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The Effect of K_3EDTA Blood Volume Variation on Complete Blood Count Results

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A B S T R A C T

This study aims to determine the effect of varying K3EDTA blood volumes on complete blood count (CBC) results. Venous blood collection was performed using a closed system method. Blood was collected into K_3EDTA vacutainer tubes with varying volumes of 1 mL, 2 mL, and 3 mL for each respondent, with a total of 10 respondents. The CBC parameters examined were WBC, RBC, Hb, Hct, MCV, MCH, MCHC, and PLT, using a quality-controlled hematology analyzer. Normally distributed data were analyzed using ANOVA, while non-normally distributed data were analyzed using the Friedman test. The results showed p-values > 0.05 for WBC, RBC, Hb, Hct, MCV, MCH, and MCHC, whereas the PLT test results showed a p-value < 0.05. The conclusion of this study is that there is an effect of varying K3EDTA blood volumes on PLT test results, but no effect on WBC, RBC, Hb, Hct, MCV, MCH, and MCHC test results.

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INTRODUCTION

Hematological examinations are generally divided into three main categories: routine or complete blood tests, hemostasis tests, and confirmatory hematological tests. (Ramdhani et al., 2019). Parameters included in a complete blood count are *hemoglobin* (Hb), *red blood cells* (RBC), *white blood cells* (WBC), *platelets* (PLT), *hematocrit* (Hct), *mean corpuscular volume* (MCV), *mean corpuscular hemoglobin* (MCH), *mean corpuscular hemoglobin concentration* (MCHC), and *leukocyte differential count (diff count*) (Maharani, 2020).

The pre-analytic, analytic, and post-analytic stages in laboratory testing significantly affect the test results, including complete blood count results. The pre-analytic stage contributes 46-68.2% to the total errors that occur in the laboratory (Plebani, 2006). The collection, handling, storage, and distribution of blood samples are crucial parts of the pre-analytic stage in complete blood count testing, as the quality of the obtained blood samples affects the accuracy of the test results. Therefore, guidelines or references for sample handling must adhere to correct and standardized procedures. (Maharani, 2020; Plebani, 2006).

The use of *Tripotassium Ethylene Diamine Tetra Acetic* (K_3EDTA) anticoagulant to maintain the stability of blood samples in complete blood count testing is highly recommended by the *National Committee for Clinical Laboratory Standards* (NCCLS). K_3EDTA vacutainer tubes typically use the anticoagulant in liquid form. While liquid K_3EDTA anticoagulant can cause sample dilution, it is easier to homogenize effectively (Barkatin & Saktiningsih, 2019; Dewi, 2017).

The correct ratio between anticoagulant and blood is crucial because an imbalance in composition can affect blood cell components. If the EDTA ratio is higher than the blood sample, RBCs can shrink as the plasma becomes hypertonic due to increased ion concentration, potentially resulting in abnormal or unclear RBC morphology, decreased Hct values, increased MCV and MCHC, while PLTs can enlarge and disintegrate. Conversely, if the EDTA anticoagulant ratio is lower than the blood sample, it can lead to coagulation and PLT microaggregation, which may result in blood clotting. Therefore, filling vacuum tubes with blood must adhere to the specified standard volume (England et al., 1993; Garini, 2013).

Ideally, the ratio between the amount of EDTA anticoagulant and the blood sample should be 1.0 - 1.5 mg of EDTA anticoagulant per 1 mL of sample for dry EDTA, and 10 - 15 µl of EDTA anticoagulant per 1 mL of blood for liquid EDTA. (Gandasoebrata, 2010; Garini, 2013; Maharani, 2020). This study aims to determine the influence of varying volumes of K3EDTA blood (1 mL, 2 mL, and 3 mL) on the results of complete blood count tests.

