

## THE DIFFERENCES OF HEMATOCRIT LEVEL IN IMMEDIATE AND DELAYED BLOOD SAMPLES ON MICROHEMATOCRIT METHOD

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### Abstract

**Background:** Hematology specimen examination should be performed at room temperature in less than 2 hours. Recommended specimen storage temperatures are in the range of 2°- 6°C. However, examining laboratory samples with uncertain storage time and temperature is often delayed. **Aims:** To determine the difference between the hematocrit values of blood samples examined immediately and those that were delayed for more than 2 hours at room temperature. **Methods:** This study was conducted from April to June 2023 at the Clinical Chemistry Laboratory of Universitas Bali Internasional. A total of 24 venous blood samples were grouped into three treatment groups that were stored for 3 hours (P1), 6 hours (P2), and 12 hours (P3) at room temperature (18-220C), and one control group was examined immediately. Data were analyzed using the Mann-Whitney U test ( $\alpha=0.05$ ). **The Results:** Statistical tests showed that the comparison of control with P1 was not significantly different ( $p=0.313$ ), while the comparison of control with P2 ( $p=0.003$ ) and the control with P3 ( $p=0.000$ ) was significantly different. **Conclusions:** There was no significant difference in hematocrit values between blood samples examined immediately and blood samples delayed for 3 hours. Significant differences in hematocrit values were shown in comparing blood samples examined immediately with blood samples delayed for 6 hours and examined immediately with blood samples delayed for 12 hours.

**Keywords:** hematocrit level, blood sample, immediate, delayed

### 1. Introduction

Hematocrit (Hct), also known as packed cell volume (PCV), is an examination of the amount in percentage (%) of red blood cell volume per milliliter contained in 100 mL of blood. This examination is done to explain the composition of red blood cells in the body. An increase or decrease in the percentage of hematocrit value can be influenced by

cellular and plasma factors such as an increase or decrease in the production and size of red blood cells or loss or addition of body fluids (1).

The Clinical Data Interpretation Guidelines state that hematocrit has diagnostic significance for detecting cases of anemia, dengue hemorrhagic fever, or burns, and a decrease in hematocrit levels

indicates anemia, leukemia, or hyperthyroidism (2).

Hematocrit values can be determined by conventional method (micro or macro method) (3), as well as by automatic method using Hematology Analyzer (4) with venous or capillary blood samples (5). In principle, all examinations must be carried out immediately after the specimen is obtained to anticipate any changes in the nature and morphology of the sample (6). Hematology specimen examination should be done in less than 2 hours at room temperature or a critical limit of up to 6 hours, depending on the examination parameters (5). The recommended storage for blood specimens in hematology examinations is generally at 2°- 6°C (7).

Control of specimen storage temperature and time significantly affects the results of hematology specimen examination. If it exceeds the recommended delay and temperature limit, there will be changes in both the quantity and quality of blood cells (5).

However, conditions in the actual situation show that there are often delays in examining laboratory samples due to the distance between the place of blood collection and the laboratory, samples stored to confirm the examination in the event of a complaint (8), a large number of samples that cause queues of examinations, delayed examinations due to busy laboratory staff performing services (8) (9), or equipment damage (9).

Related to this issue, the effect of volume, time delay, and storage temperature of blood samples on hematocrit values was examined (10). In that study, the sample delay was carried out for 1 and 2 hours at 8°C and 16°C. Another study was conducted, which examined the difference in hematocrit values of blood samples immediately and blood samples stored at 2°-6°C for 30 days (11).

Both studies showed no significant difference between immediate blood hematocrit levels and blood hematocrit levels that were delayed for 1 and 2 hours at 8°C and 16°C or for 30 days at 2°- 6°C.

In those studies, the sample examination at room temperature was delayed for a maximum of 2 hours, while samples that were delayed for more than 2 hours were stored at a temperature of 2°- 6° C. Research has not been conducted to determine the hematocrit value in blood samples stored for more than 2 hours at room temperature.

Based on this information, the author is interested in conducting a study to test the hematocrit value in blood samples stored for more than 2 hours at room temperature. This study aims to determine the difference in hematocrit values of blood samples examined immediately with blood samples delayed for 3 hours, 6 hours, and 12 hours at room temperature using the Microhematocrit method.

## 2. Research Methods

This research is an actual experimental research with a completely randomized design. The research was conducted from April to June 2023 at the Clinical Chemistry Laboratory, Universitas Bali Internasional (UNBI), Jalan Seroja, Gang Jeruk, Tonja, Kec. East Denpasar, Denpasar City.

The samples used in this study were 24 venous blood samples collected in tubes with EDTA anticoagulant. Blood samples were obtained from UNBI students who were willing to volunteer. Each six blood samples were grouped into three treatment groups, including blood samples stored for 3 hours, 6 hours, and 12 hours at room temperature (18-22°C) and one control group, which were examined immediately.

### Hematocrit Measurement by Microhematocrit Method

Microhematocrit tubes were filled with blood samples to leave 1/3 of the tube volume. Then, one end of the tube was sealed with putty. The microhematocrit tubes filled with blood samples and covered with putty are then arranged in the centrifuge with the putty-covered end positioned away from the center of the centrifuge. Centrifuge the tubes for 5 minutes at 10,000-12,000 rpm. Afterward, the microhematocrit tubes were placed on the microhematocrit scale board (figure 1) to read the results. Hematocrit examination was repeated ten times for each blood sample. The values obtained were then compiled into a table and analyzed.

