Journal of Vocational Health Studies

https://e-journal.unair.ac.id/JVHS

THE INFLUENCE OF BLOOD VOLUME AND STORAGE DURATION ON THE ERYTHROCYTE SEDIMENTATION RATE (ESR) VALUE USING THE WESTERGREN METHOD

PENGARUH VOLUME DARAH DAN LAMA SIMPAN TERHADAP NILAI LAJU ENDAP DARAH (LED) METODE WESTERGREN

Original Research Report
Penelitian

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A B S T R A C T

Background: *Hematological examination is conducted to determine the condition of blood and its components, which are used to establish a diagnosis, support a diagnosis, make a differential diagnosis, monitor disease progression, assess the severity of an illness, and determine the initial prognosis of a disease. Phlebotomy procedures in the pre-analytical stage are not always successful and sometimes encounter failure. Inappropriate anticoagulant administration can lead to erroneous hematological examination results, including Erythrocyte Sedimentation Rate (ESR) examination results.* ***Purpose:*** *This research aims to examine the influence of blood volume and storage duration on the Erythrocyte Sedimentation Rate (ESR) value using the Westergren method.* ***Method:*** *The Westergren method utilizes 5 venous blood samples with a ratio of blood volume to 3.8% anticoagulant at 4 : 1, 3 : 1, and 2 : 1, with sample storage durations of 0 and 3 hours at room temperature.* ***Result:*** *The average ESR values with a ratio of 4 : 1, at 0 hours, were 5.20 mm/hour and at 3 hours were 3.60 mm/hour. The average ESR values with a ratio of 3 : 1, at 0 hours, were 6.20 mm/hour and at 3 hours were 4.40 mm/hour. The average ESR values with a ratio of 2 : 1, at 0 hours, were 7.60 mm/hour and at 3 hours were 5.60 mm/hour.* ***Conclusion:*** *There is a significant influence of blood volume (p-value < 0.05) and storage duration (p-value = 0.05) on the Erythrocyte Sedimentation Rate (ESR) value using the Westergren method.*

A R T I C L E I N F O

Received 17 February 2024
Revised 27 February 2024
Accepted 16 June 2025
Available Online 15 November 2025

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Keywords:
Erythrocyte Sedimentation Rate (ESR), Blood volume, Storage duration, Westergren method

A B S T R A K

Latar belakang: Pemeriksaan hematologi dilakukan untuk mengetahui keadaan darah dan komponen-komponennya yang digunakan untuk menegakkan diagnosis, menunjang diagnosis, membuat diagnosis banding, memantau perjalanan penyakit, menilai beratnya suatu penyakit, dan menentukan prognosis awal suatu penyakit. Tindakan flebotomi pada tahap pra analitik tidak selalu berhasil dan terkadang mengalami kegagalan. Pemberian antikoagulan yang tidak tepat akan menyebabkan kesalahan hasil pemeriksaan hematologi, termasuk hasil pemeriksaan Laju Endap Darah (LED). **Tujuan:** Penelitian ini bertujuan untuk melihat pengaruh volume darah dengan waktu penyimpanan terhadap nilai LED metode Westergren. **Metode:** Metode Westergren menggunakan 5 sampel darah vena dengan perbandingan volume darah dan antikoagulan 3,8% yaitu 4 : 1, 3 : 1, 2 : 1 dengan lama simpan sampel 0 dan 3 jam pada suhu ruang. **Hasil:** Rata-rata nilai LED dengan perbandingan 4 : 1, 0 jam sebesar 5.20 mm/jam dan 3 jam sebesar 3.60 mm/jam. Rata-rata nilai LED perbandingan 3 : 1, 0 jam sebesar 6.20 mm/jam dan 3 jam sebesar 4.40 mm/jam. Rata-rata nilai LED perbandingan 2 : 1, 0 jam sebesar 7.60 mm/jam dan 3 jam sebesar 5.60 mm/jam. **Kesimpulan:** Terdapat pengaruh yang bermakna volume darah p (Sig) < 0.05 dan lama simpan p (Sig) 0.05 terhadap nilai LED metode Westergren.