LITERATUR REVIEW

A complete blood count involves the evaluation of blood cells, encompassing several examination parameters, including RBC, WBC, PLT, Hb, Hct, *Mean Corpuscular Volume* (MCV), *Mean Corpuscular Hemoglobin* (MCH), and *Mean Corpuscular Hemoglobin Concentration* (MCHC).(Stibis & Astuti, 2020). Quality assurance in laboratory testing, particularly in complete blood count examinations, has presented its own challenges in medical laboratory services. Quality assurance in complete blood count testing encompasses all activities

aimed at ensuring the accuracy and reliability of laboratory test results and preventing errors that could lead to inaccurate results. These activities include the pre-analytical, analytical, and post-analytical stages. (Jemani & Kurniawan, 2019; Kemenkes, 2013).

The laboratory stages, such as those in complete blood count testing, that contribute the most to error rates are the pre-analytical stages. These stages include errors in preparing the type of tube for sample collection, errors in sample collection, processing, storage, and distribution. (Plebani, 2006).

Complete blood count examinations typically utilize whole blood specimens, commonly employing EDTA as the anticoagulant. (Maharani, 2020). There are three types of EDTA: Na_2EDTA in dry form, K_2EDTA in dry form, and K_3EDTA in liquid form. ICSH (*International Council for Standardization in Hematology*) and CLSI (*Clinical and Laboratory Standards Institute*) recommend the use of K_2EDTA, while the National Committee for Clinical Laboratory Standards (NCCLS) recommends the use of K_3EDTA for complete blood count examinations because it has better stability compared to other types of EDTA and has a pH close to blood pH. (Barkatin & Saktiningsih, 2019; Dewi, 2017).

If the proportion of EDTA anticoagulant added to the blood is not appropriate, it can disrupt erythrocytes. Insufficient EDTA can cause blood to clot, while an excess of EDTA can lead to erythrocyte shrinkage (crenation), enlargement, and disintegration of platelets. (Gandasoebrata, 2010; Rosidah & Wibowo, 2018). Ideally, the ratio between the amount of EDTA anticoagulant and the blood sample should be 1.0 - 1.5 mg of EDTA anticoagulant per 1 mL of sample for dry EDTA, and 10 - 15 µL of EDTA anticoagulant per 1 mL of blood for liquid EDTA. (Garini, 2013).

METHODOLOGY

Research Desain

This research is an experimental study with a cross-sectional design. The population in this study consists of healthy respondents who meet the inclusion criteria, namely: willing to participate, not currently ill, not taking medications, having no history of hemophilia, and receiving the desired volume of blood sample. Respondents who meet the inclusion criteria undergo blood sample collection using the closed system method with K_3EDTA vacutainer tubes with volumes of 1 mL, 2 mL, and 3 mL. A total of 30 K_3EDTA blood samples were obtained.

Research Instrument

The instruments used for blood sample collection include: K_3EDTA vacutainer tubes, vacutainer needles, holder, tourniquet, sterile gauze, 70% alcohol swab, and adhesive bandage. Meanwhile, instruments for complete blood count examination utilize the *Mindray BC 2800 hematology analyzer* and control materials to ensure the quality of the equipment is in good condition.

K_3EDTA Blood Sampling Procedure

Respondents who meet the inclusion criteria undergo blood sample collection using the following procedure: placing a tourniquet around the patient's arm about 3-4 fingers above the elbow crease; palpating to locate the desired vein; disinfecting the puncture site using a 70% alcohol swab; holding the white-colored vacutainer needle cap with one hand, then twisting and removing the white-colored part with the other hand; attaching the needle to the holder by securely twisting it into place; ensuring the disinfected area is dry and avoiding repeated touching; performing a precise and correct vein puncture; once blood appears in the indicator, inserting the K_3EDTA vacutainer tube into the holder, starting with tube 1 with a volume of 3 mL, followed by tube 2 with a volume of 2 mL, and tube 3 with a volume of 1 mL; immediately releasing the tourniquet once blood flows into tube 1; homogenizing the K_3EDTA blood by inverting the tube 8-10 times with each tube change; swiftly withdrawing the needle and applying pressure to the puncture site with sterile gauze; disposing of the needle and medical waste properly; affixing an identity label to the blood-filled tube and showing it to the respondent; finally, ensuring the puncture site has stopped bleeding and covering it with a sterile adhesive bandage.

