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# THE EFFECT OF TEMPERATURE AND STORAGE DURATION OF WASHED RED CELLS ON THE NUMBER OF ERYTHROCYTES

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***Abstract***

Blood services in Indonesia, especially the processing and storage of donor blood, aim to maintain blood quality, including the Washed Red Cell (WRC) component used for patients with repeated transfusion allergies. This study aims to analyze the effect of temperature and storage time on the number of WRC erythrocytes. This research used a quasi-experimental design with serial measurements. One bag of WRC blood from PMI Bandung City was stored at room temperature (20-25°C) and refrigerator temperature (4-6°C) for 0, 3, 6, and 9 hours, with erythrocyte measurements using a Haematology Analyzer. The results showed that the average number of erythrocytes at room temperature decreased significantly from $7.54\times10^6$/µL (hour 0) to $4.56\times10^6$/µL (hour 9). In contrast, storage at refrigerator temperature showed a slower decline, from $7.65\times10^6$/µL (hour 0) to $7.02\times10^6$/µL (hour 9). The General Linear Model (GLM) statistical test confirmed the significant effect of temperature and storage time on the number of erythrocytes (sig = 0.000 < 0.05). In conclusion, storage at refrigerator temperature is more effective in maintaining the number of erythrocytes than at room temperature, indicating the importance of temperature management to ensure optimal WRC quality.

***Keywords**: Storage Temperature, Storage Time, Erythrocytes, Washed Red Cell.*

## 1. Introduction

Blood services are a vital component of the healthcare sector, aimed at providing assistance to individuals in need of blood during emergencies and medical procedures such as accidents, heart surgery, abdominal surgery, Caesarean sections, as well as for patients with conditions like leukemia, hemophilia, and thalassemia. These services are primarily facilitated through blood transfusions (1). In Indonesia, blood services are managed by the Blood Transfusion Unit (UTD), the Blood Donor Unit (UDD), and Hospital Blood Banks (BDRS). The blood service process involves several stages, from donor recruitment to blood distribution, which includes donor selection, blood collection, screening for Transfusion-Transmitted Infections (IMLTD), and blood processing and storage.

One commonly used blood product for transfusions is Washed Red Cells (WRC), which are prepared by washing Packed Red Cells with saline (0.9% NaCl) 2-3 times. However, WRC products have notable limitations, including a short shelf life and an increased risk of secondary infection during storage (2,3). WRC can be prepared either manually or using specialized machines. Storing Packed Red Cells at temperatures between 1°C and 6°C helps reduce lysis and slows down blood metabolism, thus extending the survival of red blood cells (4). Nonetheless, challenges persist in WRC storage, particularly the loss of diphosphoglycerate (DPG), a crucial molecule for red blood cell function.

Previous studies, such as those conducted by Andriyani et al. (5), have shown a 5.7% decrease in the number of erythrocytes in Whole Blood over a 30-day storage period, suggesting that storage duration negatively impacts erythrocyte count. Tumpuk et al. (4) also reported that storage temperature significantly influenced erythrocyte levels in transfused blood, with the most favorable results observed at a storage temperature of 4°C. Ningrum and Khairinisa (6) found that the number of erythrocytes in 12 WRC components averaged $7.1\times10^{6}$/µL, a result that was corroborated by Ferdi Afriasnyah et al. (7), who observed differences in erythrocyte counts depending on storage temperature.

Andriyani et al. (5) demonstrated that the erythrocyte count in Whole Blood decreased by 5.7% during a 30-day storage period, emphasizing the importance of storage duration on erythrocyte viability. Similarly, Tumpuk et al. (4) highlighted the impact of storage temperature on erythrocyte counts, with 4°C yielding the highest erythrocyte count (5.08 million/mm³), followed by 6°C (5.07 million/mm³), and 2°C (4.67 million/mm³). Ningrum and Khairinisa (6) reported that the erythrocyte count in 12 WRC components was $7.1\times10^{6}$/µL, a finding that aligns with the results of Afriasnyah et al. (7), who observed that erythrocyte counts at room temperature (20°C - 25°C) were 4.57 million/µL, compared to 4.50 million/µL at refrigerator temperatures (4°C - 8°C). These findings suggest that erythrocytes can be stored effectively at both room and refrigerator temperatures.

