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Differences In The Number Of Thrombocytes And Thrombocyte Index At Variations Of Temperature And Storage Time Using Hematology Analyzer

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Abstract, Background: *Examination of the hematology panel (hemogram) consists of leukocytes, erythrocytes, hemoglobin, hematocrit, erythrocyte index and platelets. Platelets are the smallest elements formed in the bone marrow and play an important role in the process of blood clotting. Examination of the platelet count aims to determine the number of platelets per microliter of blood. Almost all laboratories in hospitals have used a Hematology Analyzer because the examination is more practical and accurate. The specimens used were derived from venous blood with the addition of K2EDTA anticoagulant. Storage of inspection material needs to pay attention to sample stability.*Examination of the platelet count can cause changes in the platelet count, and changes in the examination of the platelet index such as the average platelet volume (MPV), platelet distribution width (PDW), and thrombocytopenia (PCT). The purpose of this study was to analyze the effect of variations in temperature and storage time on the number of platelets and the platelet index examined at various temperatures using a hematology analyzer. The study covers the field of Hematology in October-November 2021. The research is analytical in nature with a quasi-experimental design. The research subjects were 30, the sampling technique was accidental sampling with primary data. The examination method uses the impedance method with the Hematology Analyzer tool. Data were analyzed using SPSS 22 version. There was a significant difference in the number of platelets examined at variations in temperature and storage time with $p<0.05$. There was a significant difference to the platelet index value examined at variations in temperature and storage time with $p <0.05$.

Key words:*Platelets, Platelet Index, Storage Times, Storage Temperature*

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BACKGROUND

Hematological examination is one of the important tests used in the laboratory and is often requested clinically. The hematology panel examination (hemogram) consists of leukocytes, erythrocytes, hemoglobin, hematocrit, erythrocyte index and platelets [1].

Platelets are the smallest elements in blood vessels 1. Platelets are formed in the bone marrow and play an important role in the process of blood clotting. Platelets have a life span of 5-9 days with a normal value of 150,000 to 400,000 cells per microliter of blood [2].

The examination of the platelet count aims to determine the number of platelets per microliter of blood so that it can be known whether or not there are blood clotting disorders and bleeding disorders. Manual examination of the platelet count is currently relatively rarely used because almost all laboratories in hospitals have used a Hematology Analyzer because the examination is more practical and accurate even though the instrument is more expensive. Specimens for hematological examination are usually taken from venous blood with anticoagulants to prevent the blood from clotting. Anticoagulants that are often used include Ethylene Diamine Tetra Acetate (EDTA) because it can maintain cell components and blood cell morphology [3].

Anticoagulant K_2EDTA is recommended by ICSH or International Council for Standardization in Hematology and CLSI or Clinical and Laboratory Standards Institute [4].

In the storage of inspection materials, it is necessary to pay attention to the stability of the sample. Temperature and length of storage time can affect the test results. The time limit for EDTA blood tests for platelet counts is 2 hours at room temperature. During the storage period, changes in biochemistry, structure and function can occur, which is also known as a platelet storage lesion (PSL). Currently, many other tests are being carried out to see platelet viability related to PSL, such as checking the mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and microscopically observing the shape of the platelets. The delay in checking the platelet count for more than 1 hour causes a deviation in the platelet count result. EDTA blood stored for more than 1 hour at room temperature will cause platelets to easily stick to each other (aggregation) or stick to foreign objects (adhesion).

The purpose of this study was to determine the average number of platelets examined at variations in temperature and storage time using a hematology analyzer. Determine the average value of the platelet index examined at variations in temperature and storage time using a hematology analyzer. Analyzing the difference in the number of platelets in variations in temperature and storage time using a hematology analyzer. Analyzing the difference in the value of the platelet index on variations in temperature and storage time using a hematology analyzer

METHOD

The type of research used is analytic research with a quasi-experimental research design [6]. The aim is to determine the differences in platelet count and platelet index at variations in temperature and storage time with the Automatic method using a hematology analyzer.

Time and place The research was carried out in October – November 2021 at the Bandung City Advent General Hospital.

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RESULTS

In this study, the platelet count and platelet index were examined at room temperature and 2-8ºC as well as variations in storage time of 0 hours, 2 hours and 4 hours using a hematology analyzer.

Before starting the examination, internal quality assurance is carried out using 3 levels of daily control materials for hematology, namely Low, Normal, and High to guarantee the results of the examination.

