

PHOSPHATE BUFFER SALINE AS AN ALTERNATIVE DILUENT IN EXAMINATION OF ERYTHROCYTE SEDIMENTATION RATE WESTERGREEN METHOD

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Abstract

Background: Sodium Chloride (NaCl) 0.9% is the standard method for examining ESR with EDTA blood. It was found that PBS has properties comparable to 0.9% NaCl because the osmolarity and ion concentration of this solution is isotonic with human body fluids. **Aims:** This study was to determine the difference in LED results using PBS pH 7.2 and pH 7.4 with the results of examining LEDs using 0.9% NaCl Westergren method. **Methods:** This study used an experimental method with a static group comparison research design. The difference in the results of the LED examination using 0.9% NaCl as the control group and PBS pH 7.2 and pH 7.4 as the test group. **Results :** statistical tests showed that the average value of LED using 0.9% NaCl was 13.10 mm/hour, PBS pH 7.2 was 12.40 mm/hour and PBS pH 7.4 was 10.30 mm/hour. Based on the results of the One Way Anova test, the p-value was 0.528 and the Post Hoc test results on PBS pH 7.2 obtained a p-value of 0.959 and PBS pH 7.4 p-value 0.523. **Conclusion:** the results of examining the LED using both PBS pH 7.2 and PBS pH 7.4 diluents showed no difference in results with 0.9% NaCl diluent as the standard method. So there is a suggestion from researchers to use abnormal samples diluted with PBS pH 7.2 to 7.4.

Keywords: Erythrocyte sedimentation rate, westergren , phosphate buffer saline

1. Introduction

One laboratory examination to help diagnose a disease includes a hematology examination. Hematology examination is a blood examination related to blood cells and their components. Hematological examination is the most commonly performed laboratory examination (1). Therefore, hematological examination must be carried out quickly and precisely so that accurate results can be obtained (2).

Blood Sedimentation Rate (ESR) or Erythrocyte Sedimentation Rate (ESR) is a type of examination to determine the speed at which erythrocytes settle in unclotted blood (blood containing anticoagulant) in a vertical tube for one hour. Blood with anticoagulant in the LED tube that is left upright will experience separation so that it becomes two layers in the form of plasma and erythrocytes. The deposition process of blood happens in three-phase formation rouleaux, deposition, and compaction. The results of the LED

examination can be used to monitor tissue damage and inflammation and indicate the presence of disease (1).

The LED inspection method consists of two methods, namely the Wintrobe and Westergren methods. The Westergren method is a standard method that is often used for ESR examinations because it is recommended by the International Committee for Standardization in Hematology (ICSH) (3).

Method Westergren recommended using four parts blood and 1 part diluent, which is a standard test (1). Furthermore, ICSH provides an alternative use of EDTA (Ethylene Diamine Tetra Acetic Acid) blood, an anticoagulant that is often used in the laboratory, and natural LED examination Westergren method (4). EDTA prevents coagulation by binding calcium ions so that insoluble calcium salts are formed; therefore, the calcium ions that play a role in coagulation become inactive, resulting in blood clot formation not occurring (1). The use of EDTA anticoagulant is beneficial for clinicians because blood collection is a closed system, so it can increase operator safety during phlebotomy and minimize errors in the pre-analytical stage by 68% (5).

In LED examination, there are several factors that can influence the results of the examination, namely dilution. ESR examination using EDTA blood is carried out by adding physiological NaCl (Sodium Chloride) or 0.9% as a diluent. The LED diluent solution is isotonic and has the same

osmotic pressure as erythrocytes (6). An isotonic solution has the same solution concentration as body fluids so that cells will not be damaged by erythrocytes and remain stable in the same form (7). Sodium Chloride is derived from strong acids and strong bases, namely hydrochloric acid and sodium hydroxide. A strong acid and a strong base have cations and salt anions that will not be hydrolyzed when reacting with water, so the solution is neutral (8).

Phosphate Buffer Saline (PBS) has properties similar to 0.9% NaCl (9). PBS solution contains NaCl, potassium chloride, sodium dihydrogen phosphate, and potassium dihydrogen phosphate. Osmolarity and ion concentration of PBS solution because it is isotonic with body fluids. PBS is isotonic and non-toxic to cells and has the ability to maintain osmolarity. the diluent solution used pH 7.2 and pH 7.4 According to Sabolakna, the specificity of blood samples mixed with an acid solution with a pH of 7.2 has a specificity that is close to the positive control. This happens because the pH is close to the normal value of blood pH, namely 7.35 to 7.45 (10)

Based on the description above, the author is interested in examining the differences in LED examination results using 0.9% NaCl as a control group, PBS pH 7.2, and PBS pH 7.4. So, researchers want to find out what pH is similar to 0.9% NaCl.

2. Method

Study This involves as many as ten respondents without There is coercion with the agreed informed *consent*. The study has gotten agreement ethics from Commission Ethics Health University Nahdlatul Ulama Surabaya with number 0065 /EC/KEPK/UNUSA/2023.

Type study is used as a study experiment with design *Static Group Comparison*. Repetition carried out, i.e., 10 times based on a calculation using Federer's formula. The treatments used in this study had three treatments, namely four parts of EDTA blood diluted with one part of 0.9% NaCl, PBS pH 7.2, and PBS pH 7.4. Each treatment consisted of 10, so a total of 30 samples.