Although numerous studies have explored the effects of storage and temperature on donor blood, there remains a gap in understanding the specific impact of temperature and storage duration on Washed Red Cells (WRC). Further investigation into these factors is essential to improving the quality and safety of WRC products. Therefore, this study aims to determine the average erythrocyte count in WRC stored at room and refrigerator temperatures for 0, 3, 6, and 9 hours. Additionally, the study seeks

to assess the effect of temperature and storage duration on erythrocyte viability, with the goal of providing evidence-based recommendations for the optimal storage and clinical use of WRC.

## 2. Research Method

This study employed a quasi-experimental design with a time-series approach to assess the variation in storage time of Washed Red Cells (WRC). The research was conducted at the Hematology Laboratory of the Medical Laboratory Technology program at Bandung Health Polytechnic from April to May 2024. The experimental unit consisted of one bag of WRC, sourced from the Blood Transfusion Unit (UTD) of the PMI (Indonesian Red Cross) in the City of Bandung. Due to the limited availability of blood at the PMI, no specific selection criteria were applied to the blood samples used in this study. The erythrocyte count was measured four times for each sample to ensure accuracy and reliability of the data.

Primary data were collected by measuring the number of erythrocytes in WRC stored at varying time intervals (0, 3, 6, and 9 hours) under two temperature conditions: room temperature (20-25°C) and refrigerator temperature (2-6°C). A Haematology Analyzer was employed for the erythrocyte count. Due to limited blood supplies, the study utilized Packed Red Cells of blood type O, which is universally compatible and readily available at the Blood Transfusion Unit of PMI City of Bandung.

The process for preparing WRC involved washing Packed Red Cells 2-3 times with saline (0.9% NaCl) to remove preservatives, resulting in the Washed Red Cells used in this study. This procedure had to be completed within 24 hours due to the use of an open system. Erythrocyte counts were measured using the Medonic M-32 Series Haematology Analyzer, which utilizes the impedance method for blood cell counting.

The collected data were analyzed statistically using the General Linear Model (GLM) for normally distributed data, and the Friedman and Wilcoxon tests for non-normally distributed data, in order to assess the effect of temperature and storage duration on the erythrocyte count in the WRC samples.

## 3. Result and Discussion

The results of this study, which examine the effect of temperature and storage duration on the erythrocyte count in Washed Red Cells (WRC), are presented in Table 1 below:

Table 1. Results of the Examination of the Number of Erythrocytes from Washed Red Cells

| Repitition | Erythrocyte Count (x10⁶sel /µL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Room Temperature (20°C - 25°C) | | | | Refrigerator Temperature (2°C - 6°C) | | | |
| | 0 | 3 | 6 | 9 | 0 | 3 | 6 | 9 |
| | (Jam) | | | | (Jam) | | | |
| **1** | 7,52 | 6,34 | 5,37 | 4,55 | 7,68 | 7,46 | 7,25 | 7,02 |
| **2** | 7,53 | 6,36 | 5,39 | 4,57 | 7,69 | 7,47 | 7,27 | 7,03 |
| **3** | 7,58 | 6,40 | 5,44 | 4,58 | 7,63 | 7,41 | 7,29 | 7,02 |
| **4** | 7,55 | 6,38 | 5,41 | 4,56 | 7,60 | 7,38 | 7,19 | 7,01 |
| **Average** | 7,54 | 6,37 | 5,40 | 4,56 | 7,65 | 7,43 | 7,25 | 7,02 |

Source: Research's Data (2024)

