

Differences in Platelet Counts in K2edta Blood Samples with Varying Volumes Using a Hematology Analyzer

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ABSTRACT

Background: Platelet count examination is an essential hematological test that requires proper anticoagulant usage to prevent blood coagulation. The accuracy of platelet measurement is influenced by the proportion of anticoagulant and blood volume.

Method: This study used an observational cross-sectional design conducted at the Clinical Laboratory of Universitas Muhammadiyah Purwokerto. A total of 15 samples were obtained using purposive sampling. Platelet counts were measured using a hematology analyzer with three blood volume variations (0.5 mL, 1 mL, and 3 mL). Data were analyzed using normality tests and one-way ANOVA.

Results: The mean platelet counts for 0.5 mL, 1 mL, and 3 mL samples were 23486.67, 24986.67, and 25206.67, respectively. Statistical analysis showed no significant difference ($p = 0.375$; $p > 0.05$).

Conclusion: Variations in blood sample volume in K2EDTA tubes do not significantly affect platelet count results when measured using a hematology analyzer.

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1. INTRODUCTION

Platelet count testing plays a crucial role in diagnosing various hematological disorders, including those related to blood coagulation and bleeding tendencies. Platelets, or thrombocytes, are essential for hemostasis and the prevention of excessive bleeding. A reliable platelet count is therefore fundamental in clinical diagnostics. Traditionally, platelet counts have been performed manually using a microscope, but modern laboratories rely on automated hematology analyzers due to their higher efficiency and accuracy¹.

The reliability of platelet count tests depends heavily on the proper preparation of blood samples, particularly the use of anticoagulants to prevent clotting. One of the most commonly used anticoagulants in hematology is K2EDTA (Dipotassium Ethylene Diamine Tetraacetic Acid), which is recommended by the World Health Organization (WHO), the International Council for Standardization in Hematology (ICSH), and the Clinical and Laboratory Standards Institute (CLSI). K2EDTA works by binding calcium ions, which are necessary for blood coagulation, thereby preventing clot formation in the sample².

Despite the widespread use of K2EDTA in platelet count testing, challenges remain regarding the proper blood-to-anticoagulant ratio. It has been observed that variations in blood volume can significantly impact the accuracy of platelet count results. Excessive volumes of anticoagulant can lead to platelet aggregation, resulting in falsely elevated platelet counts, while insufficient blood volume may not allow for proper mixing with the anticoagulant, leading to incorrect platelet morphology and potentially lower platelet counts results³. Therefore, it is essential to investigate whether different blood volumes in K2EDTA tubes affect platelet counts when analyzed using automated hematology analyzers.

The objective of this study is to determine the effect of blood sample volume variation (0.5 mL, 1 mL, and 3 mL) on platelet counts in K2EDTA-treated samples when analyzed with an automated hematology analyzer. The rationale for this study lies in addressing the gap in the existing literature, where limited research has been conducted specifically on the influence of blood volume variation on platelet count accuracy in K2EDTA tubes. By exploring this relationship, the study aims to provide more precise guidelines for blood sample volumes, ensuring more accurate platelet count results in clinical laboratories.

The hypothesis of this study is that variations in blood sample volume will significantly affect the platelet counts, with the larger volume of 3 mL producing more accurate and reliable results compared to the smaller volumes. This hypothesis is based on previous studies that suggest a potential impact of sample volume on the measurement accuracy of platelet counts^{4,5}.

Recent studies have highlighted the significance of anticoagulant concentration and sample volume in platelet count testing. Previous research has examined the effects of sample volume and EDTA concentration on the accuracy of hematological tests⁶. Similarly, other studies have shown that inconsistencies in sample volume may lead to inaccurate platelet counts, emphasizing the need for standardization in blood sample handling⁷. In addition, maintaining an appropriate blood-to-anticoagulant ratio is crucial to avoid errors in laboratory results⁸. However, the specific impact of volume variation in K2EDTA tubes has not been thoroughly investigated, thus contributing to the novelty of this research.

This study aims to analyze the relationship between blood sample volume and platelet count accuracy in K2EDTA tubes in order to optimize clinical laboratory practices and improve diagnostic reliability.

2. RESEARCH METHOD

This study employed an observational, cross-sectional design conducted in May 2025 at the Universitas Muhammadiyah Purwokerto Clinical Laboratory. It aimed to evaluate the effect of blood sample volume variation (0.5 mL, 1 mL, and 3 mL) on platelet counts in K2EDTA-treated samples, measured with an automated hematology analyzer.

Fifteen participants from the D4 Medical Laboratory Technology program were selected using purposive sampling. Inclusion criteria: healthy, active students willing to participate. Exclusion criteria: menstruating individuals, those fasting, or with diseases affecting platelet counts (e.g., dengue fever). Blood was collected via venipuncture into 5 mL vacutainer tubes containing K2EDTA as the anticoagulant. Samples were divided into three volumes: 0.5 mL, 1 mL, and 3 mL. K2EDTA prevents clotting by binding calcium ions (Hariyanto, 2024).

Platelet counts were measured using a Mindray hematology analyzer with the impedance method. The analyzer measures platelet counts by detecting changes in electrical resistance as blood cells pass through an aperture. Each volume was measured in triplicate for accuracy. Data were analyzed using descriptive statistics and the Shapiro-Wilk test for normality. A one-way ANOVA was used to compare platelet counts across volumes, with a significance level set at 0.05. If normality was not met, the Kruskal-Wallis test was used.

