

Differences in Platelet Count Results Based on Storage Time of Modified Dyes with *Phosphate Buffer Saline* Solvent pH 7.3

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ABSTRAK

Laboratory tests must be performed properly in accordance with procedures to obtain accurate, precise, fast, and reliable results, as there is high demand from doctors for assistance in establishing diagnoses, particularly for platelet disorders. This study aims to determine the differences in platelet counts based on the storage time of modified Safranin staining reagents with PBS pH 7.3 solvent. This experimental study compared a control group using *Rees Ecker* reagent and an intervention group using modified reagent, with observations on days 0, 1, 7, and 14. The average platelet count using *Rees Ecker* reagent yielded a result of 323,000 cells/mm³. The mean platelet count using the modified reagent was 337,085 cells/mm³. The mean platelet count was calculated using the Improved Neubauer counting chamber. Statistical analysis using *One Way ANOVA* yielded a *p-value* of 0.060, indicating no significant difference between the two groups regarding reagent storage time.

INTRODUCTION

Studies on platelet counting are important in various medical applications, especially in disease diagnosis and monitoring patient health conditions (Sianipar, 2014). Laboratory examinations must be conducted properly in accordance with procedures to obtain precise, accurate, rapid, and reliable results, as there is significant demand from doctors for assistance in establishing diagnoses, particularly for platelet disorders (Raga & Gustiarni R, 2022). Understanding the differences in platelet count results based on the storage time of modified stains using PBS pH 7.3 as a solvent. This study is expected to contribute to the development of more effective and accurate staining methods for platelet counting.

Platelets are disc-shaped cells with a diameter of 1–4 micrometres and a volume of 7–8 fl. Platelets are nucleated blood cells produced in the bone marrow. Mature megakaryocytes are characterised by endomiosomal nuclear replication and an increase in plasma volume, ultimately leading to cytoplasmic granularity and platelet release (Ria Syafitri & Desty Shoumi, 2020). The quality and quantity of platelets in TC (Platelet Concentrate) are influenced by various factors, including donor selection, blood sampling, processing, storage, and distribution, all of which require special attention. TC is stored at a temperature between 20°C and 24°C with continuous and consistent agitation, enabling a shelf life of up to five days (Azimata R. et al., 2022).

The methods used to examine platelet counts consist of manual and automatic methods. Manual methods are divided into two types, namely direct and indirect methods. The direct method is carried out using a counting chamber, while the indirect method uses SADT (Peripheral Blood Smear Preparation). Meanwhile, the automatic method uses a haematology analyser. The *Rees Ecker* method is one of

the manual methods for counting platelet counts in blood. In this method, blood is diluted with *Rees Ecker* solution, which contains sodium citrate, formalin, and BCB. This solution can be used simultaneously for counting platelets and erythrocytes, thereby saving time during the examination, and it also stains the platelets so they can be counted under a microscope (Mustika, 2022).

Safranin dye, which is a counterstain for crystal violet dye (Faudiyah, 2020). The procedure discovered by Hans Christian Gram in 1884 uses differential staining with gentian violet–iodine complex and safranin counterstain to distinguish between Gram-positive and Gram-negative bacteria. After exposure to alcohol, the cell walls of Gram-positive organisms retain this complex and appear purple, while the cell walls of Gram-negative organisms change colour and become pink. Phosphate-buffered saline (PBS) is a buffer solution composed of phosphate salts and sodium chloride, designed to maintain the pH of the solution within a stable range. Phosphate-buffered saline (PBS) is a buffer solution composed of: Monohydrogen phosphate, sodium chloride (NaCl), pH 7.3. Dihydrogen phosphate functions to maintain pH stability; generally, dihydrogen phosphate is present in varying concentrations, such as 0.01 M phosphate. Sodium Chloride (NaCl) functions to mimic the ionic conditions of blood. Sodium Chloride (NaCl) is typically present at a concentration of 0.8% to 1.0%. The pH is adjusted to 7.4, but can be adjusted to other pH values such as 7.3 depending on the specific experimental requirements (Nishimura et al., 2017).

In Rahayu's (2024) study on modified dyes dissolved in PBS pH 6.8 and 7.0, it was concluded that there was no difference in the results of platelet count examinations using PBS 6.8 and 7.0 safranin solvents with *Rees Ecker* solution. A limitation of this study is the short duration of the experiment, so it is necessary to observe the

reagents over a one-month period to assess their quality and stability.