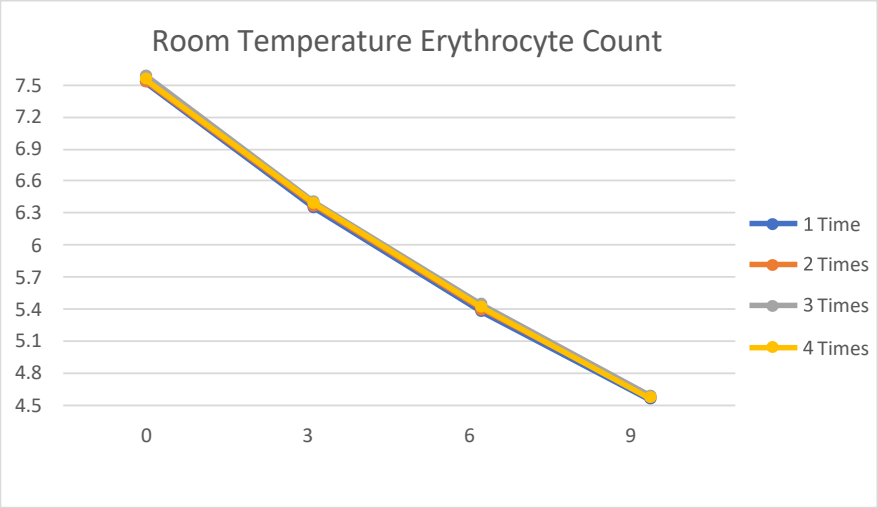


Figure 1. Number of Erythrocytes at Room Temperature

Based on Figure 1, the graph illustrating the erythrocyte count in Washed Red Cells (WRC) stored at room temperature indicates a decrease in the number of erythrocytes as storage time increases. This trend suggests that the storage duration of WRC at room temperature significantly impacts the erythrocyte count.

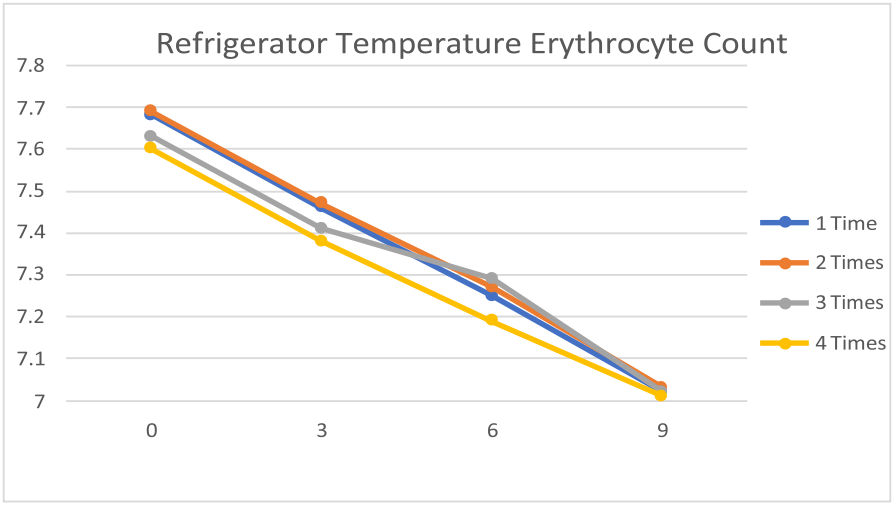


Figure 2. Refrigerator Temperature Erythrocyte Count

Based on Figure 2, the graph depicting the erythrocyte count in Washed Red Cells (WRC) stored at refrigerator temperature demonstrates a decline in erythrocyte levels as storage time increases. This trend highlights the effect of storage time on the erythrocyte count in WRC stored at refrigerator temperature.

The data from the examination of erythrocyte counts in the WRC component were analyzed descriptively. Additionally, statistical tests, including normality tests and General Linear Models (GLM), were conducted to evaluate the effect of storage duration on the erythrocyte count in WRC. Descriptive analysis was performed on the erythrocyte counts of WRC samples stored at room temperature for 0, 3, 6, and 9 hours. The results of this descriptive analysis are presented below.