Quality Control (QC) Alat Hematology Analyzer Mindray BC 2800 Procedure

The procedure for quality control (QC) of the Hematology Analyzer BC 2800 is as follows: first, ensure that the instrument is in a ready state, then press the "select" button; next, press the number 2 button and select "2. Quality Control"; after the QC screen appears, press the "sample no" button and choose the file number (normal control level), then press "enter"; next, press the number 1 button and select "1. QC Analyze", and the control analysis screen will appear; the next step is to thoroughly homogenize the control material (normal control blood) to be examined; then, open the cover of the control blood tube and place it under the aspiration probe with the probe tip touching the bottom of the control blood tube to prevent air aspiration, then press the "start switch" button to initiate the process; after hearing two "beep" sounds, remove the control blood from under the probe; then, the QC results will appear on the screen; if the QC results fall within the control range, press the number 1 button to save the QC results and press the number 3 button to print the QC results.

Prosedur Pemeriksaan Darah Lengkap

The procedure for complete blood count using the Hematology Analyzer BC 2800 is as follows: first, ensure that the instrument is ready, QC has been performed, and the QC results fall within the control range; then, press the "ID" button and enter the sample identity; before testing, homogenize the K_3EDTA blood sample tube, then place the sample tube under the aspiration probe until it touches the bottom of the tube, then press the "sampling button"; the instrument will perform calculations and wait until the results appear on the screen, then press the "print out" button to print the results; repeat this procedure for examining 30 K_3EDTA blood samples; after all samples have been tested, press the "standby" button, then press the "Center" button, and let the instrument

perform the cleaning process, finally turn off the instrument by pressing the "OFF" button on the back of the instrument.

Analisis Data

The research data was analyzed using the SPSS program. The normality of the data was tested using the Shapiro-Wilk test. If $p \geqslant 0.05$, it means that the data is normally distributed, and if $p < 0.05$, it means that the data is not normally distributed. Normally distributed data were further analyzed using parametric statistical tests, namely One-Way ANOVA. Non-normally distributed data were analyzed using non-parametric statistical tests, namely the Friedman test. One-way ANOVA and Friedman tests were used to test the research hypotheses. The hypotheses used in this study are as follows: Ho is accepted and Hi is rejected if the p-value > α (0.05), which means there is no effect of varying K_3EDTA blood volume on complete blood count results. Hi is accepted and Ho is rejected if the p-value < α (0.05), which means there is an effect of varying K_3EDTA blood volume on complete blood count results.

RESEARCH RESULTS

Quality Control Results

Table 1. *Quality Control Results of the Hematology Analyzer BC 2800 tool*

Complete Blood Parameters	QC Result	QC Target Value (Range)
WBC ($x10^3/\mu L$)	8,0	7,1 - 9,1
RBC ($x10^6/\mu L$)	4,57	4,34 - 4,82
HB (gr/dL)	14,2	13,1 - 14,3
Hct (%)	38,9	37,8 - 41,8
MCV (fL)	85,3	81,9 - 91,9
MCH (pg)	31,0	27,4 - 32,4
MCHC (%)	36,6	31,4 - 37,7
PLT ($x10^3/\mu L$)	281	221 - 301

Sumber: Data Primer, 2023

Table 1 demonstrates that the quality control (QC) results for the parameters of the complete blood count examination using the Hematology Analyzer BC 2800 fall within the target values (range), indicating that the Hematology Analyzer BC 2800 is in good condition for patient sample examination.

