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The Effect of Differences Time On The Macroscopic Picture Of Giemsa Staining Using Aquades Diluent

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ABSTRACT

Peripheral Blood Smear Preparation (SADT) is an examination used to see the structure and number of red and white blood cells, besides giemsa staining can be used for malaria examination. The quality of giemsa staining is influenced by one of which is the soaking time of the giemsa solution. The purpose of this study was to determine the quality of slides or blood smears based on the incubation duration of giemsa staining. This research method qualitatively performed giemsa staining on blood smears with different soaking times (10 minutes, 20 minutes, 30 meinit and 40 minutes) then compared with controls. Results showed that at minutes 10 and 20 the giemsa staining showed a smooth surface and no granules, at 30 and 40 minutes the giemsa staining showed a rough surface and many granules. The conclusion of this study was that 10 and 20 min giemsa soaking showed optimal staining of blood smear preparations.

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1. INTRODUCTION

Blood is a medium used for long-distance transport of various materials between the cells themselves. Blood consists of erythrocyte cells, leukocyte cells, and platelet cells as well as plasma fluid. one method of blood identification uses peripheral blood smear examination using giemsa staining which is used to observe blood cell components [1].

The blood specimen used in the manufacture of peripheral blood smears is with EDTA anticoagulant venous blood. A good thin blood preparation has a preparation that does not expand to the edge of the glass object, 2/3 long, there are thin parts, must be flat, not wavy or striped and leukocyte cells should not gather on the edges of the preparation [2].

Laboratory examination is an examination used to determine health conditions, one of the laboratory tests is an examination with hematology with the Peripheral Blood Smear Preparation (SADT) method [3]

One of the functions of blood smears is to count the type of lekocytes consisting of basophil (0-1%), eosinophil (1-3%, netrophil netrophil (2-6%), segment netrophil (40-60%). Loimocytes (20-40%), monocytes (2-8%) (4). The validity of such examination depends on the quality of the preparation.both macroscopically and microscopically [4]

Staining on peripheral blood smears that aims to see blood cells there are several kinds of staining including peroxidase staining, sudan black, rapid, BCB, wright and giemsa. Menurut Romanowsky terdapat 4 pengecatan pada hapusan darah yaitu pengecatan Liesman, Wright, pengecatan May Grundwald dan pengecatan giemsa [5]. In laboratories many use giemsa dye for peripheral blood preparations because the resistance of the dye is better and clearerStaining on peripheral blood smears that aims to see blood cells there are several kinds

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Giemsa's staining is strongly influenced by the type of diluent. Giemsa's diluent has other conditions such as having buffering, isotonic properties and has a pH of 6.8-7.0 [6]. The giemsa component consists of Azur B (trimethyl thionine) plays a role in coloring acidic-colored cell components, and eosin Y plays a role in coloring alkaline components, such as cell nuclei, granules from leukocyte cells, and a combination of eosin Y and azur B produces purple color in cells [7].

A buffer solution is a solution used to maintain pH in order not to experience significant changes. The buffer used is usually phosphate buffer. However, in some cases phosphate buffers have low stocks so they are replaced with aquadest solutions with neutral pH.

In giemsa staining, cells will appear purple, alkaline granules will look emrah, acidic granules will be blue. However, giemsa dye is affected by the diluent used and the incubation time. The results of coloring the preparation using time that is not in accordance with the dilution of giemsa will give bad results including, macroscopically the picture of the dosage form has been clearly visible. Based on this, research was conducted on the effect of time differences on macroscopic images using giemsa dyes.

2. METHOD

Sample Analysis

This research is descriptive observational, which is research conducted with the aim of knowing the picture or description of a situation objectively. (Notoatmodjo, 2002). This study will examine giemsa coloring based on different times looking at macroscopic. The sample used is a blood sample of a healthy adult. To determine the magnitude of the research unit using Federer's formul, namely $(t–1)(r–1) \geq 15$. So that the existing sample was obtained was 20 people.

Giemsa Staining

Giemas staining is carried out through several stages. First a drop of blood is done at the end of the slide, then the next slide forms a 45º angle at the end where there are blood droplets, then the second slide is pushed to the other end of the first slide. Second, a giemsa solution with a concentration of 10% was made with aquadest retailers. Third, smearing peripheral blood in the first procedure, then staining with 10% giemsa solution with different time variations, including 20 minutes, 30 minutes, and 40 minutes. Fourth, it is allowed to dry and macroscopic observation and data analysis is carried out.

3. RESULTS AND DISCUSSION

The results of this study showed that giemsa staining with different incubation times showed differences in macroscopic features in blood smear preparations. The incubation staining time of 10 minutes and 20 minutes macroscopically and qualitatively showed a smooth surface without granules in the blood smear **(Figures 1b and 1c).** Furthermore, incubation times of 30 and 40 minutes showed dirty staining surfaces and granules (Figures **1d and 1e)** when compared to incubation times of 10 minutes, 20 minutes, and controls **(Figures 1a, 1b and 1c).**