Table 2. Test Results Description Average Number of Washed Red Cell Erythrocytes at Room Temperature

| Descriptive Statistics | | | | | |
|---|---|---|---|---|---|
| | **N** | **Minimum** | **Maximum** | **Mean** | **Std. Deviation** |
| | | **Maximum (x10⁶sel / µL)** | | | |
| **0 Hour** | 4 | 7,52 | 7,58 | 7,54 | .02464 |
| **3 Hours** | 4 | 6,34 | 6,40 | 6,37 | .02582 |
| **6 Hours** | 4 | 5,37 | 5,44 | 5,40 | .02986 |
| **9 Hours** | 4 | 4,55 | 4,58 | 4,56 | .01291 |

Based on the results of the descriptive analysis presented in Table 2, the average erythrocyte count in Washed Red Cell (WRC) blood components stored at room

temperature was determined. The average erythrocyte count immediately after preparation (0 hours) was 7.54 × $10^6$/µL. After 3 hours of storage, the count decreased to 6.37 × $10^6$/µL, followed by 5.40 × $10^6$/µL at 6 hours, and 4.56 × $10^6$/µL at 9 hours. This decline in erythrocyte count aligns with the oxidative stress process during storage, wherein free radicals induce damage to red blood cell membranes, reduce cell viability, and accelerate hemolysis (8).

Previous studies corroborate these findings. Mumpuni et al. (9) demonstrated that oxidative stress caused by reactive oxygen species (ROS) damages the red blood cell membrane, hastening cell degradation during storage. Similarly, Sari (10) reported that stored red blood cells accumulate degradation products such as methemoglobin and bilirubin, which exacerbate oxidative damage and contribute to a reduction in the number of intact cells. These studies support the results of this research, showing that oxidative stress is a significant factor in the progressive decline of erythrocyte counts in WRC over time.

Additionally, the research data obtained from erythrocyte count examinations were analyzed descriptively to evaluate the average erythrocyte count in WRC stored at refrigerator temperature (2-6°C) for 0, 3, 6, and 9 hours. The results of the descriptive analysis for these samples are presented in Table 3 below.

Table 3. Descriptive Test Results of the Average Number of Washed Red Cell Erythrocytes at Refrigerator Temperature

| | Descriptive Statistics | | | | |
|---|---|---|---|---|---|
| | **N** | **Minimum** | **Maximum** | **Mean** | **Std. Deviation** |
| | | | **(x10⁶ sel / µL)** | | |
| **0 Hour** | 4 | 7,60 | 7,69 | 7,65 | .04243 |
| **3 Hours** | 4 | 7,38 | 7,47 | 7,43 | .04243 |
| **6 Hours** | 4 | 7,19 | 7,29 | 7,25 | .04320 |
| **9 Hours** | 4 | 7,01 | 7,03 | 7,02 | .00816 |

Based on the results of the descriptive analysis in Table 3, the average erythrocyte count in Washed Red Cell (WRC) blood components stored at refrigerator temperature (2-6°C) exhibited a gradual decline. The average erythrocyte count immediately after preparation (0 hours) was 7.65 × $10^6$/µL. After 3 hours of storage, the count decreased to 7.43 × $10^6$/µL, followed by 7.25 × $10^6$/µL at 6 hours, and 7.02 × $10^6$/µL after 9 hours. These results indicate a decrease in the number of erythrocytes as the storage duration of the Washed Red Cell (WRC) blood component increases.

Following the descriptive analysis, a normality test was conducted to assess

whether the data followed a normal distribution. If the data were found to be normally distributed, the General Linear Model (GLM) would be applied for statistical analysis. However, if the data were not normally distributed, the Friedman or Wilcoxon tests would be used.