According to research conducted by Ballihgoo (2023), safranin dye has better quality than gentian violet and carbol fuchsin dyes. The advantage of safranin dye is that it is relatively more stable in retaining colour. Safranin dye has characteristics similar to BCB (Brilliant Cresyl Blue), which is basic in nature and functions to stain acidic nuclei and granules (Salsabila A., 2024).

This study expects that there will be no difference in platelet count results based on the storage time of modified dye with PBS pH 7.3 solvent.

METHOD

Research Respondents

Platelet examination of venous blood samples with EDTA anticoagulant was tested using the *Rees Ecker* method as a control, then the samples were tested using alternative safranin dye, resulting in four treatment groups and one control group.

Research Procedures

Seeking information from the D-IV Health Analyst administration regarding the number of 2023 students at UNUSA Campus B. Requesting respondents to complete an informed consent form as a form of agreement that respondents are willing to participate in all stages of the research, including the grouping of questionnaire data such as respondent name, gender, age, and which cohort they belong to. Venous blood sampling, preparing the Improved Neubauer counting chamber, preparing modified staining reagents (Rahayu, 2024). Prepare a 50 ml glass beaker. Add 50 mg of safranin powder. Then dilute with 50 ml of PBS 7.3 solution. Dilute the blood using a micropipette with *Rees Ecker* control solution. Pipette 995 μ l into the *Rees Ecker* reagent tube and add 5 μ l of blood, then

homogenise. Dilute the blood using a micropipette with various alternative stains. Pipette 995 μ l into the alternative stain reagent tube and add 5 μ l of blood, then homogenise.

Instruments

The equipment used in this study included a tourniquet, needles, syringes, EDTA Vaculab anticoagulant tubes, tube racks, analytical scales, measuring cups, measuring pipettes, Petri dishes, micropipettes, Improved Neubauer Assistant haemocytometers manufactured in Germany, a binocular microscope (Olympus CX 23, Germany) and a trinocular microscope (Nikon, Japan). The materials used in this study were EDTA venous blood, cotton, 70% alcohol, *Rees Ecker* solution (Cat. No. 0090544 B, Indo Reagen, PT. Segara Husada Mandiri, Jakarta), PBS pH 7.3 (Bio Analitika Laboratory, Surabaya), distilled water, synthetic safranin powder (Sentra Kimia Labsains, Yogyakarta) intended for haematology.

Data Analysis

Data analysis in this study utilised bivariate data analysis, with parametric tests requiring data normality testing prior to data analysis. Data that was normally distributed and homogeneous was tested using the *One Way ANOVA* test. Data analysis was conducted using SPSS 25.

RESULTS AND DISCUSSION

The one-way ANOVA test yielded a p-value of 0.060 for both variables. The results show that the p-value is >0.05 , therefore the hypothesis accepted from this study is H_0 . These results conclude that there is no proven difference in the results of platelet count examinations using PBS pH 7.3 safranin solvent and *Rees Ecker* solution.

The results of the platelet count study showed that the average result in the control group using *Rees Ecker* solution was 323,000 cells/mm³ (SD 64,300 cells/mm³), while the average platelet count in the modified dye with PBS pH 7.3 solvent was 337,085 cells/mm³ (SD 79,873 cells/mm³).

The average platelet count using *Rees Ecker* reagent on day 0 was 302,000 cells/mm³, while *Rees Ecker* reagent on day 1 showed a result of 398,400 cells/mm³, *Rees Ecker* reagent 7 days yielded a result of 288,400 cells/mm³, and *Rees Ecker* reagent 14 days yielded a result of 301,200 cells/mm³. The average platelet count using the modified reagent on day 0 was 296,200 cells/mm³, on day 1 was 408,400 cells/mm³, on day 7 was 297,200 cells/mm³, and on day 14 was 349,600 cells/mm³.

The test results using *Rees Ecker* reagent showed a result of 323,000 cells/mm³, while the modified reagent with PBS pH 7.3 showed a result of 337,085 cells/mm³. As can be seen from the results, there is a difference of 14,085 cells/mm³. Platelet cells appear to have colour

differences because the cell images were captured using a digital trinocular microscope. This may occur because the trinocular microscope contains components that block blue light, allowing the cell colour to change from its actual colour.

Based on preliminary tests, it has been proven that a 0.1% concentration of modified reagent can be used to stain platelet cells. This concentration was chosen based on the control reagent, which uses a 0.1% concentration, as well as previous research stating that a 0.1% concentration is optimal for safranin (Ballihgoo, 2023). This modified stain was prepared by mixing safranin powder, which was then dissolved in PBS solution with a selected pH. This reagent consists of only two components because the PBS solution already contains stabilising agents. The solution was then stirred until homogeneous and filtered twice using filter paper to ensure clean and clear staining, thereby not interfering with microscopic observation.

