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The Difference in the Number of Platelets Calculated by the Indirect Method (Fonio) and a Hematology Analyzer

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ABSTRACT

Platelet count checks are one of the most requested tests in clinical laboratories because of their important role in helping to establish a diagnosis. Hematology Analyzer has many advantages, namely practical, fast, and reliable in treating large numbers of patients. However, because the automated method is expensive and has low yields due to the inability to read the sticky platelets. Therefore, we still use the manual edge blood swab method as a control in calculating the number of platelets This study aims to compare the results of the examination of platelet count using the Indirect Fonio indirect manual method with the automatic method using *the* Hematology Analyzer tool. This type of research is observational analytics with a cross-sectional design. The research subjects amounted to 36 respondents. The results showed that the average number of platelets obtained with the Indirect Fonio method was 290,389 µL, while with the Hematology Analyzer method it was 331,222 µL. The results of data analysis using *the Independent T-test* showed a significance value of 0.014 ($p<0.05$), which means that there is a significant difference between the two examination methods. Thus, it can be concluded that there is a difference in the results of counting platelet counts between the *manual method of Indirect* Fonio and the automatic method of Hematology Analyzer. It is recommended that the next research on sample handling procedures, especially at the pre-analytical stage such as the blood collection and shuffling process, be carried out in accordance with operational standards to prevent platelet aggregation that can interfere with the accuracy of the results.

Keywords: Platelet count, Fonio Indirect Manual, Automatic Hematology Analyzer.

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INTRODUCTION

Blood is a fluid in the body that functions to flow oxygen to all body tissues, delivers nutrients needed by cells and becomes a fortress of defense against bacteria and viruses, without enough blood a person can experience various health problems and even death [1]. Platelets are the smallest blood cells that amount to 150 to 350 10/µl of blood in healthy individuals [2]. Platelets are blood component cells that play an important role in the hemostasis process. Many platelets are produced in the bone marrow through the fragmentation of the cytoplasmic megacaryocytes [3]. The performance of platelets in the wound closure process occurs through adhesion, where contact occurs between the surface of the damaged blood vessels and induces changes in platelets spontaneously

[4]. Platelet adhesion is when the blood vessels are injured, the surface of the edotel and the underlying collagen reveal. Platelets travel to the subendothelial collagen fibers and form pseudopodia on the surface [5]. Platelet testing is widely requested in clinical laboratories. This is due to its significant role in helping to establish diagnosis, predict disease progression, and conduct follow-up [6]. The automatic method of checking platelet counts using a Hematology analyzer tool is practical and provides more accurate results. However, automated testing remains an alternative option for platelet count testing in areas with limited access to electrical resources and limited funds to purchase expensive hematology analyzers [7]. Hematology Analyzer is a digital automated tool that quickly generates results for a wide range of examination parameters, such as a complete blood test, which includes hematocrit, platelets, leukocytes, erythrocytes, erythrocyte index, and hemoglobin. In addition, the advantage of this tool is that the sample volume is not large and does not require complicated maintenance because the blood taken can be examined directly in a very short time [8]. Counting the number of platelets can be done by means of immunofluorescent, fluorescent, hematology analyzer and so on [9]. Automatic platelet count examination using Hematology Analyzer has many advantages, namely practical, fast, and reliable in handling large numbers of patients. However, because the automated method is expensive and may have low yields due to the inability to read the sticky platelets [10]. Therefore, we still use the manual edge blood swab method as a control in counting platelet counts using *a* Hematology analyzer to count cells that are automatically able to measure the number of platelets, leukocytes, and erythrocytes directly [11]. Calculating the number of platelets by the indirect method is using a blood swab as a testing medium. This method is used to determine the number of platelets in the blood [12]. Giemsa staining is Romanowsky's staining, which uses azure B (trimethylthonin, methylene blue oxidation product) and eosin (eosin B or eosin Y) which is red. The combination of these two colors can produce a variety of colors in the edge of the blood smear preparation. Giemsa staining is used to distinguish the cell nucleus and cytoplasmic shape from red blood cells, white blood cells, platelets, and parasites present in the blood [13].

MATERIALS AND METHODS

This type of research falls under the category of analytical observational research with a cross-sectional design. Cross-sectional design is a study that looks at differences or relationships between two variables at a single point in time without any intervention or treatment.A cross-sectional design is used because data from both the Fonio and Hematology Analyzer methods are taken at the same time from the same sample. Researchers compared platelet measurement results at a given time to assess whether there was a difference between the two methods. The sampling technique used is Simple random sampling.

The research population used 57 5th semester D-IV Health Analyst students at Nahdlatul Ulama University Surabaya. The sample used in this study was the blood of 5th semester D-IV Health Analyst students at Nadhlatul Ulama University Surabaya with a sample size of 36 samples.

The research time is in February 2025. The research was carried out at the hematology laboratory of Nadhlatul Ulama University Surabaya.

