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EFFECT OF DONOR BLOOD STORAGE PERIOD ON HEMOGLOBIN LEVELS AND ERYTHROCYTE CELL MORPHOLOGY

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ABSTRACT

Blood is a body fluid whose stability can be influenced by both internal and external factors such as storage period. Storing blood specimens for a long time can cause changes in the physiological condition of blood cells such as color, size, shape and cell formation. This study aims to determine the effect of the storage period for donor blood specimens at UTD RSUP dr. Kariadi Semarang on hemoglobin levels and erythrocyte morphology. The research design is an experiment using a modified Time Series design with a pre and posttest control group design. The samples in this study were CPDA-1 (Citrate Phosphate Dextrose Adenine) donor blood specimens at the Blood Transfusion Unit of RSUP dr. Kariadi Semarang. Observation of erythrocyte cell morphology using blood smear preparations (SHD). Primary data was taken from the results of measurements of hemoglobin levels and observations of erythrocyte cell morphology with blood specimens examined directly (day 0) and storage days 1, 2, and 3. Analysis of research data used the One-way Anova statistical test. The results of the One-Way Anova test showed that there was a significant difference in hemoglobin levels on the 3rd day of donor blood storage (p-value 0.000). The results of the evaluation of erythrocyte cell morphology showed that there were abnormalities in crenation morphology on the 3rd day of storage. Blood specimens should be processed immediately after they leave the body because the condition of the blood cells is still stable and optimal for use in laboratory tests.

Kata Kunci:

Darah donor;
hemoglobin;
masa penyimpanan;
morfologi eritrosit

ABSTRAK

Darah merupakan cairan tubuh yang stabilitasnya dapat dipengaruhi oleh faktor baik internal maupun eksternal seperti masa penyimpanan. Masa penyimpanan spesimen darah dalam waktu yang lama dapat menyebabkan perubahan pada kondisi fisiologis sel darah seperti warna, ukuran, dan bentuk serta formasi sel. Penelitian ini bertujuan untuk mengetahui pengaruh masa penyimpanan spesimen darah donor di UTD RSUP dr. Kariadi Semarang terhadap kadar hemoglobin dan morfologi eritrosit. Desain penelitian adalah eksperimen menggunakan rancangan modifikasi Time Series dengan pre dan posttest control group design. Sampel dalam penelitian ini yaitu spesimen darah donor CPDA-1 (Citrate Phosphate Dextrose Adenine) di Unit Transfusi Darah RSUP dr. Kariadi Semarang. Pengamatan morfologi sel eritrosit menggunakan Sediaan Hapusan Darah (SHD). Data primer diambil dari hasil pengukuran kadar hemoglobin dan pengamatan morfologi sel eritrosit dengan spesimen darah yang diperiksa langsung (hari ke-0) dan penyimpanan hari ke-1, 2, dan 3. Analisis data penelitian menggunakan uji statistic One-way Anova. Hasil uji One Way Anova menunjukkan ada perbedaan yang signifikan kadar hemoglobin pada penyimpanan darah donor hari ke-3 (p-value 0.000). Hasil evaluasi morfologi sel eritrosit menunjukkan adanya kelainan morfologi krenasi pada penyimpanan hari ke-3. Spesimen darah sebaiknya diproses segera setelah keluar dari dalam tubuh karena kondisi sel darah masih stabil dan optimal untuk digunakan dalam pemeriksaan laboratorium.

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INTRODUCTION

The need for blood has continued to increase in recent years. An increase in the need for blood is a result of handling accidents, operations or childbirth. Blood supply is needed especially for conditions such as blood cancer, burns and anemia. Developing countries such as Indonesia, the death

rate due to bleeding due to complications of pregnancy and childbirth reaches 25% of all causes of maternal death (Nurhidajat, 2018). Need and availability of blood in unit transfusion It is known that blood in Indonesia is still not sufficient to meet needs in line with the increasing frequency of medical procedures in health service facilities. According to the World Health Organization The availability of blood for a country is around 2% of its population, but it is known that it only provides less than 0.5% of this need which is managed and stored by the Blood Bank (Oktarianita et al., 2018).

