, the first of which is roulex formation. Roulex is a clump of erythrocytes that occurs not due to antibodies or covalent bonds but due to mutual attraction of cell surfaces (14). In this phase, the time required is <15 minutes (15). The second phase is the fast settling phase because after aggregation, it causes settling to take place quickly and the time required in this phase is about 30 minutes. The third phase is the slow settling or compaction phase and the time required in this phase is about 15 minutes. The sedimentation speed depends on the roulex phase. When the roulex formation is greater, the sedimentation speed is also higer (1);(15). When blood is stored, there is less roulex formation, which causes the sedimentation speed to decrease and makes the ESR value is low.

The result of this study are in accordance with the result of research concuted by Salnus et al., (2023) with a comparative study of the results of ESR examined immediately and blood stored for 5 hours which found that there was a significant comparison of ESR values in blood that was immediately examined and which was delayed for 5 hours. It can be concluded that the examination of ESR with EDTA blood should be carried out immediately, which is less than 2 hours.

In statistical data processing tha has been carried out using the General Linear Model (GLM)-Repeated Measure test regarding the effect of room temperature on the examination of ESR using Westergren method contained in Table 2. obtained Sig. 0,000 (Sig. value <0,05), which can be interpreted that there is a significant effect of the room temperature variable on the examination of ESR using Westergren method. This is because temperature can affect the settling speed. The higher the temperature used used in the examination, the higher the value of the resulting of ESR. This is because when the temperature used is high, it will cause the blood viscosity to decrease and make of ESR value increase (11);(12).

Blood viscosity will decrease about 2% with each increase in temperature, so it can be interpreted that when there is an increase in temperature higher than normal, the blood viscosity will decrease and make the ESR vakue higher (16).

The recommended temperature for ESR using Westergren method is room temperature 20-25^{0}C (1). The result of research conducted by Riana et al., (2023) regarding the temperature used when the examination of ESR is at a temperature 28^{0}C and the result showed that there was no effect of temperature on the examination of ESR using Westergren method. It can be concluded, the examination of ESR should be carried out at room temperature 20-25^{0}C and if there is an increase in temperature

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then the temperature used is not more than 28^0C.

4. Conclusion

Based on the results it can be concluded that there is an effect of EDTA blood storage time and room temperature on the examination of ESR.

The suggestion in this study is the examination of ESR should be examined immediately after blood collection and if there is a delay in the examination, it should not exceed 2 hours. In addition, the room temperature used when the examination of ESR is in accordance with the provisions at room temperature 20-25°C.

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