Table 1. Respondent Characteristics Based on Gender

Gender	Number (of people)	Percentage
Male	10 people	40%
Female	15 people	60%

Table 2. Respondent Characteristics Based on Age

Age of respondents	Number (of people)	Percentage
19	3 people	12%
20	10 people	40%
21	5 people	20%
22	6 people	24%
23	1 person	4%

Table 3. Respondent Characteristics Based on Body Weight

Weight range (kg)	Number (people)	Percentage
45-51	9 people	36%
52-58	11 people	44%
59-66	5 people	20%

Table 4. Results of Platelet Count Test

Days	Colouring reagent	Average (sel/mm ³)	SD (sel/mm ³)	Minimum number (sel/mm ³)	Maximum number (sel/mm ³)
0 Day	<i>Rees Ecker</i>	302.000	72.883	226.000	406.000
	Modification	296.200	71.869	234.000	404.000
1 Day	<i>Rees Ecker</i>	398.400	69.042	312.000	482.000
	Modification	408.400	67.549	304.000	464.000
7 Days	<i>Rees Ecker</i>	288.400	53.803	200.000	326.000
	Modification	297.200	70.747	206.000	384.000
14 Days	<i>Rees Ecker</i>	301.200	61.475	220.000	368.000
	Modification	349.600	109.329	172.000	462.000

Table 5. Data Analysis ANOVA

Variable	<i>p-value</i>	Description
<i>Rees Ecker</i> reagent platelet count, reagent modified with PBS pH 7.3	0,060	There is no difference.

Based on Tables 1, 2, and 3, the characteristics of the respondents in this study based on gender consisted of 60% female (15 respondents) and 40% male (10 respondents). The average age of the respondents was 21 years, ranging from 19 to 23 years. The average weight of the respondents was 50 kg, with 36% (9 people) weighing between 45 and 51 kg, 44% (11 people) weighing between 52 and

58 kg, and 20% (5 people) weighing between 59 and 66 kg.

Based on Table 4, the results of the platelet count study showed that the average result in the control group using *Rees Ecker* solution was 323,000 cells/mm³ (SD 64,300 cells/mm³), while the average platelet count in the modified dye with PBS pH 7.3 solvent was 337, 085 cells/mm³ (SD 79,873 cells/mm³).

Based on Table 5, the p-value of both variables is 0.060. The results show that the p-value is >0.05 , therefore the hypothesis accepted from this study is H_0 .

This result concludes that there is no proven difference in the results of platelet count examinations using PBS pH 7.3 safranin solvent and *Rees Ecker* solution

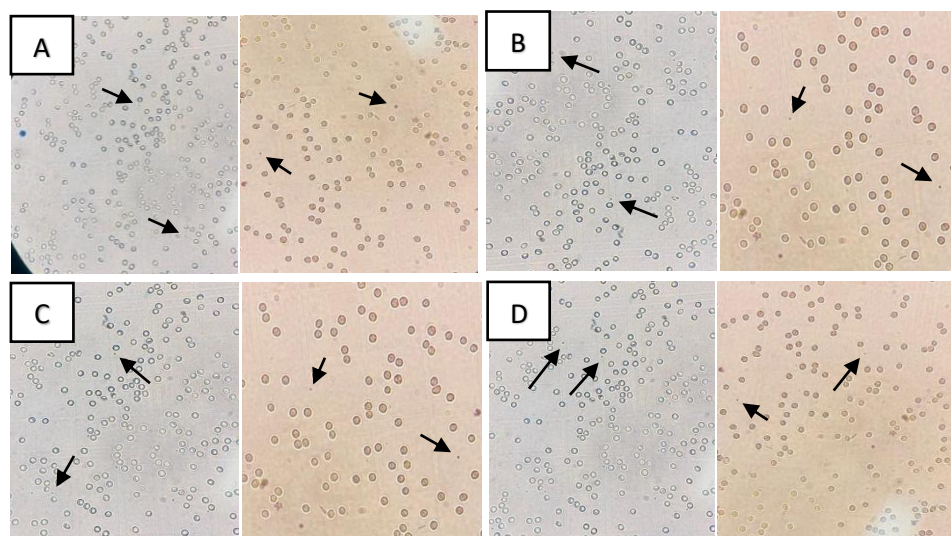


Figure 1. (A) *Rees Ecker* solution and modified solution at 0 days of storage, (B) *Rees Ecker* solution and modified solution at 1 day of storage, (C) *Rees Ecker* solution and modified solution at 7 days of storage, (D) *Rees Ecker* solution and modified solution at 14 days of storage.