Examination Results of the Average Number of Platelets at Room Temperature Examined at 0 Hours, 2 Hours, and 4 Hours

Data on the results of the examination of the platelet count in the data collection of the research results are presented in table 1 below:

Table 1. Distribution of Descriptive Statistics of Platelet Count at Room Temperature Examined at 0 Hours, 2 Hours, and 4 Hours

Platelet Count at Room Temperature Examined at Time 0 Hours, 2 hours and 4 hours (cell/µL)				
Variable	**N**	**mean**	**Min**	**Max**
Platelets 0 Hours	5	289,200	198,000	346,000
Platelets 2 hours	5	269,400	186,000	322,000
Platelets 4 hours	5	264,800	179,000	326.000

Based on table 1, after a descriptive statistical analysis of the total sample, the average number of platelets at room temperature examined at 0 hours, 2 hours, and 4 hours tends to decrease. The percentage decrease in the mean value of the platelet count from 0 hours to 2 hours was 6.85%, from 0 hours to 4 hours was 8.44% and from 2 hours to 4 hours was 1.71%.

The results of the examination of the average number of platelets at a temperature of 2-8ºC were examined at 0 Hours, 2 Hours, and 4 Hours

Table 2. Distribution of Descriptive Statistics of Platelet Count at a Temperature of 2-8ºC examined at 0 Hours, 2 Hours, and 4 Hours

Platelet Count at 2-8ºC Temperature checked at Time 0 hours, 2 hours, and 4 hours (cell/L)				
Variable	N	mean	Min	Max
Platelets 0 Hours	5	289,800	199,000	347,000

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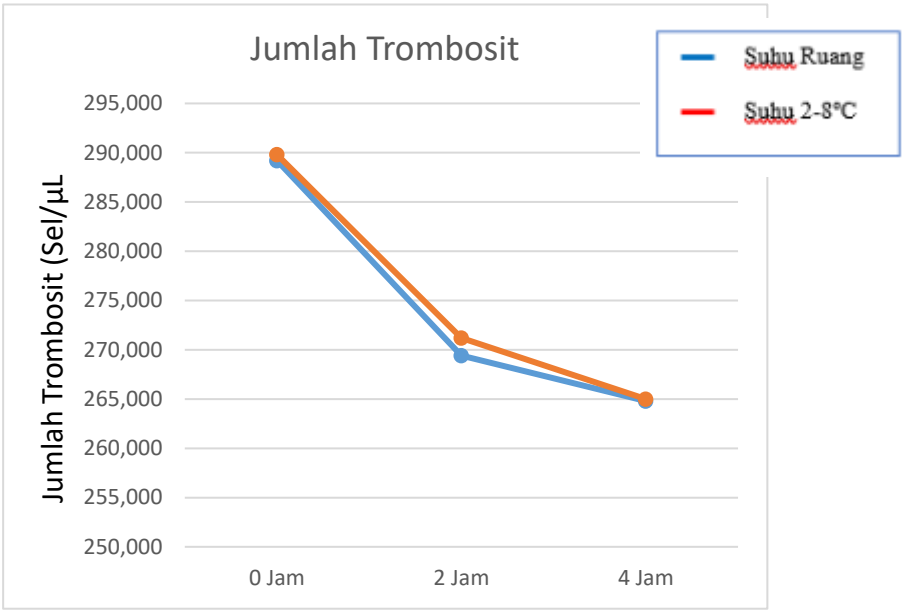
Platelets 2 hours	5	271,200	180,000	326.000
Platelets 4 hours	5	265,000	157,000	318.000

Based on table 2. after a descriptive statistical analysis of the total sample, the average number of platelets at a temperature of 2-8ºC which was examined at 0 hours, 2 hours, and 4 hours tends to decrease with a percentage decrease in the average value from 0 hours to 2 hours is 6.07%, 0 hours to 4 hours is 8.56% and from 2 hours to 4 hours is 2.65%.



Picture1. Graph of Examination of Platelet Counts at Variations in Temperature and Storage Time using the Tool*Hematology Analyzer*

In Figure 1. where the blue line is for room temperature and the red line is for a temperature of 2-8ºC. The graph shows a decrease in the number of platelets starting from 0 hours to 2 hours, 0 hours to 4 hours and 2 hours to 4 hours both from room temperature and 2-8ºC.