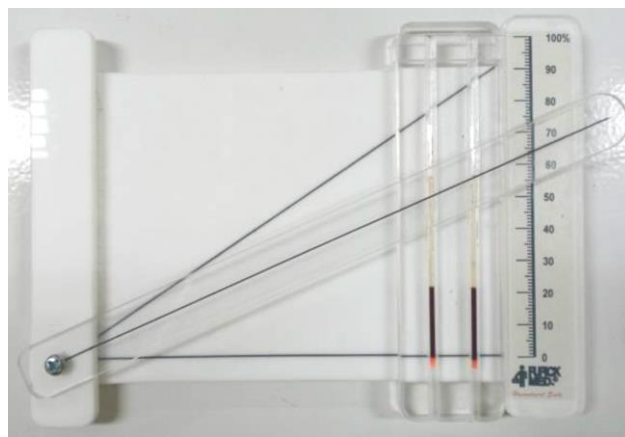


Figure 1. Microhematocrit tube on microhematocrit scale board

### Data Analysis

The Hematocrit value obtained in this study was statistically analyzed using the SPSS program with a confidence level of 95% ( $\alpha=0.05$ ). At first, the normality test

was conducted with Kolmogorov Smirnov and the homogeneity test with Levene Statistic Test. The test results showed that the data in this study were not normally distributed and heterogeneous, so to

conduct a comparison test between each treatment and control group, the non-parametric Mann-Whitney U test was used.

### 3. Results and Discussions

The results of the Hematocrit value measurements for the blood samples that were examined immediately and the blood samples that were delayed for 3 hours, 6 hours, and 12 hours by the microhematocrit method are shown in Table 1.

Table 1. Hematocrit Value of Immediate and Delayed Blood Samples

Samples	Average of Hematocrit Value			
	Immediate	3 hours delayed	6 hours delayed	12 hours delayed
<b>A</b>	35 %	34 %	34 %	33 %
<b>B</b>	37 %	36 %	35 %	34 %
<b>C</b>	36 %	36 %	34 %	34 %
<b>D</b>	33 %	33 %	31 %	31 %
<b>E</b>	35 %	35 %	34 %	34 %
<b>F</b>	32 %	34 %	33 %	31 %
<b>Average</b>	35 %	35 %	34 %	33 %

Source: Primary Data (2023)

The table shows that blood samples examined immediately and delayed for 3 hours at room temperature showed the same average hematocrit value of 35%. Blood samples that were delayed for 6 and 12 hours at room temperature showed an average hematocrit value of 34% and 33%, respectively.

The data were then statistically analyzed using the Mann-Whitney U test to see the significance of the difference in hematocrit values between each treatment and control group.

Table 2. Mann-Whitney U Test Results

Comparison	Sig
Control - 3 hours	0,313
Control - 6 hours	0,003
Control - 12 hours	0,000

Source: Primary Data (2023)

Tatistical test with Mann-Whitney U in Table 2 shows no significant difference ( $p > 0.05$ ) between blood samples examined

immediately and blood samples delayed for 3 hours. Significant differences ( $p < 0.05$ ) were shown in comparing immediate blood samples with blood samples delayed for 6

hours and the comparison of immediate samples with blood samples delayed for 12 hours.

The results of this study are similar to Jain's research (12), which showed that delaying the examination time of blood samples below 6 hours at room temperature had no significant effect on the examination results. Similar results were also shown by Afifah's research (13), which showed no significant differences in hematocrit values examined immediately and delayed for 3 hours.

Statistical test results showed a significant difference between the hematocrit values of blood samples examined immediately and those delayed for more than 3 hours. The average hematocrit value data (table 1) also showed that the longer the blood sample was delayed at room temperature, the lower the hematocrit value.

A decreased hematocrit value is usually caused by a transformation in the number and shape of erythrocytes (4). One of the triggering factors is the process of red blood cell destruction. An increase in time delay results in changes in red blood cells' components, composition, and function (11).

Long storage or delay of blood specimens causes changes in examination results because blood components will be damaged if left at an inappropriate

temperature of more than 4-8°C (14). Damage can be in the form of erythrocyte cells experiencing crenation (15), spherosis (16), or rupture of the erythrocyte membrane (hemolysis) so that the examination results decrease (17).

During storage, blood cells undergo biochemical/biomechanical change and immunological reactions, causing structural or morphological damage known as storage lesions. Erythrocytes are the blood cells most susceptible to this damage (Ekanem in Fitria [18]).

Hematocrit values are different in males and females. The hematocrit value in men ranges from 40-48%, while in women it ranges from 37-43% (19). The hematocrit value obtained in this study was slightly below the reference value.

Mathera (in Sari (20)) mentioned that the decrease in hematocrit could occur due to physical activity beyond the maximum ability limit. Senturk (in Sari (20)) also said that in several literature studies conducted by many researchers before, it is known that maximal physical exercise can cause changes in hematocrit, erythrocyte, leukocyte, and platelet values.

Generally, control of temperature and specimen storage time dramatically affects the results of hematology specimen examination. Paying attention to the laboratory specimen storage time limit based on the examination parameters is

highly necessary (5). The purpose of this control is to maintain the blood components' characteristics. Proper storage can also reduce the growth of bacteria that can contaminate blood specimens (7).

With proper treatment, the composition of the blood specimen will not change significantly so that the blood can be analyzed and show results that describe the patient's condition (11).

#### 4. Conclusions

There was no significant difference between blood samples examined immediately and blood samples delayed for 3 hours. Significant differences were shown in comparing blood samples examined immediately with blood samples delayed for 6 hours and the comparison of blood samples examined immediately with blood samples delayed for 12 hours.

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