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Optimization of Complete Blood Count Results with Variations in Specimen Handling and Whole Blood Secondary Homogenization Techniques

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ABSTRACT

Specimen homogenization and the addition of anticoagulants are pre-analytical steps that influence the accuracy of the test results. Complete blood count (CBC) is a screening test that supports disease diagnosis and aids in determining the appropriate therapy for patients. Inadequate specimens are inappropriate for subsequent testing, as they can lead to inaccurate results in the CBC examination. This study aims to determine the optimization of complete blood count results with variations in specimen handling and secondary homogenization techniques for whole blood. The type of research used is quantitative analysis using laboratory experimental methods. The study was conducted in May 2024 in the Clinical Pathology Laboratory of the Diploma-4 Medical Laboratory Technology Study Program, Faculty of Health Sciences, Muhammadiyah University of Sidoarjo, using 48 blood samples. The results of the MANOVA test indicate that the type of anticoagulant has a significant effect on the erythrocyte count (p=0.041) but does not have a significant impact on the leukocyte count (p=0.844) and platelet count (p=0.920). Meanwhile, the homogenization technique does not significantly affect the erythrocyte count (p=0.959), leukocyte count (p=0.991), or platelet count (p=0.867). This study concludes that the secondary homogenization technique has no significant effect. In contrast, the type of anticoagulant significantly impacts the results of the Complete Blood Count (CBC) test. This research suggests collecting whole blood specimens using Ethylene Diamine Tetra Acetate (EDTA) vacutainers as the anticoagulant and applying a secondary homogenization technique before performing the CBC analysis.

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INTRODUCTION

A medical laboratory is a laboratory for determining, analyzing, describing, and identifying a specimen to diagnose a patient's disease (Ministry of Health RI, 2020). Examinations in the laboratory are carried out in 3 stages, namely pre-analytical, analytical, and post-analytical. Providing anticoagulants to samples is a pre-analytical stage that influences the accuracy of the examination results (Naz et al., 2012). The optimization of complete blood count (CBC) results is intricately linked to the pre-analytical phase, which involves preparing and handling specimens. Appropriate handling of specimens can have a considerable effect on test results, especially in the context of diagnosing patients. The anticoagulant Ethylene Diamine Tetra Acetate (EDTA) is often used in hematology laboratories for complete blood counts. EDTA anticoagulants can be 10% Na_2EDTA solution (conventional EDTA) and $K3_EDTA$ in vacutainer tubes (Kuman, 2019). Anticoagulants in vacutainers are relatively more expensive, so some laboratories still use conventional anticoagulants in liquid or powder form for their examinations (Wahdaniah & Tumpuk, 2018).

Sample homogenization is also a pre-analytical stage that influences the accuracy of the examination results. The sample homogenization process is divided into two types, namely primary homogenization and secondary homogenization. Primary homogenization is the initial homogenization process after the sample is added with an anticoagulant. At the same time, secondary homogenization is the homogenization process again before the sample is read on a Hematology Analyzer (Sebayang et al., 2021). Blood samples in tubes containing anticoagulants were homogenized by inversion 10-12 times (Ministry of Health RI, 2013). Blood samples in tubes containing anticoagulants are homogenized by

inversion 8-10 times (CLSI, 2003). Blood samples in tubes containing anticoagulants were homogenized by inversion 5-10 times (Nuraeni & Septie, 2023).

The pre-analytical stage greatly influences the quality of the specimen. An incorrect specimen is unsuitable for the examination stage because it can cause invalid results. Previous research reported that most errors came from the pre-analytical stage, reaching 50-75%, including identification errors and sample problems (Plebani et al., 2014). Several errors in specimen collection and handling, namely clots in whole blood samples, inappropriate sample volumes, hemolysis, anticoagulants, and inappropriate sample storage temperatures, can affect sample quality (McPherson & Pincus, 2011).

Variations in specimen handling will have a significant impact on examination results. Several previous studies showed differences in specimen handling, particularly in the use of conventional Na2EDTA anticoagulant and K3EDTA vacutainer. Both types of anticoagulants are still widely used in clinical laboratories, community health centers, and hospitals. However, various previous studies have also found that using different anticoagulants can affect laboratory test results. Previous research showed no difference (p=0.822) in the number of erythrocytes using conventional EDTA and vacutainer (Dewi et al., 2022). However, there is a significant difference between the results of vacutainer EDTA platelet counts and conventional EDTA platelet counts (Sigit & Aini, 2013). The results of platelet counts using Na_2EDTA and K_2EDTA anticoagulants concluded that samples with K_2EDTA anticoagulant had higher results than samples with Na_2EDTA anticoagulant (Lestari et al., 2023).