From the results of data processing statistically using the GLM-Repeated Measures Two Way statistical test with variations in storage time, namely 0 hours, 2 hours and 4 hours, it is known that there is a significant difference while with temperature variations, namely at room temperature and 2-8ºC, it can be concluded there is no difference.

Examination Results of Mean Platelet Volume (MPV) at Room Temperature and Storage Time Using a Hematology Analyzer

The results of the examination of the average value of the Mean Platelet Volume (MPV) on variations in temperature and storage time using a hematology analyzer are presented in Table 3.

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Table 3. Distribution of Descriptive Statistics of MPV Value at Room Temperature Checked at 0 Hours, 2 Hours, 4 Hours

MPV Value at Checked Room Temperature at 0 Hours, 2 Hours, and 4 Hours (fL)				
Variable	N	mean	Min	Max
MPV Nilai value 0 Hours	5	9.98	9.2	10.8
MPV Nilai value 2 hours	5	10.34	9.3	11.4
MPV Nilai value 4 hours	5	10.68	9.6	11.5

Based on table 3. after a descriptive statistical analysis of the total sample, the average MPV value at room temperature examined at 0 hours, 2 hours, and 4 hours tends to increase. The percentage increase from 0 hours to 2 hours is 3.61% and from 2 hours to 4 hours is 3.29%.

Examination Results Mean Platelet Volume (MPV) at a Temperature of 2-8ºC and Storage Time Using a Hematology Analyzer

The results of the examination of the average value of the Mean Platelet Volume (MPV) at a temperature of 2-8ºC and storage time using a hematology analyzer are presented in Table 4.

Table 4. Distribution of Descriptive Statistics of MPV Value at Temperature 2-8ºC which was checked at 0 Hours, 2 Hours, 4 Hours

MPV Value at Temperature 2-8ºC checked at Time 0 Hours, 2 Hours, and 4 Hours (fL)				
variable	N	mean	Min	Max
MPV Nilai value 0 Hours	5	9.92	9.1	10.8
MPV Nilai value 2 hours	5	10.02	9.1	10.9
MPV Nilai value 4 hours	5	10.20	9.3	11.4

Based on table 4. after a descriptive statistical analysis of the total sample, the average MPV value at a temperature of 2-8ºC which was examined at 0 hours, 2 hours, and 4 hours tends to increase. The percentage increase from 0 hours to 2 hours is 1.09% and from 2 hours to 4 hours is 1.79%.

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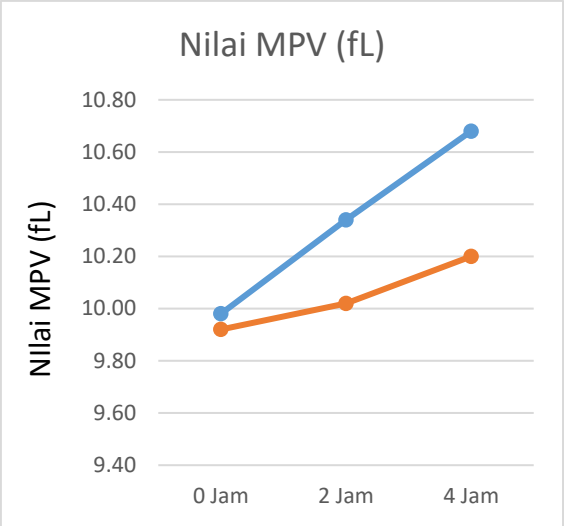

Picture2. Graph of Checking MPV values on Variations in Temperature and Storage Time using the Tool*Hematology Analyzer*

In the picture above where the blue line is for room temperature and the red line is for a temperature of 2-8ºC. The graph above shows an increase in the MPV platelet index starting from 0 hours to 2 hours and 2 hours to 4 hours both from room temperature and 2-8ºC.

From these data, it is known that there are statistically significant differences using the GLM-Repeated Measures Two Way statistical test with variations in storage time, namely 0 hours, 2 hours and 4 hours and temperature variations at room temperature and 2-8ºC. From the results of the examination, it was found that the MPV value at 0 hours, 2 hours and 4 hours of storage time tended to increase. However, when compared with the MPV reference value, it is still in the normal range. The results of MPV values at room temperature and 2-8ºC did not have a significant difference.

Examination Results Average Platelet Distribution Width (PDW) Value at Room Temperature and Storage Time Using a Hematology Analyzer

Data on the results of the examination of the average value of Platelet Distribution Width (PDW) at room temperature and storage time using a hematology analyzer are presented in Table 5.