Complete Blood Test Results

Table 2. *Data normality test, frequency distribution, and test of the effect of variations in K3EDTA blood volume on complete blood test results*

Complete Blood Parameters	Number of Respondents (N=10)			*p-value*
	Volume 3 mL	Volume 2 mL	Volume 1 mL	

WBC ($x10^3/\mu L$)	*.794	*.856	*.789	**.964
Mean±SD	9,19±2,82	9,03±2,62	8,86±2,69	
RBC ($x10^6/\mu L$)	*.395	*.350	*.391	**.957
Mean±SD	5,06±0,49	5,02±0,47	5,09±0,50	
HB (gr/dL)	*.691	*.981	*.909	**.982
Mean±SD	14,11±2,16	14,12±2,02	14,27±2,04	
Hct (%)	*.904	*.962	*.942	**.968
Mean±SD	40,75±5,64	40,33±5,40	40,94±5,29	
MCV (fL)	*.116	*.117	*.090	**.997
Mean±SD	80,59±8,13	80,38±8,00	80,61±7,74	
MCH (pg)	*.055	*.031	*.025	***.704
Mean±SD	27,95±3,03	28,04±2,98	28,02±3,10	
MCHC (%)	*.966	*.821	*.109	**.726
Mean±SD	34,69±0,68	34,93±0,60	34,77±0,74	
PLT ($x10^3/\mu L$)	*.012	*.002	*.001	***.020
Mean±SD	353,40±64,08	345,90±65,27	336,70±70,26	

Sumber: Data Primer, 2023

**Uji shapiro wilk, **Uji one-way ANOVA, ***Uji Friedman*

Table 2 indicates that from 10 respondents, blood was drawn from each respondent using 3 K_3EDTA vacutainer tubes with tube 1 volume of 3 mL, tube 2 volume of 2 mL, and tube 3 volume of 1 mL. The data of WBC, RBC, Hb, Hct, MCV, and MCHC examination results based on the Shapiro-Wilk test show that the data are normally distributed (p-value > 0.05). However, the data of MCH and PLT examination results show non-normally distributed data (p-value < 0.05).

In Table 2, it is observed that the mean WBC value in K_3EDTA blood tubes with a volume of 3 mL is 9.19x10^3/µL with a standard deviation (SD) of ±2.82, in tubes with a volume of 2 mL is 9.03x10^3/µL with SD ±2.62, and in tubes with a volume of 1 mL is 8.86x10^3/µL with SD ±2.69. The WBC examination results using K3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL show no significant difference (p-value > 0.05; p=0.964). Similarly, the mean RBC value in K_3EDTA blood tubes with a volume of 3 mL is 5.06x10^6/µL with SD ±0.49, in tubes with a volume of 2 mL is 5.02x10^6/µL with SD ±0.47, and in tubes with a volume of 1 mL is 5.09x10^6/µL with SD ±0.50. The RBC examination results using K3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL also show no significant difference (p-value > 0.05; p=0.957).

The mean examination value of Hb in K_3EDTA blood tubes with a volume of 3 mL is 14.11 g/dL with SD ±2.16, in tubes with a volume of 2 mL is 14.12 g/dL with SD ±2.02, and in tubes with a volume of 1 mL is 14.27 g/dL with SD ±2.04. The Hb examination results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL show no significant difference (p-value > 0.05; p=0.982). Similarly, the mean examination value of Hct in K_3EDTA blood tubes with a volume of 3 mL is 40.75% with SD ±5.64, in tubes with a volume of 2 mL is 40.33% with SD ±5.40, and in tubes with a volume of 1 mL is 40.94% with SD ±5.29. The Hct examination

results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL also show no significant difference (p-value > 0.05; p=0.968).

The mean examination value of MCV in K_3EDTA blood tubes with a volume of 3 mL is 80.59 fL with SD ±8.13, in tubes with a volume of 2 mL is 80.38 fL with SD ±8.00, and in tubes with a volume of 1 mL is 80.61 fL with SD ±7.74. The MCV examination results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL show no significant difference (p-value > 0.05; p=0.997). Similarly, the mean examination value of MCH in K_3EDTA blood tubes with a volume of 3 mL is 27.95 pg with SD ±3.03, in tubes with a volume of 2 mL is 28.04 pg with SD ±2.98, and in tubes with a volume of 1 mL is 28.02 pg with SD ±3.10. The MCH examination results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL also show no significant difference (p-value > 0.05; p=0.704). Furthermore, the mean examination value of MCHC in K3EDTA blood tubes with a volume of 3 mL is 34.69% with SD ±0.68, in tubes with a volume of 2 mL is 34.93% with SD ±0.60, and in tubes with a volume of 1 mL is 34.77% with SD ±0.74. The MCHC examination results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL also show no significant difference (p-value > 0.05; p=0.726).