Fonio Method Examination

Fonio Counting the number of platelets by the indirect manual method is a way of calculating the number of platelets in a blood smear preparation that has been stained with giemsa or wright. This method has its drawbacks because the uneven distribution of platelets in a blood smear can affect platelet counts [6]. Record the results of platelet count examinations. The number of platelets in the thin blood preparation is compared to 1000 erythrocytes then the absolute number can be calculated from the number of erythrocytes, Calculation of the number of platelets indirect method (Fonio) [14].

$$\text{Number of platelets (cells/ µl)} = \frac{\text{Number of platelets}}{\text{Number of Viewing Spaces}} \times 20{,}000$$

The tools used in this study include Object glass, cover glass, microscope, 3 cc syringe, tourniquet, Hematology Analyzer automatic tool, pipette, EDTA tube, blue tip, yellow tip, micropipette. The materials used in this study are Eosine Solution, Methylene Blue Solution, Cotton Alcohol, EDTA Blood, Magnesium Sulfate, Giemsa.

Data analysis was carried out to determine the difference in the number of platelets by the indirect method (Fonio) with the hematology analyzer, data analysis using paired T-test. In this study, data was analyzed using the SPSS program with an Independent Parametric T-test.

RESULTS AND DISCUSSION

This study used 36 respondents to calculate the number of platelets in D-IV Health Analysts students of UNUSA by taking venous blood with EDTA anticoagulants from patients Characteristics of respondents in the study were someone who was healthy and not undergoing any treatment Characteristics of respondents based on gender Respondent criteria based on age were 28 women (78%), and 8 men (22%).

Table 1. Characteristics of Respondents by Gender

Gender	Number of people	Percentage
Man	8	22%
Woman	28	78%
Total	36	100%

The characteristics of the respondents in this study were a healthy person aged 20-23 years, the average age of respondents was 21 years old, 4 people (11%) were 20 years old, 15 people were 21 years old (42%), 22 years old were 11 people (30%), and 23 years old were 6 people (17%).

Table 2. Characteristics of Respondents by Age

Age	Number of people	Percentage
20	4	11%
21	15	42%
22	11	30%
23	6	17%
Total	36	100%

The results of the study of calculating the number of platelets that have been carried out obtained an average result of 290,389 cells/µL in the fonio method, while the average number of platelets in the hematology analyzer method was 331,222 cells/µL.

Table 3. Average Values of Both Methods

Method	Mean (µL)	Maximum amount (µL)	Minimum amount (µL)	Normal Value
Fonio	290.389	434.000	162.000	150.000 – 450.000
Hematology analyzer	331.222	475.000	180.000	150.000 – 450.000

Because the data is normally distributed, the data analysis test uses the Independent T-test, with the test result of <0.05, H_0 is rejected and H_1 is accepted, which is that there is a difference in the number of platelets count by the indirect method (Fonio) with the hematology analyzer. If the p-value > 0.05, H_0 is accepted and H_1 is rejected, i.e. there is no difference in the number of platelets count by the indirect method (fonio) and the hematology analyzer. The results in table 4 showed a p-value of 0.014 ($p< 0.05$), meaning that H_1 was accepted and H_0 was rejected so that there was a significant difference in the calculation of platelet counts by the indirect method (fonio) with the hematology analyzer.

Table 4. T-Test Independent

Variabel	*p-value*	Information
Difference in the number of platelets *indirect* method (fonio) with *Hematology analyzer*	0,014	There are differences (Difference in the number of platelets count *by indirect* method (fonio) with *Hematology analyzer*)

When it comes to platelet counts, there are several things that must be considered. One is that the blood should be immediately mixed with the anticoagulant and mixed sufficiently to prevent platelets from clumping in the test tube wall, the purpose of homogenization is to ensure that the blood sample and anticoagulant are evenly mixed

so that the blood components remain the same in shape and condition as when distributed through the bloodstream. It also prevents clots that can affect the results of the examination. The results of the examination may change due to inadequate homogenization. At the time of sample collection for platelet count examination [15].

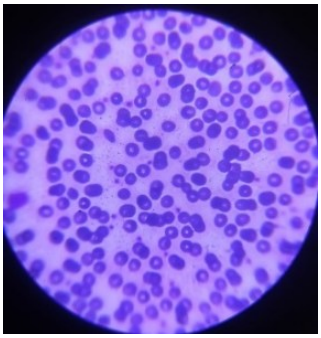

Figure 1. Platelet staining results with Giemsa Magnification 100 x Objective Lens

Based on the results of microscope observations of 100 times the objective lens, in the image above in the platelet count using an edge blood smear preparation using *Giemsa solution,* platelets stained with *Giemsa* will be purplish-blue.

CONCLUSIONS

Based on the results of the research and discussion, it can be concluded that the average number of platelets produced using the automatic method of Hematology analyzer was found to be 331,222 cells/mm^3. The average number of platelets produced using the indirect fonio method was found to be 290,389 cells/mm^3. There is a difference in the platelet count examination of the automatic method of hematology analyzer and manual indirect fonio.

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