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EFFECT OF EDTA AND 3.8% SODIUM CITRATE ON BLOOD SEDIMENTATION RATE BY WESTERGREN METHOD WITH 0.86% NACL

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ABSTRACT

Background: The erythrocyte sedimentation rate (ESR) is a hematological test used to detect inflammatory processes and blood disorders. ESR values can be influenced by the type of anticoagulant used.

Objective: To compare ESR values in venous blood samples using EDTA and 3.8% sodium citrate anticoagulants with the Westergren method modified by the addition of 0.86% NaCl.

Methods: This study employed an observational analytical approach with a cross-sectional design, conducted at the Clinical Pathology Laboratory of Politeknik Indonusa Surakarta. A total of 32 venous blood samples were examined using both anticoagulants. Data were analyzed statistically using the Mann–Whitney test.

Results: The mean ESR value in samples with EDTA anticoagulant was 18.3 mm/h, while that in samples with 3.8% sodium citrate was 13.1 mm/h. Statistical analysis yielded a p-value of 0.014 (p < 0.05), indicating a significant difference between the two groups. This difference may be attributed to the chemical properties and osmolarity of each anticoagulant, which affect erythrocyte rouleaux formation.

Conclusions: The type of anticoagulant significantly affects ESR results. 3.8% sodium citrate remains the preferred anticoagulant for the Westergren method according to standard protocols, without the addition of 0.86% NaCl dilution.

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ABSTRAK

Latar Belakang: Laju endap darah (LED) merupakan pemeriksaan hematologi yang digunakan untuk mendeteksi adanya proses peradangan dan gangguan pada darah. Nilai LED dapat dipengaruhi oleh jenis antikoagulan yang digunakan.

Tujuan: Untuk membandingkan nilai LED pada sampel darah vena yang menggunakan antikoagulan EDTA dan natrium sitrat 3,8% dengan metode Westergren yang dimodifikasi dengan penambahan NaCl 0,86%.

Metode: Penelitian ini menggunakan metode analitik observasional dengan desain *cross sectiona*l yang dilaksanakan di Laboratorium Patologi Klinik Politeknik Indonusa Surakarta. Sebanyak 32 sampel darah vena diperiksa menggunakan kedua jenis antikoagulan tersebut. Data hasil pemeriksaan dianalisis secara statistik menggunakan uji Mann–Whitney.

Hasil: Nilai rata-rata LED pada sampel dengan antikoagulan EDTA adalah 18,3 mm/jam, sedangkan pada sampel dengan antikoagulan natrium sitrat 3,8% sebesar 13,1 mm/jam. Analisis statistik menunjukkan nilai p = 0,014 (p < 0,05), yang berarti terdapat perbedaan signifikan antara kedua kelompok. Perbedaan ini kemungkinan disebabkan oleh sifat kimia dan osmolaritas masing-masing antikoagulan yang memengaruhi pembentukan rouleaux eritrosit.

Kesimpulan: Jenis antikoagulan berpengaruh signifikan terhadap hasil pemeriksaan LED. Natrium sitrat 3,8% tetap menjadi antikoagulan yang

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Kata kunci:
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direkomendasikan untuk metode Westergren sesuai dengan standar, tanpa penambahan pengenceran NaCl 0,86%.

INTRODUCTION

The erythrocyte sedimentation rate (ESR) is an important hematological parameter widely used as an early indicator of inflammatory and infectious conditions, both acute and chronic, as well as tissue damage severity **(Patmawati, 2018)**. The test remains essential in clinical decision-making because it is simple, inexpensive, and routinely needed in various clinical settings such as monitoring autoimmune diseases, infections, and malignancies **(Darras et al., 2022).** The Westergren method is the international gold standard recommended by ICSH, making accuracy and reliability of results a priority. **(Wahab et al., 2024 & Lapic et al., 2019)**.

In clinical laboratories, the validity of ESR results is significantly influenced by the type of anticoagulant used. The standard anticoagulant for the Westergren method is 3.8% sodium citrate because it maintains cell morphology. However, in routine practice, many laboratories use EDTA tubes due to practicality and efficiency, as EDTA is also required for complete blood count (CBC) examinations **(Fitriani et al., 2024)**. This substitution may lead to variability in ESR values, especially since research has shown that different anticoagulants can affect erythrocyte aggregation and plasma characteristics, which directly impact sedimentation rates **(Astuti et al., 2023)**.

A recent study by Shantika and Kusdiantini (2023) found no significant difference between ESR results using 3.8% sodium citrate and EDTA supplemented with 0.85% NaCl. However, standardized data regarding the use of EDTA and sodium citrate with the specific addition of 0.86% NaCl—aimed to equalize sample dilution in the Westergren method—are still lacking. This gap in evidence creates potential inconsistency in laboratory results, which may affect clinical interpretation and patient management **(Shantika & Kusdiantini, 2023)**.

Therefore, research is urgently needed to analyze the effect of EDTA and 3.8% sodium citrate anticoagulants with the addition of 0.86% NaCl on ESR values using the Westergren method. Findings from this study are expected to provide scientific evidence to support the selection of appropriate anticoagulants, improve examination accuracy, and enhance the standardization of ESR testing in clinical laboratories.