Table 5. Distribution of Descriptive Statistics of PDW Value at Room Temperature Checked at 0 Hours, 2 Hours, 4 Hours

PDW Value at Checked Room Temperature at 0 Hours, 2 Hours, and 4 Hours (fL)				
variable	N	mean	Min	Max
PDW value 0 Hours	5	10.96	9.8	12.9
PDW value 2 hours	5	12.08	9.6	14.6

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PDW value 4 hours	5	12.10	9.6	13.7

Based on table 5 above, after a descriptive analysis of the total sample, the average PDW value at room temperature examined at 0 hours, 2 hours, and 4 hours tends to increase. The percentage increase from 0 hours to 2 hours is 10.22% and from 2 hours to 4 hours is 10.17%.

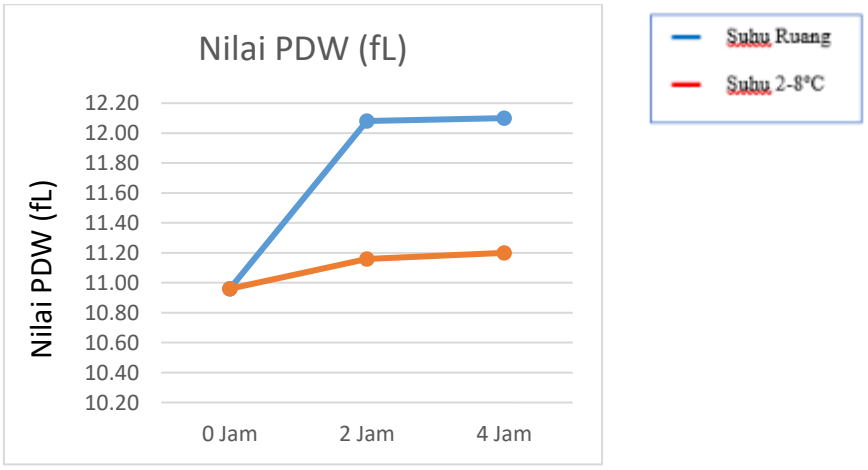
Examination Results Average Platelet Distribution Width (PDW) at a Temperature of 2-8ºC and Storage Time Using a Hematology Analyzer

The results of the examination of the average value of Platelet Distribution Width (PDW) at room temperature and storage time using a hematology analyzer are presented in Table 6.

Table 6. Distribution of Descriptive Statistics of PDW Value at Temperatures of 2-8ºC which is checked at 0 Hours, 2 Hours, and 4 Hours

PDW Value at 2-8ºC Checked Temperature at 0 Hours, 2 Hours, and 4 Hours (fL)				
Variable	N	mean	Min	Max
PDW value 0 Hours	5	10.96	9.8	12.9
PDW value 2 hours	5	11.16	9.4	12.8
PDW value 4 hours	5	11.20	9.5	13.86

Based on table 6. After a descriptive statistical analysis of the total sample, the average PDW value at a temperature of 2-8ºC which was examined at 0 hours, 2 hours, and 4 hours tends to increase. The percentage increase from 0 hours to 2 hours is 1.82% and from 2 hours to 4 hours is 0.36%



Picture3. Graph of Checking PDW Values on Variations in Temperature and Storage Time using the Tool*Hematology Analyzer*

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In Figure 3. where the blue line is for room temperature and the red line is for a temperature of 2-8ºC. The graph above shows an increase in PDW values starting from 0 hours to 2 hours and 2 hours to 4 hours both from room temperature and 2-8ºC.

From these data, it is known that there are statistically significant differences using the GLM-Repeated Measures Two Way statistical test with variations in storage time, namely 0 hours, 2 hours and 4 hours and temperature variations at room temperature and 2-8ºC. From the results of the examination, it was found that the PDW value at 0 hours, 2 hours and 4 hours of storage time tended to increase even though the increase was still within the normal value limits. At room temperature and 2-8ºC there was no difference in the examination at 2 hours but there were differences in results at 4 hours of storage.

Examination Results Average Plateletcrit Value (PCT)at Room Temperature and Storage Time Using a Hematology Analyzer

The results of the examination of the average Plateletcrit (PCT) value at room temperature and storage time using a hematology analyzer are presented in Table 7.