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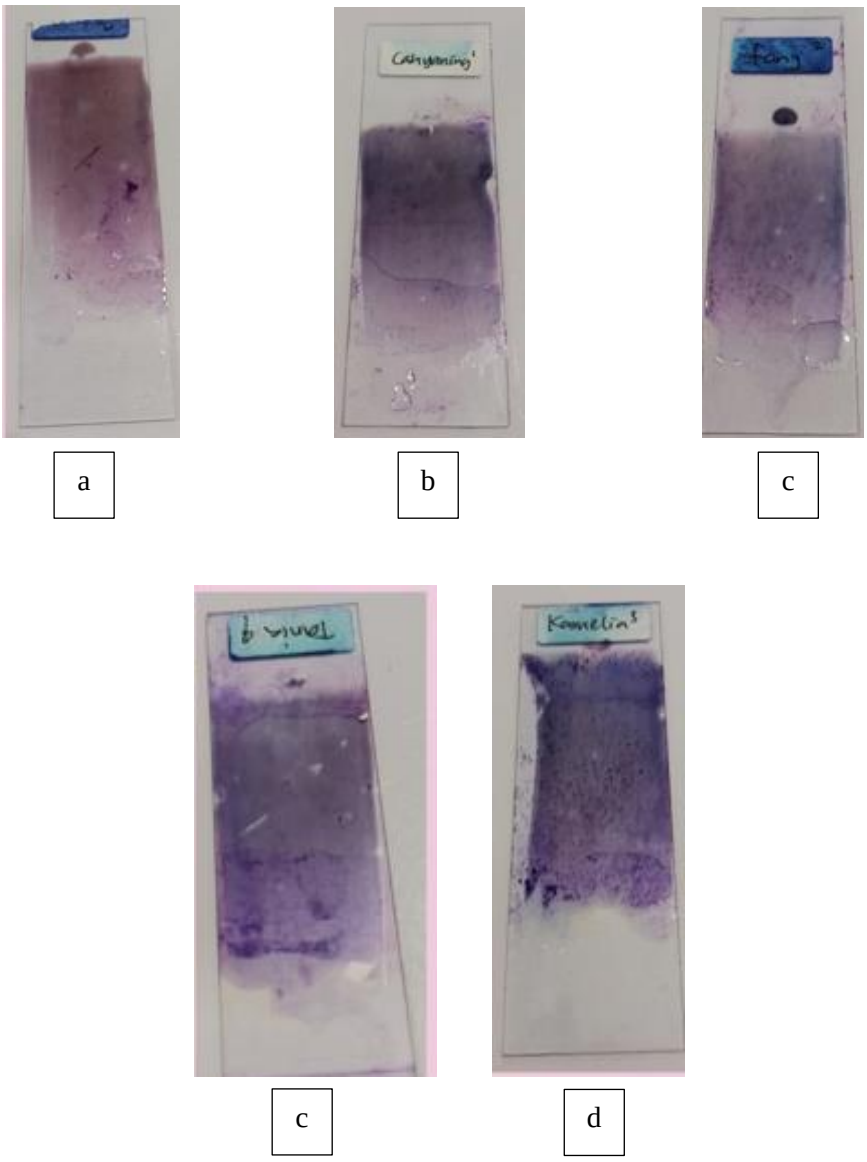

Figure 1. Differences in giemsa staining results on blood smears based on incubation time. (a) Control (b) Incubation for 10 minutes (c) Incubation for 20 minutes (d) Incubation for 30 minutes (e) Incubation for 40 minutes.

Peripheral Blood Smear Preparation (SADT) is an examination used to identify erythrocyte, leukocyte, and platelet cells both quantitatively and qualitatively, as well as to identify the presence of parasites in the blood [8]. One of the stains used to perform blood smear examination is giemsa staining.

Giemsa's staining is a method of staining cells using several dyes consisting of eosin, *methylen blue* and *methylene azzure* [9,10]. The mixture of *methylen blue* and *methylene azzure* makes the dye more stable so that it can maximize coloring in SADT [11].

Eosin dye is an acidic dye that will color the cytoplasm pink, then *methylen blue* dye will give color to the cell nucleus. So that with giemsa staining, the cytoplasm will be red and the core is blue [12], and the quality of the coloring preparation is also influenced by the length of incubation time of the giemsa dye bath.

The difference in results between the incubation time of 10 minutes, 20 minutes, 30 minutes, and 40 minutes is due to the principle of giemsa staining which is based on *Romanowsky staining*, this staining principle uses the principle of cell chemistry, namely the interaction or reaction between acid-base between cells and dye components [13]. Improper painting time in this coloring will cause imperfect, uneven paint absorption, and absorb a lot of dye [14]

The incubation time of 10 minutes and 20 minutes showed a smooth surface and no granules, when compared to the qualitative control did not show any difference, this is because the incubation time of giemsa staining was not more than 20 minutes (according to the general standard used), this is different from the incubation time of 30 minutes and 40 minutes, showing a rough surface and there are many granules causing the color bound by the cells to be too excessive and cells too thick. This result is because at the time of painting too long or above the commonly used standard, the longer the painting, the intensity becomes old, because the cells absorb dye too long [15]. In addition, there are granules in the preparation of these blood smears and can cause changes in the morphology of erythrocytes that form creation

4. CONCLUSION

The conclusion of this study is that the incubation time of 10 minutes and 20 minutes is the best time for incubation of giemsa staining on blood smear preparations, so that the color of blood smear preparations with giemsa dye becomes optimal.

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