The mean platelet count was calculated with the following formula:

$$\text{Platelet Count} = \frac{N \times P}{V}$$

The study received ethical approval from the Ethical Review Committee at Universitas Muhammadiyah Purwokerto. Informed consent was obtained from all participants, ensuring confidentiality and the right to withdraw at any time.

3. RESULT AND DISCUSSIONS

3.1. Univariate Analysis

Table 1. Average Platelet Count Results for 0.5 mL, 1 mL, and 3 mL Volumes

Blood Volume K2EDTA	Number of Samples	Average Platelet Count \pm SD (cells/ μ L)	CI 95%	P Value
0,5 mL	15	23486.67 \pm 408.199	212.261-257.472	0,375
1 mL	15	24986.67 \pm 318.453	232.232-267.502	
3 mL	15	25206.67 \pm 353.461	234.726-256.474	

Source: Primary Data, 2024.

Based on the data in the table 1, the average platelet count for the 0.5 mL K2EDTA blood sample was 23,486.67 \pm 408.199 cells/ μ L, with the highest count reaching 300,000 cells/ μ L and the lowest at 137,000 cells/ μ L. The 1 mL K2EDTA sample had an average platelet count of 24,986.67 \pm 318.453 cells/ μ L, with the highest at 304,000 cells/ μ L and the lowest at 203,000 cells/ μ L. The 3 mL K2EDTA sample showed an average platelet count of 25,206.67 \pm 353.461 cells/ μ L, with the highest at 319,000 cells/ μ L and the lowest at 188,000 cells/ μ L. The differences in platelet counts across the different blood volumes were minimal.

Table 2. Platelet Count Percentage Distribution

Category of Platelet Count	Number of Samples (N)	Percentage (%)
Low	1	2.2
Normal	44	97.8
High	0	0
Total	45	100.0

Source: Processed Primary Data, 2024.

Based on Table 2, the platelet count distribution for a total of 45 samples is as follows: 1 sample falls into the low category, 44 samples fall into the normal category, and there are no samples in the high category.

Table 3. Differences in Platelet Count for K2EDTA Samples with Varying Volumes

Blood Volume K2EDTA	Number of Samples	Average Platelet Count \pm SD (cells/ μ L)	CI 95%	P Value
0,5 mL	15	23486.67 \pm 408.199	212.261-257.472	0,375
1 mL	15	24986.67 \pm 318.453	232.232-267.502	
3 mL	15	25206.67 \pm 353.461	234.726-256.474	

Source: Processed Primary Data, 2024.

Based on Table 3, the differences in platelet counts for K2EDTA blood samples with volumes of 0.5 mL, 1 mL, and 3 mL were analyzed using the Shapiro-Wilk normality test, which indicated that the data were normally distributed. The analysis continued with one-way ANOVA, resulting in a p-value of 0.375. This p-value indicates that there were no significant differences in

platelet counts between the different blood volumes (0.5 mL, 1 mL, or 3 mL) when measured using the hematology analyzer ($p > 0.05$).

This study aimed to evaluate the differences in platelet counts in K2EDTA blood samples with varying volumes (0.5 mL, 1 mL, and 3 mL) using an automated hematology analyzer. The results showed that there were no significant differences in platelet counts among the three sample volumes, with a p-value of 0.375 ($p > 0.05$), indicating that variations in blood sample volume did not significantly affect platelet count results.

The findings of this study are consistent with previous research indicating that small variations in blood sample volume within the recommended range do not significantly influence platelet counts^{8,7}. Previous studies have demonstrated that differences in blood volume do not significantly affect platelet count results when K2EDTA is used, provided that proper mixing and handling procedures are followed⁷.

Although minor differences were observed in the average platelet counts across the different sample volumes, with the 3 mL samples showing slightly higher values than the 0.5 mL and 1 mL samples, these differences were not statistically significant. This suggests that the hematology analyzer used in this study was able to accommodate minor variations in blood volume and still produce consistent results. The impedance method used by the analyzer is known for its accuracy in cell counting, even with slight variations in sample volume⁸.

The use of K2EDTA as an anticoagulant is well-established for its effectiveness in preventing blood clotting while maintaining the integrity of blood cells, including platelets. Previous findings have shown that K2EDTA does not induce platelet swelling or fragmentation, which could otherwise affect the accuracy of platelet counts³. This supports its reliability in hematological examinations.

Another important factor influencing platelet count accuracy is the sample handling process. Delays in mixing blood with anticoagulant or prolonged storage may lead to platelet aggregation, resulting in inaccurate measurements. In this study, blood samples were processed immediately after collection, minimizing the risk of aggregation and ensuring more reliable results.

Overall, the findings indicate that variations in blood sample volume within the range of 0.5 mL to 3 mL do not significantly affect platelet count measurements when analyzed using an automated hematology analyzer, as long as proper handling procedures are followed.

Despite these findings, this study has several limitations. The relatively small sample size may limit the ability to detect subtle differences in platelet counts. Additionally, the use of a single hematology analyzer model may restrict the generalizability of the results. Future studies are recommended to include larger sample sizes and multiple analyzer types to further validate these findings.

4. CONCLUSION AND RECOMMENDATION

Based on the results of this study, it can be concluded that variations in blood sample volume (0.5 mL, 1 mL, and 3 mL) in K2EDTA tubes do not significantly affect platelet count results when measured using an automated hematology analyzer. Although slight differences were observed in the average platelet counts across the three volume groups, statistical analysis showed no significant difference ($p > 0.05$). These findings indicate that as long as the blood samples are properly mixed and processed, minor variations in volume within the recommended range do not compromise the accuracy of platelet count measurements. Future studies are recommended to involve larger sample sizes and different analyzer models to further validate these findings.

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