Kata kunci:
Laju Endap Darah (LED), Volume darah, Lama simpan, Metode westergren

Journal of Vocational Health Studies p-ISSN: 2580–7161; e-ISSN: 2580–717x
DOI: 10.20473/jvhs.V9.I2.2025.103-109

INTRODUCTION

One of the most commonly performed hematological examinations is the Erythrocyte Sedimentation Rate (ESR) test, also known as the Sedimentation Rate (Sed Rate) test. It is a blood test that reflects the rate at which red blood cells settle in blood plasma using an anticoagulant and is expressed in mm/hour (Aulia, 2017). The results of the ESR test are used to monitor inflammation processes and acute disease activity as well as for screening purposes. An increase in ESR values indicates the presence of inflammation in an individual's body, whether acute or chronic, or tissue damage (Akkiz *et al*., 2021; Patel and Gizinski, 2018; Tishkowski and Gupta, 2024; Wu *et al*., 2018).

Plasma viscosity is a measure of the thickness of blood plasma, the fluid component of blood. Plasma viscosity can affect ESR, as high viscosity can impede upward gravitational pull on red blood cells, ultimately influencing ESR (Syafa'ati *et al*., 2017). On another note, spheroid or spherical refers to a change in the shape of erythrocytes from concave to round or more rounded. The change in erythrocyte shape to more spherical can hinder the formation of rouleaux, which are clumps of red blood cells that occur due to mutual attraction and repulsion between cell surfaces, with a more spherical shape, erythrocytes cannot easily adhere to each other to form rouleaux structures, thus increasing ESR (Adhikari *et al*., 2017; Artha *et al*., 2019).

There are several methods used for manual ESR testing, but the Westergren method is recommended by the International Committee for Standardization in Hematology (ICSH) (Rahmawati and Aini, 2019) because the ESR results under normal conditions differ from those of the Wintrobe method, which do not differ significantly (Hidriyah *et al*., 2018). The Westergren method is also chosen as the reference method because it is reliable, reproducible, and sensitive (Kratz *et al*., 2017).

The principle of ESR examination involves measuring the rate of erythrocyte sedimentation and depicting the composition of plasma as well as the ratio between erythrocytes and plasma. ESR is influenced by the weight of blood cells, the surface area, and the Earth's gravity. The heavier the blood cells, the faster the sedimentation rate, and the larger the surface area of the cells, the slower the sedimentation (Dekayana, 2019; Narang *et al*., 2020).

Inappropriate administration of anticoagulants can lead to errors in hematological examination results, including ESR results (Subiyati, 2017). Based on the research outcomes of Sari in 2023, the blood volume taken did not reach the limit (<1 mL), resulting in an influence on laboratory examination errors. The procedure for using K3EDTA tubes for hematological examinations requires the blood volume taken to reach the limit, whereas in cases found in hospitals, the blood volume taken did not reach the limit (<1 mL), thus affecting hematological examinations, one of which is an increase in ESR values due to excess anticoagulants (Sari, 2023).

Based on survey results in several clinical laboratories in the Subang area, there is often a shortage of blood volumes in vacutainer tubes and samples are stored for more than 2 hours. Inappropriate blood volume, inappropriate anticoagulant, and inappropriate sample storage temperature cause hemolysis, which will affect sample stability and result in inaccurate examination results (Møller, 2006).

The ESR examination results on EDTA blood volumes that were immediately tested showed an average ESR value of 32.4 mm/hour, whereas the average ESR value for EDTA blood volumes delayed by 6 hours was 27.07 mm/hour. The examination results indicated a decrease of up to 5.33 mm/hour, attributed to the prolonged storage of EDTA blood, leading to an imbalance in the Sodium and Potassium pumps, and causing red blood cells to change shape into a more spherical form, making it difficult to form rouleaux (Dyahwisnu, 2018).