A safe blood supply is needed to support patient therapy through blood transfusions so that the benefits obtained are more effective because blood is a biological material that can be influenced by external factors such as storage time and the environment (Amalia & Sari, 2019). The storage period can cause changes in blood components, especially erythrocytes. The storage period for red blood cells that is too long causes abnormalities in the morphological condition of the cells. Changes that can occur in the morphology of red blood cells such as shape, color and size with the length of time the blood is stored. A long storage period can disrupt erythrocyte deformability and will continue throughout the storage period (Astina, 2020).

The storage period for blood causes the erythrocyte cells in the blood to become damaged and even die so that if the blood is transfused various kinds of transfusion reactions will occur, besides that storage for a long period of time will cause interactions between hemoglobin molecules and 2.3 Diphosphoglycerate (2,3-DPG) which causes the release of O_2 characterized by a shift of the oxygen dissociation curve to the left. Damage to erythrocyte cells during storage causes reduced oxygen delivered to the tissue because erythrocyte cells become lysed and possibly increases hemoglobin levels (Rosyidah et al., 2020).

Previous research conducted by Rosyidah et al., 2020 showed that there was no effect on the storage time of blood bags for 1 to 15 days on hemoglobin levels. Another study by Isti el al., 2018 showed that there was no effect of donor blood storage time on erythrocyte morphology. Action pBlood storage must comply with correct operational standards so that the quality and quality of blood is maintained as determined by the Ministry of Health, namely stored in a refrigerator at a temperature of 4-8 °C and controls are carried out every day to ensure the temperature remains stable. Blood storage system on the unit transfusion blood uses a mechanism first in first out (FIFO) which regulates the entry and exit of blood, that is, the first blood that enters is the first that is released (Oktarianita et al., 2018).

Factors supporting the success of patient treatment are passing transfusion blood, namely the availability of a safe blood supply for patients. A good and correct storage method is an appropriate standard that can give optimal benefits in treatment through transfusion blood (Audhah et al., 2021). Blood is a body fluid that is very sensitive to time and environmental conditions. Previous research shows that there is a significant influence on the storage time of donor blood on hemoglobin levels and erythrocyte cell morphology. The aim of this study was to determine the effect of long storage time for donor blood specimens with storage times of 1, 2 and 3 days on hemoglobin levels and erythrocyte morphology.

METHOD

This research is an observational analytic with time series design by comparing the hemoglobin levels in donor blood between the control group (0 hours storage time) with the treatment groups of one day (24 hour), two days (48 hours) and three days (72 hours) of storage time. The donor blood is stored at temperature around 4-6℃ and monitored every 8 hours based on variations in storage time. The research was carried out at the Hematology Laboratory of Muhammadiyah University Semarang in May – June 2022.

Research Sample

The sample used in this study was one bag fresh Packed Red Cell (PRC) with the anticoagulant CPDA-1 (Citrate Phosphate Dextrose Adenine) taken from the Blood Transfusion Unit of RSUP dr. Kariadi Semarang. The research samples were divided into 4 groups consisting of control (examined immediately), DD1 (1st day of storage), DD2 (2nd day of storage), and DD3 (3rd day of storage).

Donor Blood Storage Time

Donor blood specimens are stored inside the refrigerator at a temperature of 4-6º. Storage time specimen Donor blood is stored on day 1, day 2 and day 3, then the hemoglobin level is checked and the morphology of the erythrocyte cells is observed.

Examination of Hemoglobin (Hb) Levels

Donor blood specimens that have been stored are then measured for hemoglobin levels, namely on the 1st, 2nd and 3rd day of storage. The method for measuring hemoglobin levels is an automatic method using a Hematology Analyzer Mindray BC 2600. Measurement results in g/dl units.

