Jurnal Analis Medika Biosains (JAMBS)
Vol.11, No.01, March 2024, pp. 60-65 http://jambs.poltekkes-mataram.ac.id/index.php/home
E-ISSN: 2656-2456 DOI: https://doi.org/10.32807/jambs.v11i1.347

Differences In Storage Time In Safranin Dye On Counting The Number Of Platbocytes By Rees Ecker Method

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Article Info

Article history

Received : March 18, 2024
Revised : April 05, 2024
Accepted : June 09, 2024

Keyword :

Platelet count
Storage time
Safranin stability

ABSTRACT

Platelet count examination is important to assess hemostasis function. One of the commonly used manual methods is the Rees Ecker method. However, during the examination, impurities were found that were difficult to distinguish from platelets. Therefore, this study aimed to determine the difference in platelet count results using the Rees Ecker method against storage time with safranin dye. This study used an experimental method on 10 normal blood samples. Platelets were counted with an Improved Neubeauer hemocytometer using safranin dye stored for 0 days, 1 day, 7 days, 14 days, and then compared with the platelet count results using Rees Ecker as a control solution. The Mann Whitney Test results showed no significant difference between the control group and the safranin group in terms of platelet count at 0 days (sig. 0.850), 1 day (sig. 0.820), 7 days (sig. 0.130), and 14 days (sig. 0.121) of storage. Safranin is safe and effective for counting platelets using the Rees Ecker method, as shown by the stability test results for 14 days.

INTRODUCTION

Platelet count examination is one type of examination that is very important and highly requested in clinical laboratories. This is because the examination has a crucial role in establishing diagnosis, determining therapy, and monitoring the treatment process of various diseases (Handini, 2022). One of the methods used in this examination is the manual method with the Rees Ecker method. The principle is to dilute the blood using Brilliant Cresyl Blue (BCB) which can stain platelets so that they appear more clearly. Although able to stain platelets well, this method has weaknesses such as being unable to lyse red blood cells, so clumped red blood cells can cover platelets and interfere with observation. In addition, the diluent used is also more expensive compared to other diluents (Garini et al., 2019).

Previous research conducted by Ballihgoo (2023) found that safranin has the potential to be used as an alternative to BCB in the Rees Ecker solution for platelet count examination. Safranin is able to stain platelet cells so that they can be distinguished from impurities or other cells. This is because safranin has similar characteristics to BCB, which is a basic dye that can stain the acidic nucleus of granules. However, it was also found that after storage for several days, there was a change in color stability in the safranin reagent.

Safranin has the ability to stain platelets because it has the same characteristics as BCB. The dye is basic in nature and functions to stain the acidic nucleus and granules

How to cite:
Salsabila, A., & Nugraha, G. (2024). Differences In Storage Time In Safranin Dye On Counting The Number Of Platbocytes By Rees Ecker Method. *Jurnal Analis Medika Biosains (JAMBS), 11*(1). doi:https://doi.org/10.32807/jambs.v11i1.347

(Rahmadhanty et al.,2018). According to Abukhadra et al., (2021), safranin is a basic and cationic synthetic dye with a positively charged molecule, and has similarities with Malachite Green. In a study conducted by Oktari et al., (2018), it was mentioned that staining using Malachite Green remained stable for 2 weeks of storage. This study aims to prove the difference in storage time on safranin dye for platelet counting, as well as to determine the stability of safranin dye as an alternative reagent in platelet count examination.

MATERIALS/METHOD

This research uses an experimental research type with a Static Group Comparison research design. To obtain the research sample, the method used in this study is Simple Random Sampling, which is a random sampling technique that provides an equal opportunity for every member of the population to become a research sample. The research data were analyzed using SPSS with the non-parametric Mann-Whitney Test to determine the difference between the control group and the treatment group.