The innovative aspect of this research lies in assessing the ratio of blood volume to anticoagulant, particularly for patients in whom blood collection is difficult (pediatrics or baby). Thus, the results of this study can provide information on whether variations in the ratio of blood volumes to their storage duration influence the outcome (4 : 1, 3 : 1, and 2 : 1). This research aims to determine the effect of storage duration with various ratios of blood volumes and anticoagulants on ESR test results. Furthermore, it aims to provide recommendations for appropriate storage duration and optimal ratios of blood volumes and anticoagulants for ESR testing in clinical laboratories.

MATERIAL AND METHOD

The research design employed in this research is a quasi-experimental research, measuring the ESR using the Westergren method. The design in this research design is a time series design. In this research design, the experimental group received different treatments by being given variations in blood volume and storage duration of 0 hours (checked immediately) and 3 hours after blood was drawn, then the ESR value was checked. The principle is that blood with an anticoagulant in a certain ratio and put into a special tube (Westergen) which is placed upright and left for 1 hour, the erythrocytes will settle. The rate of erythrocyte precipitation reflects the final blood velocity and is expressed in mm/hour. The design utilized in this research is a time-series design. In this research design, the experimental group received different treatments with variations in blood volume and storage duration.

The samples were collected from 5 normal blood donors, each donating 10 mL of blood, who were students of the Medical Laboratory Technology Department at Poltekkes Kemenkes Bandung. Subsequently, the research data were analyzed using IBM SPSS Statistics 26.

The research began with taking venous blood volumes and then varying the blood volumes with citrate blood 3.8%. After that, each blood volume was stored at room temperature (20 – 25^{o}C) for 0 hours (immediately) and 3 hours before the ESR examination. Followed by checking the ESR in accordance with Standard Operating Procedures (SOP), this is done by mixing 3.8% sodium citrate with venous blood, then drawing it into a Westergren tube and allowing it to stand upright for 1 hour. After that, the distance the red blood cells have settled to the bottom of the tube is measured.

RESULT

The research results regarding the influence of blood volume and storage duration on the ESR using the Westergren method are presented in Table 1. The research outcomes show that the range of ESR values using the Westergreen method with a blood volume ratio of 4 : 1, stored for 0 hours, ranges from a minimum of 4 mm/hour to a maximum of 7 mm/hour, while for storage duration of 3 hours, the range is from a minimum of 2 mm/hour to a maximum of 5 mm/hour. A blood volume ratio of 3 : 1, stored for 0 hours, the range is from a minimum of 5 mm/hour to a maximum of 8 mm/hour, whereas for a storage duration of 3 hours, the range is from a minimum of 3 mm/hour to a maximum of 6 mm/hour. A blood volume ratio of 2 : 1, stored for 0 hours, the range is from a minimum of 6 mm/hour to a maximum of 10 mm/hour, and for a storage duration of 3 hours, the range is from a minimum of 5 mm/hour to a maximum of 7 mm/hour.

Data processing results using SPSS

Normality test results

The results of the normality test are presented in Table 2. The obtained p-value (Sig) is greater than 0.05, indicating that the data are normally distributed, with these results, the statistical test used is the General Linear Model (GLM) Repeated Measures test.

Homogeneity test results

The outcomes of both the platelet count homogeneity and platelet aggregation tests are displayed in Table 3. The homogeneity test results above yielded a p-value (Sig) > 0.05, indicating that the data overall are homogeneously distributed. with these results, the statistical test used is the GLM repeated measures test.

General Linear Model (GLM) test results

Based on Table 4, the GLM test results above yielded Sig values from the output for the influence of blood volume ratios of 4 : 1, 3 : 1, and 2 : 1 showing Sig values of 0.000, indicating p (Sig) < 0.05. Additionally, for storage durations of 0 hours and 3 hours, Sig values of 0.000 were obtained, also indicating p (Sig) < 0.05. Therefore, it can be concluded that there is a significant difference in the distribution of blood volume examination data concerning the Westergren method's ESR values.