There was a difference in the number of platelets (p=0.001) in EDTA blood homogenized using the inversion technique and the figure of eight technique (Hartina et al., 2018). In other studies, there was a difference (p=0.000) in the number of erythrocytes in the blood that were secondarily homogenized using the 4 and 8 times inversion technique (Nuraeni & Septie., 2023). Meanwhile, the results showed no difference (p=0.416) in the number of erythrocytes with the secondary homogenization technique 5 and 8 times (Haiti et al., 2021). However, there was no difference (p=0.938) in hemoglobin levels in the secondary homogenization process 3, 5, 7, and 8 times (Sebayang et al., 2021). In several previous studies, it has been found that specimen homogenization techniques also influence examination results. Inversion homogenization techniques in various literatures employ different secondary homogenization methods for vacutainer specimens, with ranges of 8-10 times, 10-12 times, and 5-10 times. Research conducted using various inversion homogenization techniques, such as 4 and 8 times, 5 and 8 times, and 3, 5, 7, and 8 times, has shown that these differing frequencies yield varying results in the examinations.

Based on this explanation, it is known that variations in specimen handling will have a significant influence on the examination results. Several previous studies showed variations in specimen handling, namely the use of conventional Na_2EDTA anticoagulants and K_3EDTA vacutainers. These two types of anticoagulants are still widely used in clinical laboratories, public health centers, and hospitals. However, in various previous studies, it was also known that using different anticoagulants can affect the results of laboratory tests. This study compares various specimen handling variations, specifically conventional Na2EDTA anticoagulant and K3EDTA vacutainer, using secondary homogenization techniques of four, eight, and twelve cycles. This is necessary to determine the optimization CBC results with variations in specimen handling and secondary whole blood homogenization techniques.

METHOD

This research has received approval from the Health Research Ethics Committee of Nahdlatul Ulama University Surabaya with ethical certificate Number 0214/EC/KEPK/UNUSA/2024. This type of research is laboratory experimental. The research sample was the blood of students from the Diploma-4 Medical Laboratory Technology Study Program, Faculty of Health Sciences, Muhammadiyah University of Sidoarjo, with the criteria of being male and willing to become a research subject by signing informed consent. Based on Federer's formula, the minimum sample size for each treatment is four. In this study, eight subjects were used per group. Therefore, the total number of samples used is 8x6=48. This research used eight respondents. Each respondent had 12ml of blood drawn, which was divided into six treatments: 3 tubes of blood in K_3EDTA vacutainer tubes (for secondary homogenization 4 times, 8 times, and 12 times), and three tubes of blood in conventional Na_2EDTA tubes (for secondary homogenization

4 times, 8 times, and 12 times). So, a total of 48 blood samples were obtained. The research was conducted in the Clinical Pathology Laboratory at Muhammadiyah University of Sidoarjo in May 2024. The complete blood count examination was done automatically with a hematology analyzer (Medonic M32). The normality test conducted using the Shapiro-Wilk method indicated that the data are typically distributed ($p>0.05$). Subsequently, the data were statistically analyzed using MANOVA to determine the effect of one or more independent variables on two or more dependent variables.

RESULTS

The research data showed that the lowest erythrocyte count was 4,740,000cells/µl and the highest was 6,140,000cells/µl. The number of leukocytes ranges from 4,700cells/µl to 12,800cells/µl. The platelet count is around 234,000cells/µl to 458,000cells/µl. Based on Table 1, the results of the complete blood count examination showed that the average number of K_3EDTA anticoagulant erythrocytes was 5,497,083cells/µl, the average number of Na_2EDTA anticoagulant erythrocytes was 5,326,250cells/µl. The mean number of K_3EDTA anticoagulant leukocytes was 7,579cells/µl, and the mean Na_2EDTA anticoagulant leukocyte count was 7,729cells/µl. The mean platelet count for the K_3EDTA anticoagulant was 297,208cells/µl, and the mean platelet count for the Na_2EDTA anticoagulant was 295,375cells/µl.