The tools used in this research include tourniquets, syringes, plaster, gloves, test tubes, German Assistant brand *Westergren tube pipettes*, *Westergren tube racks* (Onemed, PT. Jayamas Medica Industri, Indonesia), bulbs, and Vaculab tube EDTA 3 ml. The materials used in the research were EDTA venous blood samples, 70% alcohol, cotton, 0.9% NaCl infusion fluid, PBS pH 7.2, and PBS pH 7.4.

Taking blood specimens and making dilution with 4-part comparison blood And

For every treatment added, 1 part thinner NaCl 0.9% (control), PBS 7.2, and PBS 7.4. Pipette the EDTA blood specimen that has been given diluent using a *Westergren tube* to the 0 mark. Place the tube on a tube rack or *Westergren pipette stand* upright. Leave it for 1 hour. Measuring plasma height in mm, from sign limit 0 to sign limit erythrocytes settles. Note That the LED value is in mm/hour.

Data collected from the LED examination results were analyzed using the SPSS (*Statistical Program for Social Science*) statistical test. version 21.0. If the research data is normally distributed and the population and sample used in the research are homogeneous, then proceed using the one way ANOVA *test*. The basis for decision-making in the One Way Anova test is that *if the p-value > 0.05, then the average is the same, and vice versa. If the p-value < 0.05, then the average is different.*

3. Result and Discussion

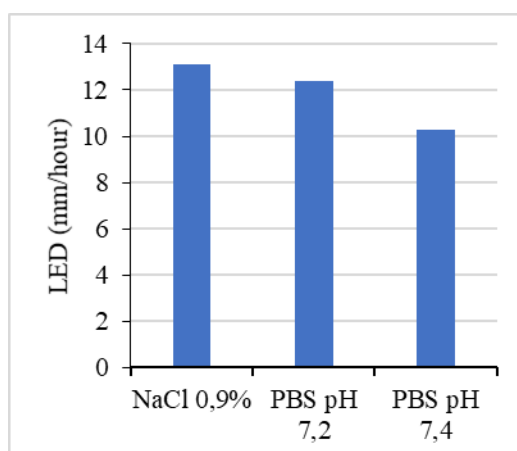
Study This with titled the difference in the results of the LED examination using PBS diluent with a pH of 7.2 and 7.4 held on month April 2023 in the Laboratory Faculty Health University Nahdlatul Surabaya Ulama.

Table 1. Average LED method results *Westergren*

LED thinner	Mean (mm/hour)
NaCl 0.9%	13,10
PBS pH 7.2	12.40
PBS pH 7.4	10.30

Based on Table 1. The mean and SD value of the LED results using the *Westergren method* using 0.9% NaCl dilution obtained an average of 13.10 mm/hour. PBS pH 7.2 dilution

obtained an average of 12.40 mm/hour. PBS dilution pH 7.4 obtained an average of 10.30 mm/hour with an SD of 5.417 mm/hour.



Picture 1. Bar Chart of Average LED Results

Data analysis

One Way Anova statistical test using SPSS on the results of the LED examination using 0.9% NaCl, PBS pH 7.2, and PBS pH 7.4, the significant value of the output was

0.528, meaning the significance value was more than 0.05. In conclusion, there was no difference in the results of the LED examination using PBS diluent pH 7.2 and 7.4. Next, the *Post Hoc Turkey's Honestly*

Significant Difference (HSD) test was carried out to determine which group, PBS pH 7.2 and pH 7.4, were the same as the control group.

Table 3. *Post Hoc* Test Results

Group		<i>p-value</i>
NaCl 0.9%	PBS pH 7.2	0.959
	PBS pH 7.4	0.523

The Post Hoc test results table shows the results of the LED examination data using 0.9% NaCl as a control group compared to the PBS pH 7.2 treatment group, which had a *p-value* of 0.959, and PBS pH 7.4, which had a *p-value* of 0.523. Because of the two *p-values* > 0.05. So, this shows that there is no difference in the results of the LED examination of the two diluent solutions using PBS pH 7.2 and PBS pH 7.4 compared to 0.9% NaCl diluent as the standard method.

The results of this research show that there is no difference in the results of the LED examination using PBS pH 7.2 and PBS pH 7.4 diluents with 0.9% NaCl diluent. This is in line with the results of research conducted by Diarti, who found that 0.9% NaCl has the same quality as phosphate buffer solution as a diluent because phosphate buffer solution has isotonic properties and is able to withstand changes in pH when hydrogen or hydroxide ions are added

or when the solution is diluted it is called a buffer solution (9).

According to Amelia, PBS is isotonic and non-toxic to cells and has the ability to maintain osmolarity. diluent solution used pH 7.2 and pH 7.4 (11).

According to another research, the specificity of blood samples mixed with an acid solution with a pH of 7.2 has a specificity that is close to the positive control. This happens because the pH is close to the normal value of 8blood pH, namely 7.35 to 7.45 (10).

4. Conclusion

Based on the research results, it was concluded that LED examination using PBS pH 7.2 and 7.4 had results that were no different from LED examination using 0.9% NaCl, which is the conventional method.

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