MATERIALS AND METHODS

This study used an observational analytical method with a cross-sectional design conducted at the Clinical Pathology Laboratory of Indonusa Polytechnic Surakarta. Venous blood samples were collected in two tubes, namely EDTA and 3.8% sodium citrate, from medical laboratory technology students who voluntarily participated in this study. Each blood sample in both tubes was taken 1.6 ml and added with 0.4 ml of 0.86% NaCl solution to maintain isotonic conditions and minimize plasma osmolality variation. The ratio of anticoagulated blood in the tubes was 4:1 (1.6 mL of blood with anticoagulant to 0.4 mL of 0.86% NaCl). ESR measurements were performed using the Westergren method after 1 hour of sedimentation at room temperature (25 ± 2 °C) with the tubes placed vertically. The data obtained were analyzed using SPSS with appropriate statistical tests. The sample size was determined using the Slovin formula **(Amin et al., 2017)**.

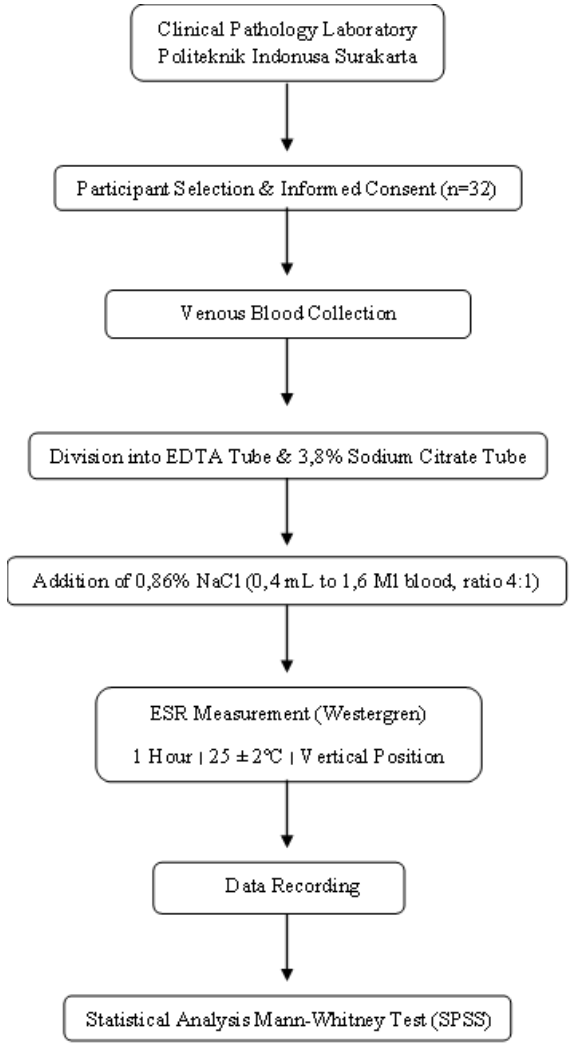

Figure 1. Research workflow model using an observational analytical method with a cross-sectional design.

CODE OF ETHICS IN HEALTH

This study was approved by the Ethics Committee of Muhammadiyah University Purwokerto, approval number KEPK/UMP/333/VI/2025.

RESULT AND DISCUSSIONS

Blood sedimentation rate tests using the Westergren method with EDTA and 3.8% sodium citrate anticoagulants are presented in Table 1.

Table 1. Frequency distribution of erythrocyte sedimentation rate values based on anticoagulants

Anticoagulant	N	Minimum Value (mm/h)	Maximum Value (mm/h)	Mean ± SD (mm/h)	p-value
EDTA	32	1	55	18.3 ± 12.68	0.014
Natrium Sitrat 3.8%	32	1	41	13.1 ± 9.38	

Statistical analysis was performed using the Mann–Whitney test.

The data in Table 1 show the erythrocyte sedimentation rate (ESR) results for both anticoagulants. The mean ESR value for samples treated with EDTA was 18.38 ± 12.68 mm/h, while that for samples treated with 3.8% sodium citrate was 13.16 ± 9.38 mm/h. Statistical analysis using the Mann–Whitney test yielded a p-value of 0.014 ($p < 0.05$), indicating a significant difference between the two groups. These results suggest that sedimentation in blood samples treated with sodium citrate anticoagulant occurs more slowly than in those treated with EDTA.

This study involved 32 students from the D4 Medical Laboratory Technology program at Indonusa Polytechnic Surakarta who voluntarily participated as research subjects. The data were analyzed using SPSS with the Shapiro–Wilk normality test, since the sample size was less than 50. The results of the Shapiro–Wilk test are presented in Table 2.