Table 1. Mean blood cell count ± Standard Deviation

Blood cell count (µl)	Mean (µl) ± Standard Deviation
Erythrocyte anticoagulant K_3EDTA	$5{,}497x10^6$ ± 276.350
Erythrocyte anticoagulant Na_2EDTA	$5{,}326x10^6$ ± 274.983
Leukocyte anticoagulant K_3EDTA	$7{,}579x10^3$ ± 2.524
Leukocyte anticoagulant Na_2EDTA	$7{,}729x10^3$ ± 2.615
Platelet anticoagulant K_3EDTA	$2{,}972x10^5$ ± 57.565
Platelet anticoagulant Na_2EDTA	$2{,}953x10^5$ ± 65.279

Figure 1 shows that the number of erythrocytes and platelets in samples with anticoagulant K_3EDTA is higher than that of erythrocytes and several platelets with anticoagulant Na_2EDTA. Meanwhile, the number of leukocytes in samples with K_3EDTA anticoagulant was lower than that of leukocytes with Na_2EDTA anticoagulant.

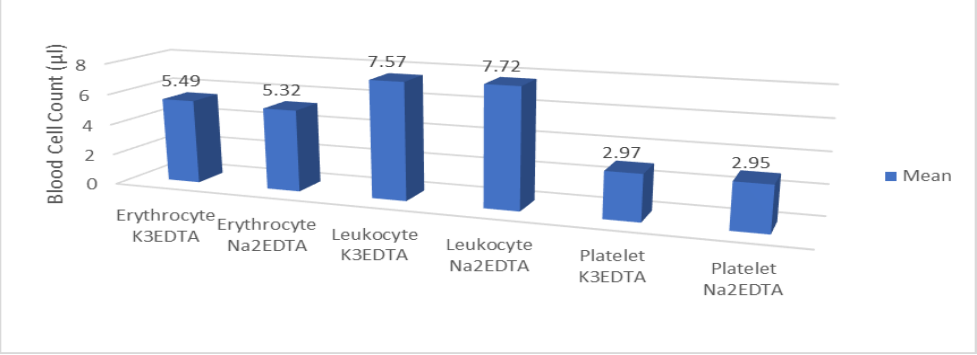

Figure 1. Average blood cell count in anticoagulant K_3EDTA and Na_2EDTA

Table 2. MANOVA test statistical results

Fixed factor	Variable dependent	Significance (p)
Anticoagulant	Erythrocyte count	0,041
	Leukocyte count	0,844
	Platelet count	0,920
Homogenization	Erythrocyte count	0,959
	Leukocyte count	0,991
	Platelet count	0,867

The research data were tested statistically using the Manova test. Based on Table 2, the results showed that the type of anticoagulant had an effect on the erythrocyte count ($p=0.041$) but did not have a significant effect on the leukocyte count ($p=0.844$) and platelets count ($p=0.920$). Meanwhile, the homogenization technique did not have a significant effect on the erythrocyte count ($p=0.959$), leukocyte count ($p=0.991$), and platelets count ($p=0.867$).

DISCUSSION

The pre-analytical stage plays an essential role in sample quality in clinical laboratories. The pre-analytical stage is the stage before analyzing a sample in the laboratory, which includes patient preparation, sampling, labeling, sample transportation, sample storage, and sample preservation, which may affect laboratory results

(Sianipar, 2019). The pre-analytical phase is the most vulnerable stage of the entire laboratory examination process because the stages involved are directly dependent on humans and outside the direct control of the laboratory. The pre-analytical phase's quality can affect the test results' accuracy. The pre-analytical phase can be categorized into (1) the conventional phase and (2) the analytical phase. The conventional phase involves laboratory processes starting from patient identification, tube selection, transportation, storage, and sample preparation. At the same time, the analytical phase occurs outside the laboratory. It consists of selecting appropriate tests based on clinical questions, ordering, collection and handling, transportation, and receipt of samples prior to testing (Sengupta et al., 2016).

One of the essential pre-analytical steps is sample preparation prior to examination. The choice of anticoagulant, tube type, and homogenization technique is essential to sample quality. Inappropriate blood-to-anicoagulant ratio (inappropriate specimen volume), specimen clotting or hemolysis due to improper tube homogenization, and inappropriate specimen container are examples of errors in the pre-analytical stage (Grover & Gadhavi, 2024). Achieving proper sample mixing is essential and crucial in obtaining reliable, accurate, and representative results in the laboratory. This increases the sample inspection process's efficiency and ensures the validity of the inspection results. So, obtaining a homogeneous sample is very important in the analysis (Dezhakam et al., 2024).