The mean examination value of PLT in K_3EDTA blood tubes with a volume of 3 mL is 353.40x10^3/µL with SD ±64.08, in tubes with a volume of 2 mL is 345.90x10^3/µL with SD ±65.27, and in tubes with a volume of 1 mL is 336.70x10^3/µL with SD ±70.26. The PLT examination results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL show a significant difference (p-value < 0.05; p=0.020).

DISCUSSION

The mean examination value of PLT in K_3EDTA blood tubes with a volume of 3 mL is 353.40x10^3/µL with SD ±64.08, in tubes with a volume of 2 mL is 345.90x10^3/µL with SD ±65.27, and in tubes with a volume of 1 mL is 336.70x10^3/µL with SD ±70.26. The PLT examination results using K3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL show a significant difference (p-value < 0.05; p=0.020).

This study involved 10 respondents, where venous blood was collected using the closed system method. Blood was collected using three K_3EDTA vacutainer tubes, with the filling sequence starting from 3 mL, followed by 2 mL, and finally 1 mL. The closed system venous blood collection method is considered the safest method for both patients and healthcare workers, especially when a large volume of blood sample is required, and it helps to minimize the risk of contamination. (Davis, 2011; Himesch, 2018).

The handling of K_3EDTA blood samples must be done accurately and properly. Homogenization of blood and K_3EDTA is achieved by inverting the sample 8-10 times. (Nugraha, 2022). Before examining the blood and K_3EDTA samples, internal quality control (QC) is performed on the hematology analyzer used for complete blood count examination, including WBC, RBC, Hb, Hct, MCV, MCH, MCHC, and PLT. In this study, QC is conducted using normal control-level material. The QC results for the parameters of the complete blood count examination generated by the hematology analyzer fall within the target

range, indicating that the hematology analyzer is in good condition for use in examining research samples. The purpose of this QC is to obtain quality and reliable laboratory examination results and ensure that these values are acceptable (Acceptable True Value), so every stage of laboratory examination must undergo QC to minimize potential errors. (Kesuma et al., 2020; Praptomo, 2021).

The examination results for WBC showed a mean WBC value of 9.19x10^3/µL with SD ±2.82 for the 3 mL K_3EDTA blood tube, 9.03x10^3/µL with SD ±2.62 for the 2 mL tube, and 8.86x10^3/µL with SD ±2.69 for the 1 mL tube. The highest mean WBC value was observed in the 3 mL blood volume tube, while the lowest was in the 1 mL blood volume tube. However, statistically, there was no significant difference in WBC examination results when using K_3EDTA blood volumes of 3 mL, 2 mL, and 1 mL (p-value > 0.05; p=0.964). This indicates that there is no influence of varying blood volumes of 3 mL, 2 mL, and 1 mL when using K3EDTA vacutainer tubes.

The mean examination value for RBC in the 3 mL K_3EDTA blood tube was 5.06x10^6/µL with SD ±0.49, in the 2 mL blood tube was 5.02x10^6/µL with SD ±0.47, and in the 1 mL blood tube was 5.09x10^6/µL with SD ±0.50. Statistically, there was no significant difference (p-value > 0.05; p=0.957), indicating that the RBC examination using K_3EDTA blood volumes of 3 mL, 2 mL, and 1 mL does not affect the RBC count. This is consistent with the findings of a study byHidayatulloh et al., (2021) which reported no difference in RBC examination results using K_3EDTA blood volumes of 1 mL, 2 mL, and 3 mL after storage for 2 hours at a temperature of 18-22°C. The difference in the studies lies in the duration of sample storage, which was immediately examined and stored for 2 hours at a temperature of 18-22°C.