The results of the normality tests for both room temperature and refrigerator temperature storage conditions are presented in Table 4 below:

Table 4. Normality Test of the Effect of Temperature and Storage Time of Washed Red Cells on the Number of Erythrocytes

| Test Of Normality | | | | |
|---|---|---|---|---|
| **Variation Data Group** | **Historical Data Set** | **Shapiro-Wilk** | **Sig Value Result** | **Conclusion** |
| | | **Dif** | **Sig** | |
| **0 Hour** | Room Temperature | 4 | 0.689 | Normal Distribution |
| | Refrigerator Temperature | 4 | 0.492 | Normal Distribution |
| **3 Hours** | Room Temperature | 4 | 0.972 | Normal Distribution |
| | Refrigerator Temperature | 4 | 0.492 | Normal Distribution |
| **6 Hours** | Room Temperature | 4 | 0.952 | Normal Distribution |
| | Refrigerator Temperature | 4 | 0.577 | Normal Distribution |
| **9 Hours** | Room Temperature | 4 | 0.972 | Normal Distribution |
| | Refrigerator Temperature | 4 | 0.945 | Normal Distribution |

The results of the normality test for the eight groups showed significance values of 0.689, 0.492, 0.972, 0.492, 0.952, 0.577, 0.972, and 0.945, respectively. Since all significance values are greater than 0.05, the data are normally distributed. Therefore, the General Linear Model (GLM) test was applied.

Once the normality of the data was confirmed, the GLM test was conducted to assess the effect of the storage temperature of Washed Red Cells on the erythrocyte count. The results of the GLM test for the Washed Red Cell samples are presented in Table 5 below, which shows the effect of storage temperature on the number of erythrocytes.

Table 5. General Linear Model Test of the Effect of Washed Red Cell Storage Temperature on the Number of Erythrocytes

| | | **Sig** | **Result** | **Conclusion** |
|---|---|---|---|---|
| **Room Temperature** | 3 Hours<br>6 Hours | .000 | < α (0.05) | There are Changes |



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| | | | | |
|---|---|---|---|---|
| | 9 Hours | | | |
| **Refrigerator Temperature** | 3 Hours<br>6 Hours<br>9 Hours | .000 | < α (0.05) | There are Changes |

The test results indicate that the significance values (sig) for the Washed Red Cell samples stored at 3, 6, and 9 hours are all less than 0.000, which is smaller than the significance level α (0.05). This suggests that the storage temperature significantly affects the number of erythrocytes in Washed Red Cells.

Following the confirmation of normality through the normality test, the General Linear Model (GLM) test was conducted to assess the effect of storage time on the erythrocyte count. The results of the GLM test for the Washed Red Cell samples are presented in Table 4.6 below.

Table 4. 6. General Linear Model Test of the Effect of Washed Red Cell Storage Time on the Number of Erythrocytes

| | | Sig | Result | Conclusion |
|---|---|---|---|---|
| **Storage Time** | 3 Hours<br>6 Hours<br>9 Hours | .000 | < α (0.05) | There are Changes |

The test results showed that the significance value of the Washed Red Cell (WRC) sample at 3, 6, and 9 hours of storage was **sig < 0.000**, which is less than the significance level α (0.05). This indicates a significant effect of storage duration on the erythrocyte count in Washed Red Cells. This decrease in erythrocyte count is consistent with previous findings (11), which reported that the storage of red blood cells leads to damage to the cell membrane and a reduction in cell viability over time. Furthermore, other studies have also shown that a decrease in red blood cell count occurs due to the accumulation of degradation products and oxidative damage during storage (12).

Packed Red Cells (PRC) have a shelf life of 35 days, but once washed into Washed Red Cells (WRC), the shelf life is reduced to a maximum of 4-6 hours because the preservative has been removed (Permenkes, 2015). The washing process, which uses an open system and saline solution (0.9% NaCl) 2-3 times, removes the residual plasma (3).