Morphological Assessment of Erythrocyte Shape

Assessment of the shape of erythrocyte cells using the Preparation method Smear Thin Blood (SHDT). 10 µ blood specimens were taken from each storage treatment, then placed on a glass object, then removed with a glass pusher and dried. SHDT fixation using methanol solution until dry then painted with Giemsa 10%. Observation of the morphology of erythrocytes using a binocular microscope with an objective magnification of 100 times.

Processing and analysis of data

Data on examination of hemoglobin levels and erythrocyte morphology are presented in tables and graphs. Hemoglobin level data descriptive analysis to determine the mean, maximum and minimum hemoglobin levels, then statistical analysis using the One-way ANOVA test to determine the effect of storage time for donor blood specimens on hemoglobin levels and assessment of morphological abnormalities in the shape of erythrocyte cells.

RESEARCH RESULT

Table 1. Effect of donor blood storage time on hemoglobin levels

Storage Time	Hb Level (g/dl)					Average of Hb Levels (g/dl)	SD	Sig.
	1	2	3	4	5			
Control	25.4	25.6	25.5	25.3	25.3	25.42	0.073	0.000
Day 1	25.3	25.3	25.1	24.2	25.2	25.02	1.600	
Day 2	25.6	25.5	25.8	25.6	25.6	25.62	0.100	
Day 3	26.3	26.5	26.5	26.2	26.2	26.34	0.600	

Based on Table 1, the effect of donor blood storage time on hemoglobin levels on days 1 and 2 showed a no significant increase in hemoglobin levels compared to the control, while the day 3 storage time showed a significant increase in hemoglobin levels compared to the control with a standard deviation of 0.600 and p-value <0.05 (0.000). This showed that there was a difference in hemoglobin levels on blood donor storage time period.

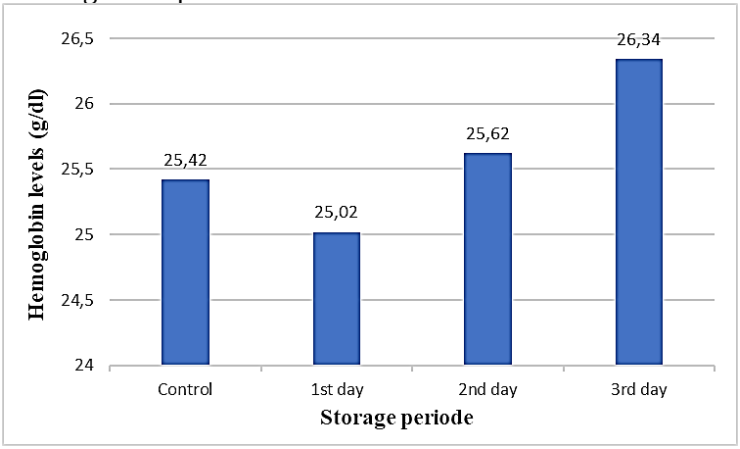

Figure 1. Graph of average hemoglobin levels based on blood storage time

Based on Figure 1, the highest average hemoglobin level occurred on the 3rd day of storage, namely 26.34 g/dl and the lowest on the 1st day of storage, namely 25.02 g/dl. Results showed an increase in hemoglobin levels that were stored for three days.

Table 2. Effect of blood storage time on erythrocyte morphology

Storage Time	Erythrocyte Morphology Examination Results		
	Color	Size	Form
Control	Normokrom	Normosister	No abnormalities were found
Day 1	Normocrom	Normosister	No abnormalities were found
Day 2	Normokrom	Normosister	No abnormalities were found
Day 3	Normokrom	Normosister	Crenation deformities

Based on Table 2, observations of erythrocyte morphology abnormalities regarding storage time show that no color and size abnormalities were found on the 0th, 1st, 2nd and 3rd days of storage, whereas on the 3rd day of storage, deformities were found, namely crenation cells (Figure 2(d)). The results of observations of erythrocyte morphology consisting of color, size and shape in blood stored for three days showed that there were no significant changes in either color or cell size, however, on the third day of storage, abnormalities were found in the morphology of the shape, namely crenation cells.

