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Platelet Counts with Modified Stains Using PBS Solution pH 6,8 and 7,0

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ABSTRACT

The platelet count examination in this study was performed manually using the direct counting method with an Improved Neubauer counting chamber and Rees Ecker reagent. This study was conducted on 15 respondents using experimental method with simple random sampling technique. The results the Rees Ecker reagent was prepared by mixing 3.8 g of sodium citrate, 0.1 g of brilliant cresyl blue, and 100 mL of distilled water. For each sample, 20 µL of EDTA-anticoagulated blood was diluted with 380 µL of Rees Ecker reagent (1:20 dilution). The mixture was gently homogenized and allowed to stand for 10 minutes at room temperature to ensure adequate staining and platelet stabilization. The modified reagents used phosphate-buffered saline (PBS) with pH 6.8 and pH 7.0 as solvents for the same staining components. The same dilution ratio and protocol were applied to ensure consistency across all groups. of this study were obtained in the control.

INTRODUCTION

The platelet a derivative of megakaryocytes derived from cytoplasm of megakaryocytes and formed from bone marrow. Platelet examination is one of the examinations that is widely carried out in clinical laboratories. This is due to the important role of platelets in efforts to establish a diagnosis, provide therapy, illustrate prognosis anda monitor patients (Nurseha, 2021). Due to the ability of platelets to easily aggregate and adhesion, platelet examination must be carried out no more than 1 hour. In addition, other factors that affect the results of platelet count are pathological and laboratory factors such as the time of examination and also the anticoagulant used (Gandasoebrata, 2013).

The platelet count was performed manually using the direct method. The direct platelet count is made using the Improved Neubauer counting chamber. This examination is usually made using Rees Ecker reagent, which contains BSB (Brilliant Cresyl Blue). BCB is a dye that can be absorbed by platelet, causing them to appear blue when viewed under a microscope. However, Rees Ecker reagent still has disadvantages, that is the erythrocytes are not lysed, so it's still possible to find errors in the calculation (Mustika *et al*., 2022).

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Based on previous research conducted by Ballihgoo (2023), platelet count examination calculated using alternative reagents, with the best results obtained using Safranin. This study was conducted by dissolving Safranin with distilled water to be used as an alternative stain. However, after the research there were shortcomings, that is the prepared reagents were still less stable. So it was still possible that the stability of reagents had decreased. Therefore, in this study, researchers conducted a renewal by using PBS as a solvent to maintain to pH and stability of alternative reagents, which were then modified in the solvent.

MATERIALS/METHOD

This study used 15 respondents and was approved as ethically feasible by the Ethics Committee of Nahdlatul Ulama University Surabaya with No. 0364/EC/KEPK/UNUSA/2023. This research is included in experimental research with the sampling technique used is simple random sampling. The tools and materials used include Improved Neubauer counting chamber, microscope, micropipette, glass beaker, measuring glass, stirring stick, spatula, serology tube, tube rack, analytical balance, blue and yellow tip, syringe, tourniquet, alcohol swab, plaster, tissue, thrombogel, EDTA vacutainer tube, Safranin, PBS solution pH 6,8 and 7,0, filter paper, petri dish, and Rees Ecker reagent.

Prepare a modified staining concentration of 0,1% by weighing Safranin O first. Then dissolve it in PBS of varying pH in a ratio 1:1. Stir with a stirring stick and then filter the modified stain so that no impurities remain when it's used to stain platelets. Using a micropipette, pipette the reagent or modified stain up to 995 µL and add 5 µL of blood. Homogenize the solution in the tube so that it's mixed, and then add sufficient solution to the counting chamber. Place the filled counting chamber in a petri dish containing wet tissue and allow to stand for 5-15 minutes.

Platelet count examination was performed on 10-25 medium erythrocyte boxes at 400x magnification on a microscope. The results of the platelet count examination were calculated using the equation: Platelet count: N x P x KV (Nugraha, 2017). The platelet count examination was counted 3 times to reduce the bias of the examination results. The data were collected and then analyzed using the One-Way Anova test.

RESULTS AND DISCUSSION

Table 1 Descriptive Analysis of Platelet Counts (Cell/mm^3)

Reagent Staining	N	Minimum Amount	Maximum Amount	Average	SD
Rees Ecker	15	195.000	405.000	283.330	70.500
PBS pH 6,8	15	195.000	465.000	332.330	85.958
PBS pH 7,0	15	165.000	465.000	321.000	99.878

Base on table 1 above, it represents the value of the platelet count results in each treatment. The results shown in each treatment are not much different either using a modified reagent with PBS pH 6,8 solvent or with PBS pH 7,0. These two modified reagents can still be used if they are to be used as alternative reagents in place of the Rees Ecker reagent for the determination of platelet count.

Table 2 One-Way Anova Test Result

Variable	One-Way Anova Test
Platelet Count Rees Ecker reagent, Modified reagent with PBS pH 6,8 and 7,0	0,275

Based on table 2, it can be seen that the test value obtained in the One-Way Anova test is 0,275, and this result is greather than the p-value (α) 0,05. From the result shown H0 is accepted, which means that there is no significant difference between the control solution (Rees Ecker) and the modified staining solution using PBS pH 6,8 and 7,0 solvent.

Based on the data presented above, the results of platelet counts using control reagents and using modified reagents were not found to have significant differences. The results of platelets in the control reagent, cells appear blue in color and round or oval in shape without nuclei with a size of 1-4 μm. in addition, erythrocytes in the control reagent are not lysed, so when viewed under a microscope, two cells are readable, platelet cells and erythrocytes (Suryatama *et al*., 2023).

Whereas in both modified reagents, platelet cells appear red, round, and have no nucleus (Grott *et al*., 2023). In the modified reagent, the erythrocytes appear used so that when viewed under the microscope, the platelets appear clearly without erythrocytes that may be covering the platelets. This may occur because the PBS solution used as the solvent is capable of lysing erythrocytes. PBS solution contains NaCl, which is isotonic, so it causes lysis of erythrocytes due to the difference in concentration on substances between extracellular and intracellular (Ifada *et al*., 2023).

The lysis of erythrocyte is one of the advantages not found in control reagents such as Rees Ecker. With erythrocyte lysis, platelet cells appear clearly, making them easier to read under a microscope and minimizing errors in platelet readings. The isotonic nature of PBS is the primary trigger for erythrocyte cell lysis. The isotonic nature of PBS is also useful in maintaining pH and osmotic balance in reagents as well as in cells and tissues (Viratikul *et al*., 2022).

The results of platelet counts performed with control reagents and modified stain with PBS pH 6,8 and 7,0 solvents did not show significant differences. The results between the three treatments showed results that were more or less in the same range. Therefore, these two modified reagents can be used as alternative reagents to replace the Rees Ecker reagent in platelet counting using the Improved Neubauer counting chamber method.

CONCLUSIONS

The modified reagents demonstrated comparable results to the Rees Ecker reagent in this limited sample, suggesting their potential as alternatives pending further validation.

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