General Linear Model (GLM) test for storage duration

Furthermore, a GLM test was also conducted for storage duration to determine whether there is a difference in storage duration concerning the values of the Westergren method ESR, presented in Table 5.

Based on Table 5, the influence of storage duration on the Westergren method ESR values can be observed. The GLM test results for the storage duration groups of 0 hours and 3 hours show a Sig value of 0.000, indicating that p (Sig) < 0.05. Therefore, H0 is rejected, and H1 is accepted, meaning that there is an influence of storage duration on the Westergren method ESR values.

Results of the Wilcoxon test

Based on the GLM test in Table 5, it was found that there is a significant influence between blood volume and sample storage duration on the Westergren method ESR values. Therefore, the Wilcoxon test was conducted to observe the differences in ESR values between blood volume ratios of 4 : 1 with a storage duration of 0 hours as control, compared to blood volume ratios of 4 : 1 with a storage duration of 3 hours, blood volume ratios of 3 : 1 with a storage duration of 0 hours, blood volume ratios of 3 : 1 with a storage duration of 3 hours, blood volume ratios of 2 : 1 with a storage duration of 0 hours, and blood volume ratios of 2 : 1 with a storage duration of 3 hours. These comparisons are presented in Table 6.

Based on Table 6, the Wilcoxon test results above indicate that the ESR volume 4 : 1 value with 0 hours of storage duration compared to the ESR volume 4:1 value with 3 hours of storage duration yielded a significant result of 0.038 p (Sig) < 0.05. It can be concluded that there is a significant difference between the ESR volume 4 : 1 value with 0 hours of storage duration and the volume 4 : 1 with 3 hours of storage duration. Furthermore, comparing the ESR volume 4 : 1 value with 0 hours of storage duration to the ESR volume 3 : 1 value with 0 hours of storage duration, a significant result of 0.025 p (Sig) < 0.05 was obtained. It can be concluded that there is a significant difference between the ESR volume 4 : 1 value with 0 hours of storage duration and the volume 3 : 1 with 0 hours of storage duration.

The ESR volume 4 : 1 value with 0 hours of storage duration compared to the ESR volume 3 : 1 value with 3 hours of storage duration yielded a significant result of 0.046 p (Sig) < 0.05, indicating a significant difference

between the ESR volume 4 : 1 value with 0 hours of storage duration and the volume 3 : 1 with 3 hours of storage duration. Additionally, comparing the ESR volume 4 : 1 value with 0 hours of storage duration to the ESR volume 2 : 1 value with 0 hours of storage duration, a significant result of 0.038 p (Sig) < 0.05 was obtained. It can be concluded that there is a significant difference between the ESR volume 4 : 1 value with 0 hours of storage duration and the volume 2 : 1 with 0 hours of storage duration.

The ESR volume 4 : 1 value with 0 hours of storage duration compared to the ESR volume 2 : 1 value with 3 hours of storage duration yielded a significant result of 0.047 p (Sig) < 0.05, indicating a significant difference between the ESR volume 4 : 1 value with 0 hours of storage duration and the volume 2 : 1 with 3 hours of storage duration, from the results, it can be concluded that there is an influence of blood volume and storage duration on the ESR value in the Westergren method.