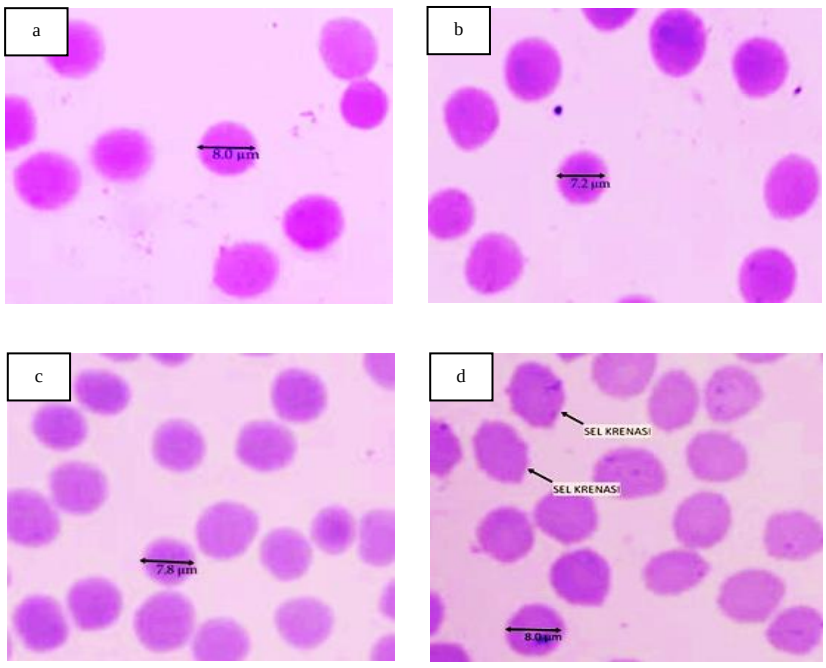

Figure 2. Erythrocyte morphology on blood specimen storage time

Information:

a) Storage day 0: Normochrome color, size 8.0 µm, no deformities on erythrocyte cells in the control group.
b) Storage day 1: Normochrome color, size 7.2 µm, no deformities in erythrocytes in the storage group day 1.
c) 2nd day of storage: Normochrome color, size 7.8 µm, no deformities in erythrocytes in the 2nd day group.
d) 3rd day of storage: Normochrome color, size 8.0 µm, had crenation deformities in erythrocyte cells in the 3rd day of storage group.

DISCUSSION

Hemoglobin is a protein in red blood cells which plays a role in transporting oxygen from the lungs to the tissues and transporting carbon dioxide contained in the body's tissues to the lungs (Nurjanah et al., 2023). Cell components found in blood include red blood cells (erythrocytes), white blood cells (leukocytes), and platelets. Blood cells can experience changes both macroscopically and microscopically, including shape, size, color and formation (Yuliarti, 2021). The results of this study are in line with research conducted by Sugireng et al, 2021 which shows the influence of storage time of one day, one week, two weeks, three weeks and four weeks on hemoglobin levels. Another study conducted by Saidjao et al, 2019 showed the influence of blood bag storage time Packed Red Cell (PRC) day one and day fifteen on hemoglobin levels. The long storage time for blood will cause a decrease in ATP (Adenosine Tri Phosphate) levels in erythrocyte cells in the blood so that cell metabolism is not optimal and becomes damaged (Situmorang et al., 2023). Packed Red Cell blood bags experienced a significant increase in hemoglobin levels during storage days 0, 1, 2 and 3. Blood that is stored using a blood bag, the condition and stability will be different from blood that is still in the body. This condition can increase the hemoglobin levels in the blood due to lysis of erythrocyte cells. The effect of blood storage will cause many erythrocytes to die immediately due to a decrease in ATP levels. Blood samples should be checked immediately so that stability is maintained. Blood specimens with delayed examination should be stored according to operational standards (Rosyida et al., 2020). The maximum temperature for storing blood is 6º C, if the temperature is above 6º C will occurs bacterial growth and if the temperature is below 2º C it can damage cell membranes due to blood clotting which causes hemolysis and this condition can increase the hemoglobin levels the blood specimens (Situmorang et al., 2023).