Table 7. Distribution of Descriptive Statistics of PCT Values at Room Temperature Checked at 0 Hours, 2 Hours, and 4 Hours

PCT Value at Room Temperature Checked at 0 Hours, 2 Hours, and 4 Hours (%)				
variable	N	mean	Min	Max
PCT value 0 Hours	5	0.258	0.20	0.29
PCT value 2 hours	5	0.254	0.20	0.28
PCT value 4 hours	5	0.250	0.20	0.27

Based on table 7. above, after a descriptive statistical analysis of the total sample, the average PCT value at room temperature examined at 0 hours, 2 hours, and 4 hours tends to decrease. The percentage decrease from 0 hours to 2 hours is 1.55% and from 2 hours to 4 hours is 1.57%.

Examination Results Average Plateletcrit Value (PCT) at a Temperature of 2-8ºC and Storage Time Using a Hematology Analyzer

The results of the examination of the average Plateletcrit (PCT) value at room temperature and storage time using a hematology analyzer are presented in Table 8.

Table 8. Distribution of Descriptive Statistics of PCT Values at Temperatures of 2-8ºC Checked at 0 Hours, 2 Hours, and 4 Hours

PCT Value at 2-8ºC Temperature Checked at 0 Hours, 2 Hours, and 4 Hours (%)				
variable	N	mean	Min	Max

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PCT value 0 Hours	5	0.256	0.20	0.29
PCT value 2 hours	5	0.248	0.18	0.29
PCT value 4 hours	5	0.240	0.17	0.29

Based on table 8. above, after a descriptive statistical analysis of the total sample, the average PCT value at room temperature examined at 0 hours, 2 hours, and 4 hours tends to decrease. The percentage decrease from 0 hours to 2 hours is 3.13% and from 2 hours to 4 hours by 1.61%. The description of the tendency of increasing the number of PCT values in variations in temperature and storage time is shown in Figure 4. below.

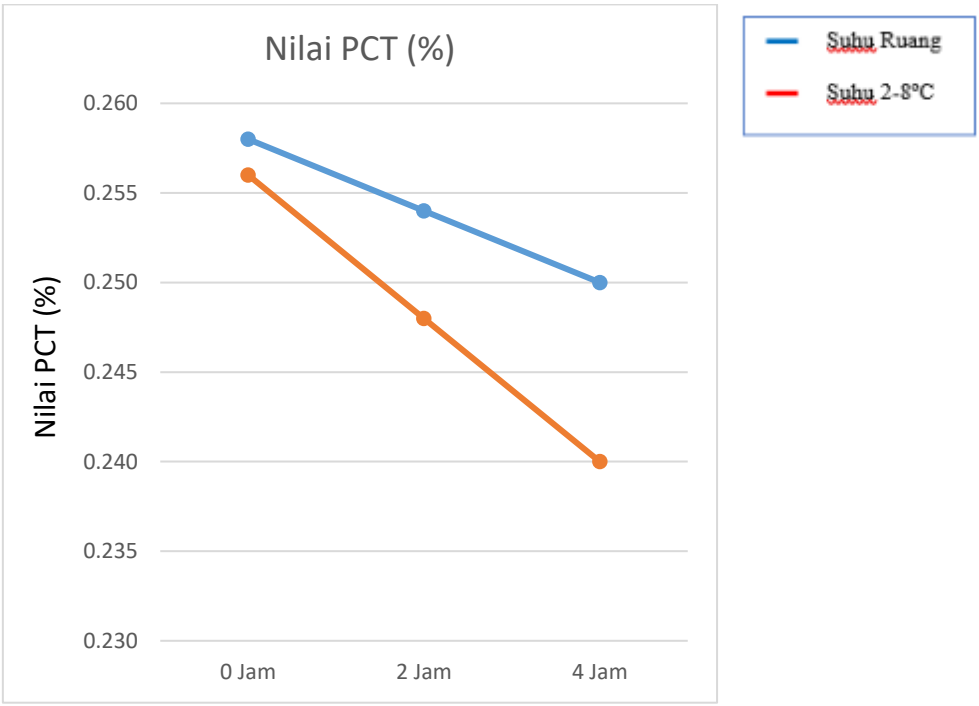

Figure 4. Graph of Checking PCT Values on Variations in Temperature and Storage Time using the Tool*Hematology Analyzer*

In Figure 4. where the blue line is for room temperature and the red line is for a temperature of 2-8ºC. The graph above shows a decrease in the PCT platelet index starting from 0 hours to 2 hours and 2 hours to 4 hours both at room temperature and 2-8ºC.