Figure 1 shows the difference in platelet staining over storage time, namely 0 days, 1 day, 7 days, and 14 days. There was no difference in staining and platelet count at 0 days, 1 day, 7 days, and 14 days of storage. In Figure 1 (A), platelets stained with *Rees Ecker* at 0 days appear blue, and modified staining at 0 days appears red under the microscope. This is also seen in Figure 1 (B), (C), (D), where platelet staining with *Rees Ecker* solution appears blue and modified solution appears red at 1 day, 7 days, and 14 days of storage. There were no differences in platelet counts between the *Rees Ecker* solution and the modified solution at storage times of 0 days, 1 day, 7 days, and 14 days, as listed in Table 4.

CONCLUSION AND RECOMMENDATIONS

Based on the results of the study, it can be concluded that:

1. The average platelet count using *Rees Ecker* reagent on day 0 was 302,000 cells/mm³, while *Rees Ecker* reagent on day 1 showed a result of 398,400 cells/mm³, the *Rees Ecker* reagent at 7 days yielded a result of 288,400 cells/mm³, and the *Rees Ecker* reagent at 14 days yielded a result of 301,200 cells/mm³.
2. The average platelet count using the 0-day modified reagent was 296,200 cells/mm³, while the 1-day modified reagent yielded a result of 408,400 cells/mm³, the modified reagent at 7 days yielded a result of 297,200 cells/mm³, and the modified reagent at 14 days yielded a result of 349,600 cells/mm³.

3. There was no difference in platelet count results based on storage time with *Rees Ecker* reagent and modified dye.

Recommendations

Further research should consider the use of microscopes in observing platelets using different types of microscopes. If you wish to conduct further research, it is recommended to use more blood samples, use abnormal samples, or compare them with automatic devices.

BIBLIOGRAPHY

- Azimata Rosyidah, R., Murni Hartini, W., Sumoko, E., Ciwi Makawara, I., (2022). Studi, P. D., Bank Darah, T., & Kesehatan Bhakti Setya Indonesia, P. Health Journal “Love That Renewed” Pengaruh Lama Masa Simpan Thrombocyte Concentrate (Tc) Terhadap Jumlah Trombosit Dengan Metode Manual Improved Neubauer. *Jurnal Kesehatan*, 10(1).
- Ballihgoo H. (2023). Perbedaan Hitung Jumlah Trombosit Pewarna Safranin, Gentian Violet Dan Karbol Fuksin Dengan Larutan Rees Ecker. Surabaya: Universitas Nahdlatul Ulama Surabaya (Unusa).
- Faudiyah, N. N. (2020). Identifikasi Bakteri *Vibrio Cholerae* Pada Tubuh Lalat Hijau (*Chrysomya Megacephala*) Di Pasar Legi.
- Mustika, Y. S., dkk., (2022). Perbandingan Hasil Hitung Jumlah Trombosit Menggunakan Metode Manual Dan Automatic di Klinik Dr. Fakhrurozi Depok. *Jurnal Analisis Biologi*, 06(02), 1-5.
- Nishimura, dkk., (2017) AHA/ACC Focused Update of the 2014 AHA/ACC Guideline for the Management of Patients with Valvular Heart Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*, 135, e1159-e1195.
- Raga, E., Gustiarni R, D. (2022). Perbandingan Pemeriksaan Trombosit Cara *Rees Ecker* Dan Amonium Oxalate Dengan Gold Standard Hematology Analyzer. *Cerdika: Jurnal Ilmiah Indonesia*, <https://Doi.Org/10.36418/Cerdika.V.2i3.351>
- Rahayu. (2024). Perbedaan Hasil Hitung Jumlah Trombosit Dengan Pewarna Modifikasi Menggunakan Pelarut PBS pH 6,8 dan 7,0. Universitas Nahdlatul Ulama Surabaya
- Ria Syafitri, Desty Shoumi. (2020). Perbedaan Kadar Trombosit Pada Pasien Trombositopenia Sebelum Dan Sesudah Transfusi Trombosit Konsentrat Di Rsud Karawang Tahun 2019 – 2020. Perbedaan Kadar Trombosit Pada Pasien Trombositopenia Sebelum Dan Sesudah Transfusi Trombosit Konsentrat Di Rsud Karawang Tahun 2019 – 2020.
- Salsabila A, Nugraha G. (2024). Differences In Storage Time In Safranin Dye On Counting The Number Of Platbocytes By *Rees Ecker* Method. *Jurnal Analisis Medika Biosains (Jambs)*, Vol.11, No.01.