Blood storage must be carried out according to standards because blood is a biological material that can be influenced by many factors such as the environment and temperature. The temperature used for storage ranges from 2-6ºC (Pramudita et al., 2024). Storing blood at temperatures above 6ºC can trigger the growth of bad microorganisms in the blood, while storing blood at temperatures below 2ºC can result in damage to the blood cell membrane due to blood clotting, causing the red blood cells to burst (hemolysis). The condition of hemolyzed blood specimens can affect hemoglobin levels in the blood (Fajriyani et al., 2019). The storage period can also affect the morphology of blood cells, especially erythrocytes. Biomechanical changes that can occur in erythrocyte cells in blood during the storage period are changes in shape, size, color, deformability and osmotic fragility, as well as the ability to aggregate (Afriansyah et al., 2021). Storage for a certain period of time can trigger changes in blood components, especially erythrocyte cells. The storage period for blood can affect the physiological condition of erythrocyte cells, which is characterized by significant changes in shape and becomes uncontrollable with the length of time the blood is stored (Fauziyah et al., 2019).

The erythrocyte cells in fresh blood are still stable because they have just left the body under standard conditions so there have been no physiological changes, however storing blood for days can trigger morphological changes such as crenation cells, namely erythrocyte cells that shrink with bulges on the cell surface due to loss of intracorpuscular fluid (Asiffa et al., 2020). Erythrocyte cells that become created as a result of cell shrinkage with protrusions on the cell surface. Research conducted by Isti et al, 2018 showed that the storage time for PRC specimens caused changes in the morphology of erythrocytes to sphero-echinocytes. Erythrocyte cells in fresh blood or without storage have not experienced a change in shape, but shape changes occur after storage for 72 hours (Maulidan et al., 2022).

Changes in the shape of erythrocytes can be influenced by intrinsic factors such as reduction of adenosin triphosphat (ATP) or due to extrinsic factors such as increasing the pH of anticoagulants (Ridwan et al., 2021). The concentration of intracellular and extracellular fluids also influences erythrocyte cell morphology. Hypertonic concentrations cause fluid inside the cells to come out to maintain the cells' osmotic pressure. The fluid that comes out of the cells causes cell shrinkage due to hemodilution which causes an increase in the concentration of plasma fluid compared to the concentration of blood cells, resulting in the formation of crenation cells (Yuliarti, 2021). Apart from that, the appearance of crenation cells can also be caused by technical factors such as errors in the process of making blood smears (Astina, 2020).

Biomechanical changes during the storage period occur due to changes in blood cell components, especially erythrocytes, which will experience changes in shape along with the length of storage time (Ghenong, 2020). Erythrocyte cells stored in vitro require energy to maintain the shape and function of the cells. This energy is obtained from glucose metabolism, namely ATP and oxygen (Amalia & Sari, 2019). Metabolism that occurs continuously during the storage period causes a decrease in ATP levels which results in the loss of lipid components in the erythrocyte cell membrane which triggers a loss of cell elasticity so that the cells become stiff and a rapid change in morphology from biconcave to echinocytes with a bulge that eventually becomes sphero echinocyte and crenation (Wahdaniah et al., 2020). Another factor such as high concentrations of anticoagulants can cause hypertonicity of plasma fluid, which causes intracellular fluid to move out of cells due to extracellular osmotic pressure being higher than intracellular osmotic pressure so that erythrocyte cells experience shrinkage. This can trigger changes in cell morphology in an abnormal direction (Aridya et al., 2023).