From these data, it is known that there are statistically significant differences using the GLM-Repeated Measures Two Way statistical test with variations in storage time, namely 0 hours, 2 hours and 4 hours and temperature variations at room temperature and 2-8ºC. From

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the results of the examination, it was found that the PCT value at storage time of 0 hours, 2 hours and 4 hours with variations in storage temperature at room temperature and 2-8ºC tends to decrease even though the decrease that occurs is still within the normal value limits. However, the results of temperature variations both at room temperature and at 2-8ºC did not show a significant difference.

DISCUSSION

DIFFERENCES IN THE RESULTS OF THE EXAMINATION OF THE NUMBER OF THROMBCYTES AT VARIATION OF TEMPERATURE AND STORAGE TIME

From the results of the above data processing statistically using the GLM-Repeated Measures Two Way statistical test with variations in storage time, namely 0 hours, 2 hours and 4 hours, it is known that there are significant differences while with temperature variations, namely at room temperature and 2-8ºC. concluded that there was no difference.

These results are in accordance with the theory that if blood with anticoagulants is stored at room temperature, platelets will continue to actively carry out metabolism, causing a decrease in platelet resistance. These changes can be slowed down if the blood sample is stored in the refrigerator 7.

The number of platelets that have decreased during storage can be caused by certain factors. One of the pre-analytic stages in laboratory examination includes blood sampling and sample homogenization. Difficulty in drawing blood causes platelets to stick together resulting in false low results, and poor homogenization also causes aggregation. In this study, the researchers controlled these factors by taking blood samples from patients according to the SOP, not puncturing the vein multiple times and ensuring perfect homogenization. The factors that come from the hematology analyzer include not being able to count abnormal cells, for example immature cells in leukemia, bacterial infections, sepsis, aggregating platelets and so on. and unable to count when the cell count is very high. Cross check using peripheral blood smear is very meaningful [8].

K2EDTA blood samples stored at 2-8ºC prior to examination were allowed to stand for approximately 15 minutes at room temperature to avoid falsely low platelet results.

Platelets will continue to actively perform metabolism if stored at room temperature. The result of this metabolism is the accumulation of lactate and a decrease in pH. Platelets that have a pH below 6.0 – 6.2 will cause platelet resistance to decrease. In addition, it will cause platelet cells to enlarge and disintegrate.

Upon activation, the platelets lose their discoid morphology and become more spherical with some pseudopods. The conformational change in the GPIIb/IIIa complex reveals the binding site for the adhesive protein (fibrinogen, vWF) that produces platelet aggregates. Subsequent activation stimulates release of granular contents and expression of sequestered membrane proteins on the outer surface.

Storage of platelets over time leads to a decrease in product quality and functionality; this is called a "platelet storage lesion." Platelet storage lesions are characterized by loss of discoid morphology, degranulation, lactic acidosis, loss of aggregation function, and expression of activation markers. Maintenance of these functional characteristics requires continuous production of adenosine triphosphate (ATP), and metabolic substrates can be rapidly depleted over time under room temperature storage conditions[11].

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Platelets lack the capacity for mitochondrial biogenesis or repair, and during in vitro storage they are limited to available physiological substrates. Thus, to slow down metabolic fatigue through refrigerated storage has been shown to be an effective way to prolong the shelf life and function of platelets[12].

Sustainable research resultsyear 2019about the difference in the number of platelets in the storage of blood samples at room temperature and in the refrigerator for 24 hours*result*sthere is a significant difference between the results of the platelet count at the beginning of the examination with a delay in the refrigerator storage temperature (2-8ºC) and room storage temperature (18-24ºC) for 24 hours [13].

The same result, according to Gunawerdana In 2016 the results showed that with a delay of 6 hours, 24 hours, and 48 hours, there was a significant decrease in the examination of the platelet count both at room temperature and at 4 degrees Celsius [14].

Based on the results of the examination, it was found that the number of platelets at storage time of 0 hours, 2 hours and 4 hours with variations in storage temperature at room temperature and 2-8ºC tends to decrease.