Storing K_3EDTA blood samples for 2 hours at a temperature of 18-22°C in volumes of 1 mL and 2 mL resulted in a decrease of 100,000 cells/µL and 70,000 cells/µL, respectively, compared to the 3 mL volume. This indicates the importance of ensuring the correct ratio between blood and anticoagulant during the blood sampling process. Inaccuracy in this ratio can lead to hypertonicity in the RBCs. Increased hypertonicity causes fluids to leave the cells to maintain osmotic pressure. Consequently, the fluid leaving the blood cells causes them to shrink, resulting in hemodilution and ultimately a decrease in the number of RBCs. (Hidayatulloh et al., 2021).

Furthermore, the results of the Hb examination showed a mean value in the K_3EDTA blood tube of 14.11 g/dL with an SD of ±2.16 for a volume of 3 mL, 14.12 g/dL with an SD of ±2.02 for a volume of 2 mL, and 14.27 g/dL with an SD of ±2.04 for a volume of 1 mL. These results indicate that the mean Hb level in the 1 mL blood volume tube is higher than in the 3 mL blood volume tube. However, statistically, the Hb examination results using K_3EDTA blood volumes of 3 mL, 2 mL, and 1 mL did not show any significant differences (p-value > 0.05; p=0.982).

The findings of this study are consistent with previous research conducted by Arrafi et al., (2022). Their study found that in a blood volume of 1 ml, the average Hb level was 13.03 g/dL with a standard deviation of 1.71, while in a

blood volume of 3 ml, the average Hb level was 12.63 g/dL with a standard deviation of 1.29. From these results, it was concluded that there were no significant differences in Hb levels between the two blood volumes.

Variation in measured Hb levels between EDTA blood volumes of 3 mL, 2 mL, and 1 mL can be attributed to differences in the proportion of anticoagulant, sample homogeneity, red blood cell stability, and technical variability in the measurement procedure. In tubes with smaller blood volumes (1 mL), the proportion of anticoagulant (EDTA) to blood may be higher. This could lead to more significant dilution, resulting in lower measured Hb levels. Conversely, larger blood volumes (3 mL) may allow for better and more homogeneous mixing. This could provide more accurate results in measuring Hb levels, which may appear higher compared to smaller volumes where mixing may be less optimal. (Gandasoebrata, 2010; Maharani, 2020).

The mean values of Hct examination in K_3EDTA blood tubes with volumes of 3 mL, 2 mL, and 1 mL were 40.75% with SD ±5.64, 40.33% with SD ±5.40, and 40.94% with SD ±5.29, respectively. The statistical test results showed no significant difference (p-value > 0.05; p = 0.968) between the Hct examination results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL. The variation in Hct values is likely caused by the proportion of blood volume and sample homogeneity. A smaller blood volume compared to the anticoagulant can lead to sample dilution and RBC shrinkage, resulting in a decrease in Hct values. (Dewi, 2017; Gandasoebrata, 2010).

In this research, based on statistical analysis, the mean values of MCV examination results using K_3EDTA blood with volumes of 3 mL, 2 mL, and 1 mL showed no significant difference (p-value > 0.05; p = 0.997). This result indicates that there is no influence of variation in K_3EDTA blood volume, whether 3 mL, 2 mL, or 1 mL, on the MCV examination results. This finding is consistent with the research byRamdhani et al., (2019) which also found no difference in MCV values for blood volumes of 1 mL, 2 mL, and 3 mL using EDTA vacuum tubes (p-value = 0.995 > 0.05).

The variation of Mean Corpuscular Volume (MCV) examination results using K_3EDTA tubes with blood volumes of 1 mL, 2 mL, and 3 mL can be influenced by several factors, including the proportion of anticoagulant, sample homogeneity, and sample collection and measurement techniques. Less sample volume compared to the anticoagulant will cause the RBCs to shrink the homogenization process is less than optimal, causing variations in MCV results and even decreasing the MCV. (Gandasoebrata, 2010; Maharani, 2020).