Table 1. Erythrocyte Sedimentation Rate (ESR) of different storage durations with various blood volumes

	Erythrocyte Sedimentation Rate (ESR)(mm/hour)														
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Blood volume / Storage duration (hour)	4 : 1					3 : 1					2 : 1				
0	4	6	4	5	7	5	7	5	6	8	7	8	6	7	10
3	3	5	2	3	5	4	5	3	4	6	5	6	5	5	7

Repetition: 5 times on both of blood storage durations and blood volumes (1,2,3,4, and 5)

Table 2. Normality test results of different storage durations

Storage duration (hour)	Blood volume	p-value	Results	Conclusion
0	4 : 1	0.421	p > 0.05	Normal distribution
	3 : 1	0.421	p > 0.05	Normal distribution
	2 : 1	0.492	p > 0.05	Normal distribution
3	4 : 1	0.201	p > 0.05	Normal distribution
	3 : 1	0.814	p > 0.05	Normal distribution
	2 : 1	0.546	p > 0.05	Normal distribution

Table 3. Homogeneity test result of blood volume and storage duration

Variabels	Significance value	Results	Conclusion
Blood volume	0.993	p > 0.05	Homogeneous distribution
Storage duration	0.534	p > 0.05	Homogeneous distribution

Table 4. Test of between subject effect in storage durationss to blood volume

Storage duration (hour)	Blood volume	Significance value	Results	Conclusion
0	4 : 1	0.000	p < 0.05	There is a difference
	3 : 1	0.000	p < 0.05	There is a difference
	2 : 1	0.000	p < 0.05	There is a difference
3	4 : 1	0.000	p < 0.05	There is a difference
	3 : 1	0.000	p < 0.05	There is a difference
	2 : 1	0.000	p < 0.05	There is a difference

Table 5. Results of the General Linear Model (GLM) test for storage durations

Storage duration (hour)	Significance value	Results	Conclusion
0	0.000	p < 0.05	There is a difference
3	0.000	p < 0.05	There is a difference

Table 6. Results of the Wilcoxon test

	Test statistics[a]				
	Ratio 4 : 1 at 0 hours storage compared to 4 : 1 at 3 hours storage.	Ratio 4 : 1 at 0 hours storage compared to 3 :1 at 0 hours storage.	Ratio 4 : 1 at 0 hours storage compared to 3 : 1 at 3 hours storage.	Ratio 4 : 1 at 0 hours storage compared to 2 : 1 at 0 hours storage.	Ratio 4 : 1 at 0 hours storage compared to 2 : 1 at 3 hours storage.
Z	-2.070[b]	-2.236[c]	-2.000[b]	-2.070[c]	-2.414[c]
Asymp. Sig. (2-taiESR)	0.038	0.025	0.046	0.038	0.047

DISCUSSION

In this study, venous blood was taken using a 10 mL syringe and divided into three tubes based on the ratio of blood volumes to citrate blood anticoagulant (4 : 1, 3 : 1, and 2 : 1) and carried out with immediate storage duration and 3 hours. Previous research only examined the effect of blood volume, not the ratio of citrate blood to 3.8%. In addition, this study also investigated the effect of a 3 hour delay, as the 2018 study by Dyahwisnu (2018) showed that a 6-hours delay had a significant impact.

After conducting statistical tests for normality and homogeneity, a GLM test was performed on the variations in blood volume and storage duration. The obtained p-value (Sig) was < 0.05. This indicates that statistically, there are differences in ESR values using the Westergren method between storage duration of 0 hours and 3 hours for blood volumes of 4 : 1, 3 : 1, and 2 : 1. Therefore, it is concluded that variations in blood volume and storage duration have an effect on the examination of ESR using the Westergren method. This may lead to an increase in plasma viscosity, however, in this study no viscosity examination was carried out, which in turn can cause an increase in ESR. High plasma viscosity can lead to faster sedimentation of red blood cells, reflected in increased ESR. Thus, if the blood volume to anticoagulant ratio is 3 : 1 or 2 : 1, this may result in an increase in ESR due to increased plasma viscosity affecting red blood cell sedimentation (Harrison, 2015; Yin *et al*., 2017).

When the volume of blood collected is incorrect, reducing the concentration of anticoagulant, and if more blood is collected than the designated volume, it can lead to crenation. Crenation can also be caused by changes in the shape of erythrocytes, which may result from a lack of ATP in erythrocytes. When the volume of blood inserted into the vacutainer is less than the specified amount, it causes the blood cells to shrink and hemodilution occurs, as hemodilution reduces the concentration of anticoagulant and increases the concentration of erythrocytes and leukocytes. Therefore, it is important to ensure the appropriate volume of blood collected to avoid crenation and errors in blood testing, and excessive blood volume can lead to blood clotting (Cahya, 2021).