RESULTS AND DISCUSSION

The platelet count was performed using a microscope with 400x magnification. The platelet count examination was carried out using two groups of color reagents, namely using the Rees Ecker reagent as a control and using the safranin reagent that had been stored for 0 days, 1 day, 7 days, and 14 days. Based on the observations under the microscope, the appearance of platelet cells using the Rees Ecker solution from 0 days to 14 days was round, and the platelet cells were stained blue. The microscopic result images are shown in Figure A (0 day storage), Figure B (1 day storage), Figure C (7 day storage), and Figure D (14 day storage), in the following images:

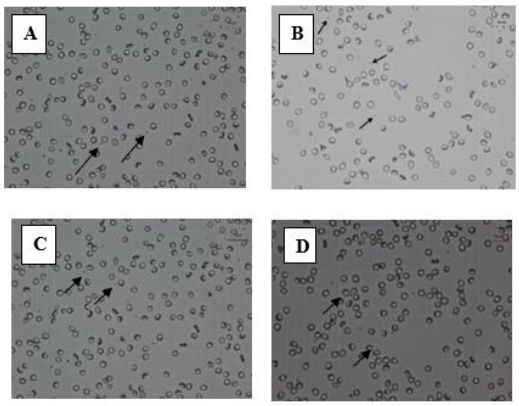

Figure 1. Microscopic observation results using Rees-Ecker

For the safranin dye, the research results showed that after 0 days of storage, the platelet cell morphology appeared round with slightly oblong shape and red color with a red background color as well. This can interfere with the visibility of the field of view due to the dominant red color, as shown in the following image:

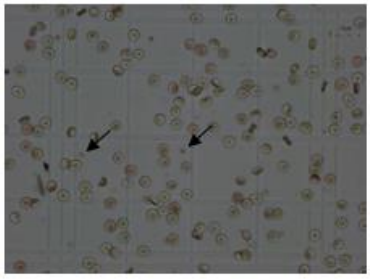

Figure 2. Microscopic observation results using Safranin stain, 0-day storage

For safranin staining with 1 day of storage, the platelet cell morphology appeared round with a slightly oblong shape and red color, with a background color that was a relatively softer shade of red. This allowed the researcher to perform cell counting more easily, as shown in the following image:

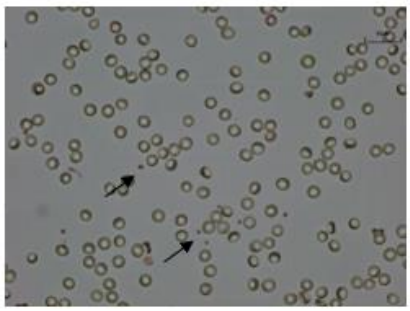

Figure 3. Microscopic observation results using Safranin stain, 1-day storage

Platelet cells stained with 7 days of safranin storage showed a round, slightly oblong morphology and red color, with the background no longer being red, making it easier for the researcher to count the number of platelet cells, as shown in the following image:

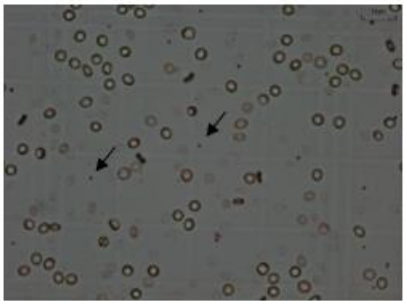

Figure 4. Microscopic observation results using Safranin stain, 7-day storage

For the 14-day safranin dye storage, the platelet cell morphology appeared round with a slightly oblong shape and red color, with the background no longer having a dominant red color. However, there were some impurities such as safranin crystal fragments visible in the field of view area, making it slightly difficult for the researcher to perform the platelet count, as shown in the following image:

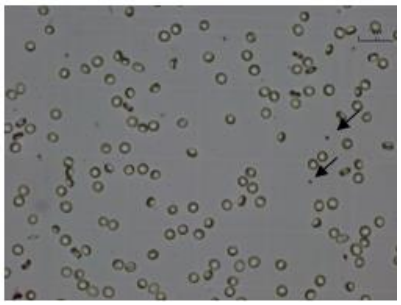

Figure 5. Microscopic observation results using Safranin stain, 14-day storage

The results of the platelet count research conducted in the UNUSA hematology laboratory can be seen in the following table:

Table 1. Platelet count examination results using Rees Ecker reagent and Safranin with 0 days, 1 day, 7 days, and 14 days of storage.