The results indicated that the average erythrocyte count in WRC stored at room temperature was $7.54\times10^6/\mu L$ immediately after washing, $6.37\times10^6/\mu L$ after 3 hours, $5.40\times10^6/\mu L$ after 6 hours, and $4.56\times10^6/\mu L$ after 9 hours. At refrigerator temperature, the erythrocyte counts were $7.65\times10^6/\mu L$

immediately after washing, $7.43\times10^6$/µL after 3 hours, $7.25\times10^6$/µL after 6 hours, and $7.02\times10^6$/µL after 9 hours. Based on the General Linear Model (GLM) test, there is a significant effect of storage time on erythrocyte count (sig < 0.05), which is attributed to hemolysis that reduces the quality and morphology of red blood cells, including decreased ATP levels, leading to changes in the erythrocyte membrane (5).

Moreover, storage at room temperature accelerates cellular degradation, impairs blood oxygenation, and exacerbates the decline in cellular functions, such as iron deficiency (13,14). Andriyani et al. (5) found a 5.7% decrease in erythrocyte count after 30 days of storage, further supporting the effect of storage time on erythrocyte count.

Storage lesions encompass all changes in red blood cells during storage caused by oxidative stress and the accumulation of metabolite residues (3,15,16). Oxidative stress plays a crucial role in the formation of lesions in red blood cells during storage. Free radicals generated during storage can damage the cell membrane, leading to lipid oxidation and hemolysis, thereby reducing the integrity of the red blood cells. Anggraini et al. (17) demonstrated that oxidative stress increases lipid peroxide accumulation, which damages cell membranes and reduces cell viability. The accumulation of residual metabolites such as methemoglobin and bilirubin also contributes to lesion formation, further degrading the quality of stored red blood cells (18).

Citrate Phosphate Dextrose (CPD), as a preservative, helps maintain erythrocyte viability during storage. However, a decrease in Adenosine Triphosphate (ATP) leads to morphological changes and increases the risk of red blood cell lysis (14,19). ATP is crucial for maintaining the integrity of the red blood cell membrane, and its depletion results in structural damage to the cells. Puspitaningrum et al. (20) reported that ATP levels decreased by 50% during long-term storage, leading to reduced cell viability. Furthermore, Kusbianti (21) noted that decreased ATP exacerbates oxidative stress, increases membrane permeability, and accelerates red blood cell lysis. These findings support the results of this study, where a decrease in ATP contributed to the reduction in erythrocyte count in stored Washed Red Cells.

## 4. Conclusion

Based on the results of this study, it can be concluded that both temperature and storage time significantly affect the number of erythrocytes in Washed Red Cells (WRC). At room temperature, the average erythrocyte count immediately after washing was $7.54\times10^6$/µL, decreasing to $6.37\times10^6$/µL after 3 hours, $5.40\times10^6$/µL after 6 hours, and $4.56\times10^6$/µL after 9 hours.



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At refrigerator temperature, the average erythrocyte count immediately after washing was $7.65\times10^6/\mu L$, decreasing slightly to $7.43\times10^6/\mu L$ after 3 hours, $7.25\times10^6/\mu L$ after 6 hours, and $7.02\times10^6/\mu L$ after 9 hours. The General Linear Model (GLM) test yielded a significance value (sig) < 0.05, indicating that both storage temperature and duration significantly influence the number of erythrocytes.

These findings are consistent with previous research by Aliviameita and Puspitasari (12), which noted that the storage of red blood cells leads to membrane damage and a reduction in cell viability over time. Additionally, Andriyani et al. (5) found that the decrease in erythrocyte count is associated with hemolysis and the reduction of ATP levels, which cause structural changes and damage to the erythrocyte membrane.

The results further support the conclusion that storage at room temperature accelerates cellular degradation, adversely affecting red blood cell function. This degradation potentially reduces the quality of stored blood and diminishes its oxygen-carrying capacity (13,14).

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