CONCLUSIONS AND RECOMMENDATIONS

The donor blood storage time on days 1 and 2 did not have a significant effect on hemoglobin levels and erythrocyte cells morphology, while the day 3 storage time showed a significant effect on increasing hemoglobin levels and the shape of erythrocyte cells. Blood specimens should be processed immediately after leaving the body because the condition of the blood cells is still stable and optimal for use in laboratory tests.

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BIBLIOGRAPHY

Afriansyah, F., Bastian, B., Sari, I., & Juraijin, D. (2021). Pengaruh Lamanhya Penyimpanan dan Suhu Terhadap Jumlah Eritrosit. *Journal of Indonesian Medical Laboratory and Science (JoIMedLabS)*, *2*(2), 108–114. https://doi.org/10.53699/joimedlabs.v2i2.51

Amalia, N., & Sari, P. K. (2019). Description of the Effect of Storage Time for Blood Bags on the Number of Platelets in Blood Bags (PRC) with CPDA-1 Anticoagulant at BHHB Kandangan Regional Hospital. *Ergasterio Journal*, *06*(02), 1-6. Available at: https://jurnalstikesborneolestari.ac.id/index.php/analisborles/article/view/407/216

Asiffa, E., Ruliati, R., & Umaysaroh, U. (2020). Gambaran Kadar Hemoglobin Pada Mahasiswi (Studi di Program Studi D III Analis Kesehatan Sekolah Tinggi Ilmu Kesehatan Insan Cedekia Medika Jombang). Jurnal Insan Cendekia, 7(1, Maret), 6-10. Retrieved from https://digilib.itskesicme.ac.id/ojs/index.php/jic/article/view/549

Asriyani Ridwan, Nina Agustina, & Andi Suswani. (2021). Description of Erythrocytes in Peripheral Blood Smears of Alcohol Drinkers in Gattareng Village, Bulukumba Regency. *Jurnal TLM Blood Smear*, *2*(1), 1–5. https://doi.org/10.37362/jmlt.v2i1.432

Astina, F. (2020). Qualitative Analysis of Erythrocyte Morphology in EDTA (Ethylene Diamine Tetraacetic Acid) Blood Smears for Immediate Examination (0 Hours) and Postponed Examination (2 Hours). *Borneo Journal of Medical Laboratory Technology*, *5(1)*, 326–334. Doi: https://doi.org/10.33084/bjmlt.v5i1.4430

Audhah, N. A., Suhartono, E, Sardjono, T. W, & Fitri, L. E. (2021). Diration of Storage Reduced Erythrocytes Profiles and Plasmodium Viability in Donor Blood. *Journal of Blood Medicine*, 12, 87-99. Doi: http://doi.org/10.2147/JBM.S276069

Dwi Aridya, N., Yuniarti, E., Atifah, Y., & Alicia Farma, S. (2023). The Differences in Erythrocyte and Hemoglobin Levels of Biology Students and Sports Students at Padang State University. Differences in Erythrocyte and Hemoglobin Levels of Biology Students and Sports Students at Padang State University. *Porch Biology Journal*, *8*(1), 38–43. Available at: https://serambibiologi.ppj.unp.ac.id/index.php/srmb/article/view/167

Fajriyani, F., Eem, H., Nina, Marliana & Betty, N. (2019). The Role of Temperature and Storage Time of Liquid Fresh Frozen Plasma (Ffp) on Prothrombin Time (Pt) Values. *Bandung Department of Health Polytechnic Health Research Journal*, *11*(1), 244–252.

https://juriskes.com/index.php/jrk/article/download/790/145/1757

Fauziyah, Z., Hayati, E., Nurhayati, B., & Marliana, N. (2019). Stability of PRC in Artificial Alsever Solution on Erythrocyte Morphology and Osmotic Fragility. *Bandung Department of Health Polytechnic Health Research Journal*, *11*(1), 277–284. https://doi.org/10.34011/juriskesbdg.v11i1.779