Good homogenization techniques not only maintain an effective anticoagulant-to-blood ratio but also provide specimens without blood clots. Homogenization is carried out immediately after blood collection, using several manual inversion techniques performed with the wrist to ensure the anticoagulant is mixed with the blood sample. Some manual inversion techniques are influenced by the back-and-forth wrist movements required to achieve homogeneity of the blood sample. In some laboratories, homogenization techniques are carried out using mechanical mixers, consisting of 2 types: rocking-type and rotary-type mixers (Mehndiratta et al., 2021).

Complete blood count (CBC) is clinical laboratories' most commonly performed routine hematology test. CBC is often used as a screening test to determine a patient's health status. CBCs are several tests that evaluate the cells circulating in the blood, including erythrocytes, leukocytes, and platelets. CBC helps to diagnose various conditions such as anemia, infection, inflammation, bleeding disorders or leukemia, acute hemorrhagic conditions, and allergies and is essential in monitoring the effectiveness of treatment or therapy response. CBC contains a basic assessment of various blood components by determining the number, variety, percentage, concentration, and quality of various blood components (Yucel et al., 2017).

Currently, many clinical laboratories are equipped with automated devices for checking CBC capable of processing a large number of blood samples quickly and efficiently. CBC examination using a Hematology Analyzer has advantages, namely short examination time, small number of samples, and accurate results. However, when inaccurate results are found, pre-analytical factors such as venous blood collection, insufficient blood volume collection, and sample storage should be given more attention because they are the most common factors that affect CBC results (Zandecki et al., 2007). Ethylene diamine tetra-acetic (EDTA) anticoagulant is an organic compound that functions as a metal ion binder; in the blood, it functions to bind plasma calcium to inhibit the coagulation cascade. EDTA anticoagulant is commonly used in hematological examinations because it does not distort blood cells (Patel, 2009).

In this study, it was found that anticoagulants affected erythrocyte count ($p=0.041$). This is because the vacutainer anticoagulant in liquid form may cause slight dilution of the sample. In the case of prolonged storage, changes in the anticoagulant/blood ratio may lead to changes in the mean erythrocyte size. An appropriate volume of EDTA is required to prevent coagulation, but excessive amounts can cause changes in blood cell morphology. In addition, vacutainer tubes should be homogenized several times (8-10) to ensure thorough mixing and proper anticoagulation (Patel, 2009). Another study found that excess EDTA can decrease hematocrit and Mean Corpuscular Volume (MCV) levels because hypertonic plasma, due to increased ion concentration, can cause an increase in Mean Corpuscular Hemoglobin Concentration (MCHC). Blood samples with inappropriate K_3EDTA volume can increase cell damage, causing erythrocytes to deform into crenation and spherocytes, causing differences in results on the hematology analyzer (Riba et al., 2020).

If the vacutainer tube is filled with a smaller blood sample, the ratio between the blood

sample and the anticoagulant becomes inaccurate. This results in an excessively high concentration of EDTA, causing erythrocytes to shrink due to hypertonic plasma, thereby creating artifacts that make erythrocyte morphology challenging to interpret. Other studies also show that excess EDTA causes membrane damage to erythrocytes and leukocytes (National Committee for Clinical Laboratory Standards, 2003). Incorrect mixing of anticoagulants (too little or too slow) can cause small blood clots. Additionally, improper blood sample collection can lead to thrombin release and falsely low platelet counts due to platelet aggregation (Patel, 2009).

Research data indicates no effect of secondary homogenization 4 times, 8 times, and 12 times on the number of erythrocytes, leukocytes, and platelets. This is because the blood specimen has been well-mixed using the secondary homogenization technique. Mixing blood and anticoagulant using the secondary homogenization technique 4 times, 8 times, and 12 times can provide examination results that do not differ statistically. This is consistent with previous research, where there was no difference (p=0.416) in the number of erythrocytes using the secondary homogenization technique 5 and 8 times (Haiti et al., 2021).

The limitations of this study include the limited number of specimens and the fact that it did not account for the time between blood collection and reading on the hematology analyzer. Although all specimens were examined within less than 2 hours, the time difference in readings could also affect the examination results. For further research, increasing the sample size and incorporating a greater variety of anticoagulant vacutainers would be beneficial, including variations in anticoagulant volumes (1 ml, 2 ml, and 3 ml).

CONCLUSION

This study concludes that the anticoagulant affects the number of erythrocytes but does not have a significant effect on the number of leukocytes and platelets. Meanwhile, the homogenization technique does not have a significant effect on the number of erythrocytes, the number of leukocytes, and the number of platelets. This research suggests collecting whole blood specimens using EDTA vacutainers as the anticoagulant and applying a secondary homogenization technique before performing the CBC analysis.

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