Table 2. Shapiro–Wilk normality test results

Variable	Anticoagulant	*Shapiro-Wilk*		
		Statistic	df	Sig.
LED Value	EDTA	0.858	32	0.001
	Natrium Sitrat 3.8%	0.817	32	0.000

From the table above, the Shapiro–Wilk normality test results for EDTA yielded a significance value of 0.001, while for 3.8% sodium citrate the significance value was 0.000. Because the significance values (Sig.) for both anticoagulants were smaller than 0.05 ($p < 0.05$), the data were considered not normally distributed.

Statistical analysis was continued using a non-parametric test, namely the Mann–Whitney test, because this test is appropriate for comparing two independent groups with data that are not normally distributed **(Mufarrikoh, 2024)**. The results of the Mann–Whitney test are presented in Table 3.

Table 3. Mann-Whitney test results

	Nilai LED
Mann-Whitney U	329.500
Wilcoxon W	857.500
Z	-2.456
Asymp. Sig. (2-tailed)	0.014

The results in Table 3 show a significance value (p) of 0.014, which is less than the significance level of 0.05 ($p < 0.05$). Therefore, it can be concluded that there is a significant difference between the use of EDTA and 3.8% sodium citrate anticoagulants in the Westergren method blood sedimentation rate (BSR) test with the addition of 0.86% NaCl.

The Westergren method for measuring the erythrocyte sedimentation rate (ESR) generally uses 3.8% sodium citrate as the standard anticoagulant. EDTA is another anticoagulant widely used in clinical laboratories for complete blood count testing; however, EDTA supplemented with 0.85% NaCl can also be used as a modified form of the Westergren method **(Shantika & Kusdiantini, 2023)**. Isotonic 3.8% sodium citrate is typically used in a ratio of 4 parts blood to 1 part anticoagulant **(Muyasaroh, 2017)**. Sodium citrate acts by chelating calcium ions (Ca^{2+}) into an inactive complex, thereby preventing the coagulation process **(Amtiran, 2019).**

In this study, the addition of 0.86% NaCl solution played an important role in maintaining isotonic conditions in both anticoagulant groups. A volume of 0.4 mL NaCl was added to each sample to reduce the hyperosmolar effect of EDTA and to stabilize the sample environment so that it more closely resembled physiological conditions **(Salvagno et al., 2020)**. Under these conditions, EDTA blood diluted with 0.86% NaCl can be used for ESR testing. This difference is likely due to the ability of EDTA to enhance rouleaux formation more significantly than 3.8% sodium citrate, resulting in higher ESR values in EDTA-anticoagulated blood. Another contributing factor may be the difference in viscosity, where citrate-anticoagulated blood tends to be less viscous, leading to lower ESR values **(Getaneh et al., 2019)**. This observation highlights how the biochemical properties of the anticoagulants influence ESR measurements and supports careful selection of anticoagulants in clinical practice. This approach aligns with the recommendation of the International Committee for Standardization in Hematology (ICSH), which states that a 0.85–0.86% NaCl solution can be used as a diluent for EDTA blood in ESR testing using the Westergren method **(Pratama et al., 2019)**.

However, when NaCl dilution is applied to tubes containing 3.8% sodium citrate, the isotonic balance may be slightly disturbed, potentially lowering the effective concentration of the anticoagulant and altering the standard blood-to-citrate ratio. Since sodium citrate is already more isotonic and maintains cell morphology effectively, further dilution may reduce plasma viscosity and slow down erythrocyte sedimentation. Consequently, the measured ESR values may appear lower than the true value. Even minor variations in dilution can cause differences in ESR results exceeding 20%, which may have a direct impact on clinical interpretation **(Kratz1 et al., 2017)**.

In addition, erythrocyte sedimentation rate (ESR) results are influenced by various internal and external factors. External factors include vibration or shaking of the sample tube, which can accelerate the erythrocyte sedimentation process **(Ayunawati, 2016)**, as well as the position of the tube during examination. Tubes that are not placed completely upright, for example with an inclination of about 3°, can increase the sedimentation rate by up to 3% **(Siwalette, 2022)**. Internal factors include blood components themselves, such as increased fibrinogen levels, erythrocyte count, and high globulin levels, which can accelerate rouleaux formation and increase ESR values **(Nazarudin & Kartika Sari, 2021)**. Therefore, careful control of technical and biological factors is crucial to maintaining the reliability of test results. Significant differences between the use of EDTA anticoagulants and 3.8% sodium citrate also emphasize the need for standardized methods and appropriate anticoagulant selection to obtain valid and reproducible ESR results.

CONCLUSIONS

This study analyzed the effect of anticoagulant type on erythrocyte sedimentation rate (ESR) values measured by the Westergren method with the addition of 0.86% NaCl. A total of 32 venous blood samples were examined using EDTA and 3.8% sodium citrate anticoagulants. The mean ESR value for EDTA was 18.3 mm/h, whereas that for 3.8% sodium citrate was 13.1 mm/h. The Mann–Whitney test showed a significant difference ($p = 0.014$), with EDTA producing higher ESR values than sodium citrate. These findings confirm that the type of anticoagulant significantly influences ESR results. In accordance with standard guidelines, 3.8% sodium citrate remains the recommended anticoagulant for the Westergren method, as it provides more stable and physiologically reliable results.

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