Day	Dye Reagent	N	Minimum (sel/mm³)	Maksimum (sel/mm³)	Mean (sel/mm³)	Std. Deviation (sel/mm³)
0 Days	Control	10	272.000	430.000	343.000	62.772
	Safranin	10	286.000	505.000	359.600	79.791
1 Day	Control	10	213.000	480.000	356.200	99.325
	Safranin	10	188.000	448.000	343.500	103.593
7 Days	Control	10	242.00	485.000	301.500	68.634
	Safranin	10	181.000	474.000	274.100	88.670
14 Day	Control	10	225.000	373.000	286.500	46.073
	Safranin	10	181.000	373.000	257.200	57.632

Based on Table 1, the platelet count results using freshly stored safranin dye (day 0 and day 1) were close to the control value using the Rees Ecker solution. However, after a longer storage period, namely on day 7 and day 14, there was a decrease in platelet count values compared to the control. The average data in the table showed a decrease in platelet count values if the safranin dye was stored for too long.

Table 2. Results of the Mann-Whitney Test statistical test

Group	Variable	*P-Value*	Information
0 Days	Rees Ecker	0,850	There is no difference
	Safranin		
1 Day	Rees Ecker	0,820	There is no difference
	Safranin		
7 Days	Rees Ecker	0,130	There is no difference
	Safranin		
14 Days	Rees Ecker	0,121	There is no difference
	Safranin		

Based on the research results that have been carried out using the Rees Ecker reagent and safranin dye reagent with storage time variations of 0 days, 1 day, 7 days, and 14 days, on day 0 storage, the average result obtained by the safranin dye was 359,600 cells/mm³ while the platelet count result using Rees Ecker was 343,000 cells/mm³. On day 1 storage, the average result obtained by the safranin dye was 343,500 cells/mm³ and the result obtained by Rees Ecker was 356,200 cells/mm³. On day 7 storage, the average result obtained by the safranin dye was 274,100 cells/mm³ and the average obtained by Rees Ecker was 301,500. On day 14 storage, the average obtained by the safranin dye was 257,200 cells/mm³ and the average obtained by Rees Ecker was 286,500 cells/mm³.

Based on the Mann-Whitney statistical test performed to compare the platelet counts using the Rees Ecker reagent and Safranin at 0, 1, 7, and 14 days of storage, the results showed that the P-Value for 0 days (0.850), 1 day (0.820), 7 days (0.130), and 14 days (0.121) of storage was greater than 0.05. Based on the test criteria, if the P-Value is greater than 0.05, then the null hypothesis (H0) is accepted. This means that there is no significant difference in the platelet counts using Safranin at 0, 1, 7, and 14 days of storage compared to the platelet counts using the Rees Ecker reagent.

The stability of the safranin reagent is very important to produce a quality color. Pure safranin is generally more stable compared to modified safranin reagents. The storage duration of the safranin reagent is also influenced by the pH stability of the reagent. Safranin is known to be more stable in neutral pH conditions (pH 7) (Babashahi et al., 2013). Therefore, this study used pure safranin dissolved in distilled water which has a neutral pH.

This research supports the findings of Ballihgoo (2023) that safranin is an effective positively charged dye that can stain platelet cells red. This is in line with the research by Abukhadra et al. (2021) which stated that safranin is a basic cationic synthetic dye with a positive charge, similar to Malachite Green. Oktari et al. (2018) showed the stability of Malachite Green for 2 weeks of storage. This study investigated the stability of safranin as a substitute for BCB in the Rees Ecker solution for staining platelet cells in the direct platelet count using an Improved Neubauer counting chamber, with storage time variations of 0, 1, 7, and 14 days.

CONCLUSIONS

This study shows that the safranin dye solution remains stable and safe for use in platelet counting by the Rees Ecker method, even after being stored for 14 days.

ACKNOWLEDGEMENTS

Gilang Nugraha, S.Si., M.Si, as the supervisor who has provided criticism, advice, and guidance to the author in completing the writing of this journal. The laboratory technicians from UNUSA's hematology laboratory who have assisted in the process of the research that has been conducted.

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