In blood volumes with a lower ratio, namely 3 : 1 and 2 : 1, blood clots will occur, because the blood volume is greater than the SOP, so as a result there will be small clots which can create falsely low ESR values. Apart from that, delaying citrate samples can also cause blood hemolysis and high ESR values (Tutwiler *et al*., 2016).

The examination of ESR using blood with anticoagulants needs attention to the storage duration limit to avoid in vitro changes during storage or due to the influence of anticoagulants. In vitro changes occur when blood is stored for a long time, resulting in a decrease in ESR. The longer the blood is stored, the more red blood cells undergo changes and affect the ESR value. Ideally, ESR testing on citrated blood volumes should be done within a maximum of 2 hours (Gandasoebrata, 1968).

Hadi's theory in 2011 states that in blood stored or not immediately examined more than 1 - 2 hours after sample collection, red blood cells will change shape to become more spherical and have difficulty forming rouleaux, resulting in slower ESR and causing ESR values to tend to decrease (Sitepu, 2019). The change in shape of red blood cells to spherical and difficulty in forming rouleaux is due to a reduction in the amount of ATP or energy in the cells, causing disruption in the function of Na^+ and K^+ pumps in maintaining or regulating volume (Nugraha, 2017).

Rouleaux is a term used to describe a condition in which red blood cells clump together like stacks of coins. This occurs when proteins in the blood plasma, especially fibrinogen, increase, causing red blood cells to stick together and form structures resembling stacks of coins (Masito, 2020). This condition can occur when blood is stored or not immediately examined more than 1 - 2 hours after sample collection, due to changes in the condition of the blood plasma. Red blood cells that change shape to become more spherical and have difficulty forming rouleaux can affect the results of ESR tests.

This research is in line with the results of research conducted by Dyahwisnu in 2018, where the results of the ESR examination on EDTA blood volumes which were immediately examined had an average ESR value of 32.4 mm/hour and the average ESR value with EDTA blood volumes which was delayed 6 hours had an average 27.07 mm/hour. The results of the examination showed a decrease of up to 5.33 mm/hour, which was caused by storing EDTA blood for too long, resulting in an imbalance in the Sodium Potassium pump and the blood cells would experience a change in shape to become more spherical, making it difficult to form a rouleaux (Dyahwisnu, 2018).

CONCLUSION

There is a significant influence of blood volume and storage duration on the ESR values in the Westergren method. The purpose of providing advice is to advise readers to use appropriate blood and anticoagulant ratios to obtain accurate ESR values or levels.

ACKNOWLEDGMENTS

The author expresses gratitude to all parties involved who have facilitated this research.

AUTHOR CONTRIBUTION

I. M. as the lead author, I. M. conceived the idea, developed the manuscript, conducted data analysis, prepared the manuscript, and provided funding. E. H., A.D., Z. R., and G. N. acted as supervisors, providing consultation and full direction for the manuscript.

FUNDING SUPPORT

Not applicable.

DATA AVAILABILITY

Not applicable.

CONFLICT OF INTEREST

The authors state there is no conflict of interest with the parties involved in this study.

ETHICAL APPROVAL

This research has been approved by the ethics committee with number 80/KEPK/EC/XII/2023 issued on 8th December 2023 by the Health Research Ethics Commission of the Bandung Ministry of Health Polytechnic.

INFORMED CONSENT

This study is voluntary for participants from the Medical Laboratory Technology Department of the Bandung Ministry of Health Polytechnic. Respondents will have 10 mL of venous blood drawn using a sterile syringe and will undergo an ESR examination using the Westergren method. The participants have obtained publication consent by signing an informed consent form.

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