Ghenong, K. N. (2020). *Description of Donor Blood Erythrocyte Morphology Based on Male and Female Gender in Packed Red Cell Blood with a Shelf Life of 30 Days*. [Skripsi, STIKes Guna Bangsa Yogyakarta]. Retrieved from: https://repository.gunabangsa.ac.id/index.php?p=show_detail&id=187&keywords=2020

Isti R., Rofinda D. Z., & Husni. (2018). Morphological Description of Packed Red Cell Erythrocytes Based on Storage Time in the Blood Bank of RSUP Dr. M. Djamil Padang. *Jurnal Kesehatan Andalas, 7(2)*, 17–20. Doi: http://dx.doi.org/10.25077/jka.v7i0.819

Maulidan, E. B., Tambunan, B. A., & Hajat, A. (2023). The Effect of Storage Time on the Whole Blood (WB) Quality at the Blood Bank of Dr. Soetomo General Hospital. *International Journal of Health Sciences*, 6(2), 396-405. Doi: https://doi.org/10.53730/ijhs.v6nS2.5023

Nurhidadajat. (2018). Effectiveness of Implementing the Acceleration of Reducing Maternal Mortality Rates in Indonesia. *Journal of Public Sector Budgeting*, *2*(1), 1–28. https://anggaran.e-journal.id/akurasi/article/view/32/17

Nurjanah, M., Tilarso, D., & Rahmawati, S. (2023). Gambaran Kadar Hemoglobin Pada Ibu Hamil Desa Sumberdadi Kabupaten Tulungagung. *Indonesian Journal of Biomedical Science and Health, 3*(1), 32–38. https://e-journal.ivet.ac.id/index.php/IJBSH/article/view/2596

Oktarianita, Angraini, W., Febriawati, H., & Auliani, A. (2018). Analysis of the Hospital Blood Bank Service System at Dr. M. Yunus Bengkulu 2018. *Avicenna: Jurnal Ilmiah*, *13*(3), 29–39. https://jurnal.umb.ac.id/index.php/avicena/article/view/178

Pramudita, J. J., Widjanarko, B., Munadi, Suwondo, A. (2024). Effect of Storage Period on Platelet Count and Erythrocyte Morphology in Whole Blood Bags. *Modern Medicine,* 31(1), 67-72. Doi: https://doi.org/10.31689/rmm.2024.31.1.67

Rosyidah, R. A., Mustapa, A. N., & Sumoko, E. (2020). The Differences in Hemoglobin Levels in Packed Red Cell Donor Blood Components Based on Storage Time. *Jurnal Ilmu Kesehatan Bhakti Setya Medika*, *5*(1), 34–41. https://www.researchgate.net/publication/371570834_Perbedaan_Kadar_Hemoglobin_Pada_Komponen_Darah_Donor_Packed_Red_Cell_PRC_Berdasarkan_Lama_Penyimpanan

Situmorang, P. R., Tampubolon, R., & Tarigan, R. V. B. (2023). Analysis of Erythrocyte Packed Red Cell (PRC) Morphology Based on Storage Time at UDD PMI Medan. *PRIME Saelmakers Health Journal*, *6*(2), 417–431. https://journal.ukmc.ac.id/index.php/joh/article/view/1009/949

Wahdaniah, W., Tumpuk, S., Setiawan, A., Suryani, E., W., Saleh, R., Dwiyana, A., Parno, Ratih, R. H., Purnama, T., Fety, Y., Atri, Perwitasari, E., Pada, K., Pharmacy, M., Force, U., Nuryati, A. & S., Nuraeni, M., Novasiska, Wilujeng, R. (2020). Gambaran Morfologi Eritrosit Pada Pekerja Bengkel Motor Yang Sering Terpapar LB3 (Limbah Bahan Bakar Beracun). *Mandala Waluya MediLab Journal*, *5*(1), 034–038.

Yuliarti, L. (2021). *Delay in painting peripheral blood smear after fixation of erythrocyte morphology*. [Skripsi, STIKes Nasional Surakarta]. Retrieved from: https://librepo.stikesnas.ac.id/841/2/KTI.pdf