The examination results of MCH and MCHC in this study showed that the mean values of MCH and MCHC with blood volume of K_3EDTA 3 mL, 2 mL, and 1 mL did not have a significant difference (p-value > 0.05; p=0.704 and p=0.726). These results indicate that there is no influence of variations in blood volume of K_3EDTA 3 mL, 2 mL, and 1 mL on the examination results of MCH and MCHC.

The values of MCH and MCHC reflect the proportion of hemoglobin content in erythrocytes, so a decrease or increase in MCH and MCHC values can lead to hypochromic or hyperchromic RBCs (Maharani, 2020). A higher

proportion of anticoagulant to blood volume can cause more significant blood dilution and decrease MCH values, whereas a higher proportion of anticoagulant to blood volume can cause more shrinkage of red blood cells due to the osmotic effect of K_3EDTA, leading to increased MCHC values. (Gandasoebrata, 2010; Maharani, 2020).

The mean value of PLT examination on K3EDTA blood tubes with a volume of 3 mL was obtained as much as 353.40x103/µL with SD ±64.08, the volume of 2 mL was obtained as much as 345.90x103/µL with SD ±65.27, and the volume of 1 mL was obtained as much as 336.70x103/µL with SD ±70.26. The results of the statistical test found that the Mean PLT value with K3EDTA blood volumes of 3 mL, 2 mL, and 1 mL had a significant difference (p-value < 0.05; p=0.020). These results showed that there was an effect of variations in K3EDTA blood volumes of 3 mL, 2 mL, and 1 mL on the results of PLT examinations.

In contrast to research conducted byRamdhani et al., (2019) which reported no significant differences in platelet count results among blood volumes of 1 mL, 2 mL, and 3 mL using EDTA vacuum tubes. In line with this research by Syuhada et al., (2021) that there was no significant difference in the results of PLT examination between blood volumes of 1 mL, 2 mL, and 3 mL using K_2EDTA vacuum tubes.

The difference between the results of this research and previous studies is the use of EDTA anticoagulant. There are three types of EDTA tubes, namely Na2EDTA (Dinatrium EDTA), K_2EDTA (Dipotassium EDTA), and K3EDTA (Tripotassium EDTA). Na2EDTA and K2EDTA are available in dry or powder form, although some products are also sold in liquid (spray) form. Meanwhile, K3EDTA is available in liquid form. The advantage of K_2EDTA in powder form is that it does not cause a dilution effect on the sample volume. In contrast, K_3EDTA in liquid form can cause a dilution effect on the sample volume, but this anticoagulant is easier to homogenize well. (Dewi, 2017; Retnoningrum & Saktiningsih, 2019).

The significant difference in the number of PLT between blood volumes of 3 mL, 2 mL, and 1 mL in K_3EDTA tubes is likely due to the difference in the proportion of anticoagulant because the proportion of anticoagulant (K_3EDTA) to blood volume is higher, which can cause greater dilution and damage or activation of PLT, thus reducing the number of platelets measured.

CONCLUSIONS AND RECOMMENDATIONS

This study concluded that there was an effect of K_3EDTA blood volume variation on the PLT examination results and there was no effect of K_3EDTA blood volume variation on the WBC, RBC, Hb, Hct, MCV, MCH, and MCHC examination results. Based on the conclusions of the results of this study, it is recommended that for PLT examination, the ratio between blood volume and K_3EDTA anticoagulant must be correct and the use of K3EDTA vacutainer tubes for PLT examination, filling blood must be right at the upper limit of the tube volume.

CONTINUED RESEARCH

This study has several limitations, namely the number of samples that are less than 50 samples, not using K_3EDTA blood volume of 4 mL or exceeding the upper limit of tube volume as a comparison of complete blood test parameters other than PLT. It is recommended for further research to use a larger number of samples and use K_3EDTA blood volume variations $\geqslant$ 3 mL.

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