Although the results of the descriptive test showed a decrease in the number of platelets and statistically there were differences in the number of platelets with variations in storage time, namely 0 hours with 2 hours and 0 hours with 4 hours, but when viewed clinically based on the Total Error Allowable (TEA) value, the results were not there are differences both stored at room temperature and stored at a temperature of 2-8oC. Total Error Allowable (TEa) is defined as bias(%) + 2CV. The bias value is obtained by subtracting the average value of the control material examination results with the true value, then divided by the true value, then multiplied by 100% and CV is obtained by dividing the SD and the mean then multiplied by 100%. The TE value for the platelet count 2 hours is 6.892% and 4 hours is 8.488%, if TE < Tea (25%), then there is no difference between the platelet count at room temperature examined at 0 hours, 2 hours, and 4 hours clinically. Likewise the same thing when stored at a temperature of 2-8oC. The TE value for the platelet count 2 hours is 6.464% and 4 hours 8.61%, If TE < Tea (25%), then there is no difference between the platelet count at a temperature of 2-8ºC which is checked at 0 hours, 2 hours, and 4 hours clinically.

Differences in Platelet Index Value Examination Results on Variations in Temperature and Storage Time

Platelet index examination includes Mean Platelet Volume (MPV), Mean Platelet Volume (PDW), and Platelet Crit (PCT).

DIFFERENCES IN MPV VALUE EXAMINATION RESULTS

Mean platelet volume(MPV) is the average volume of platelets that describes the function and activity of platelets, namely the higher the MPV indicates the number of large platelets which is a sign of increased platelet turnover. Due to the excessive destruction of platelets, there will be compensation with the release of young megakaryocytes to come out early, this is what causes an increase in the MPV value of 15.

Platelet volume is known to be related to cytokines (thrombopoetin, interleukin-6, and interleukin-3) which regulate megakaryocyte ploidy, platelet count and greater platelet production. When platelet production decreases, young platelets become larger and more active, and MPV values increase. An increase in MPV value indicates an increase in platelet diameter, which can be used as a marker of the level of production and activation of platelets. During activation, the shape of the platelets changed from a biconcave shape to a spherical shape and a pseudopodia

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shape, thereby increasing the MPV 16 value.

On the hematology analyzer, the MVP readings look at the size and volume of platelets where the normal platelet size is 1-4 m and the normal platelet volume is 5-7 fL. In delaying the examination of platelets, platelets will experience aggregation which causes the size of the platelets to become larger, exceeding 4 m and the number of platelets will decrease so that the MPV value on the hematology analyzer will increase due to the size of the aggregating platelets and the decreased number of platelets.

The results of this study are in line with research conducted by Dewi Astuti (2020) with the title Platelet Index Value as a Quality Control of Platelet Concentrate Components It is known that the average MPV value tends to increase during the five-day storage period which indicates an increase in platelet volume during the storage period.

Differences in PDW Value Check Results

Platelet distribution width (PDW) measures variations in the size of platelets circulating in peripheral blood, young platelets are larger and old platelets are smaller. (Kickler, 1999)So, in the blood circulation there are biphasic platelets, young platelets have a larger size and the size of the platelets will decrease with increasing age. As a result of increasing the proportion of young platelets, there is also an increase in PDW18.

During the storage period of platelet components, metabolic changes occur, including decreased glucose levels, decreased pCO2, increased pO2 and cessation of lactate production, adenosine triphosphate and decreased bicarbonate concentration, and decreased pH. This causes the platelets to start to enlarge and the MPV value to increase. An increase in MPV indicates an increase in platelet diameter which can be used as a marker of platelet activation. During the activation process, the shape of the platelets changes from biconcave to spherical and pseudopodia which causes the shape of the platelets to become more varied so that the PDW value will increase.

The results of this study are in line with research conducted by Dewi Astuti with the title Platelet Index Value as Quality Control of Platelet Concentrate Components It is known that the average PDW value tends to increase during the five days of storage and this result is in line with the observational data from microscopic analysis conducted by the Kunichi method which found an image of discoid changes into spherical and pseudopodia forms.

Differences in PCT Value Examination Results

PCT is the percentage of the number of platelets in the blood. This PCT value is influenced by the platelet value of 16. This can indicate the decreasing number of platelets, the PCT value will also decrease which is proven in this study.

CONCLUSION

There was a significant difference in the number of platelets examined at variations in temperature and storage time with $p<0.05$. There was a significant difference to the platelet index value examined at variations in temperature and storage time with $p <0.05$.

It is recommended for further researchers to examine the platelet count and platelet index with a storage time of more than 4 hours.
For further researchers, it is recommended to conduct research on differences in platelet count and platelet index at variations in temperature and storage time

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using a hematology analyzer and confirming platelet morphology examination with SADT or electron microscopy (SEM).

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