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COMPARISON OF LEUKOCYTE TYPE MORPHOLOGY USING AIR CONDITIONER WASTE AND PHOSPHATE BUFFER IN PBS PREPARATION

Regita Nurulliza[1], Hadits Lissentiya Armal[1*], Hanny Siti Nuraeni[1], Cecep Dani Sucipto[1]

[1]Medical Laboratory Technology, Poltekkes Kemenkes Banten
Corresponding Author: *hadits.lissentiya@poltekkesbanten.ac.id*

ABSTRACT

In a health laboratory, Giemsa staining is routinely performed. The Giemsa staining procedure is carried out to identify various cell types, one of which is leukocytes in PBS (a common acronym likely referring to a preparation technique, such as a smear or differential count). Before staining, the Giemsa stain must first be diluted using a buffer. The use of Air Conditioners (AC) in daily life has become common among Indonesian society. The commonly used AC often produces waste water or effluent that is directly discharged into drainage systems or dedicated pipes without being utilized for other purposes. Based on previous research, it has been shown that AC waste water has properties similar to deionized water (aquadest) and can be used as an alternative substitute for it. This study aims to determine whether there is a difference in the quality of the slides for examining leukocyte type morphology when using Air Conditioner (AC) waste as a buffer substitute in Giemsa staining. The research method is experimental, involving taking blood samples from respondents. The research data was analyzed using the Wilcoxon test. The results obtained an Asymp. sig > 0.05 for all leukocyte cell nuclei and monocyte cytoplasm. However, the cytoplasm of eosinophils, eosinophil granules, cytoplasm of neutrophils, neutrophil granules, and cytoplasm of lymphocytes received a value of < 0.05, which indicates that there is a difference in the quality of the slides for leukocyte morphology when using phosphate buffer versus AC waste The abstract should be a maximum of 250 words and contain the following substance Background: A brief explanation of the problem or issue raised in the research. Research Objective: Explain the main objective of this research. Research Method: Briefly describe the methods or approaches used in the research. Research Results: Present the main findings or results obtained from the research. Conclusion: Provide the main conclusions from the research results and their implications

INTRODUCTION

The system or device often referred to as an Air Conditioner (AC) is specifically designed to maintain the temperature and humidity of the air in a certain area, aiming to provide cooling or heating as needed, depending on the air conditions at the time. AC generally uses the refrigeration cycle process, although it occasionally also utilizes evaporation, especially in buildings, to create a comfortable environment (Danang and Mat, 2022).

The use of AC in daily life has become common among Indonesian society, especially because the country has a tropical climate with relatively high temperatures. AC serves as an air-cooling device used in various places, ranging from homes and offices to special events. The primary reason behind the use of AC is to create cool or cold air temperature and maintain smooth air circulation to provide comfort (Ginting et al., 2022).

The commonly used AC often produces waste water or effluent that is directly discharged into drainage systems or dedicated pipes without being utilized for other purposes. AC waste water is usually not reprocessed but is immediately disposed of into the environment through the discharge pipe. Ideally, AC waste water could be recycled, considering its significant quantity. Currently, AC waste water is often neglected and simply

discarded, even though the water produced through air condensation is pure water with impurities originating only from the air. This waste has the potential for further utilization (Sumarwanto et al., 2019).

Based on research conducted by Padya, Yuni, and Mukhamad Irfan (2019), the characterization of AC waste water and deionized water (aquadest) was performed through acidity and specific gravity analysis. The analysis results showed no significant difference in specific gravity between AC waste water and aquadest. Although AC waste water has the potential to replace aquadest due to its low mineral content, its utilization is still limited. The acidity of AC waste water is also not far different compared to aquadest (Sumarwanto et al., 2019). Referring to preliminary water quality tests conducted by Minarni, Dian, and Herda (2023), the AC waste demonstrated adequate quality. However, the nitrite parameter yielded a value of 3.77 mg/L, which exceeded the quality threshold. PERMENKES No. 32 of 2017 stipulates that the maximum permissible nitrite level in water content is 1 mg/L (Minarni et al., 2023).

A laboratory experiment involves the use of staining agents. Giemsa staining is recommended for thick and thin smears of PBS (Peripheral Blood Smear or *Sediaan Apus Darah Tepi*). The purpose of using Giemsa is to diagnose and identify malaria and microfilaria. The Giemsa solution consists of eosin and methylene blue, where the eosin component gives a red color to the parasite nucleus, while the methylene blue component gives a blue color to the parasite cytoplasm (WHO, 2016). Giemsa staining on PBS can also be used for observing erythrocyte morphology, platelet morphology and count, and leukocyte morphology and differential count (Nugraha, 2015).

Before the staining process, Giemsa needs to be diluted using a buffer solution. However, buffer solutions are often relatively expensive, and the availability of materials in the laboratory can sometimes be limited. Therefore, other diluting solutions that are more affordable and readily available are often used as substitutes for phosphate buffer, provided that the diluting solution has a pH suitable for the buffer solution. The appropriate buffer pH as a blood smear diluent ranges between 6.8 and 7.2 (Yati et al., 2019).

Referring to the research conducted by (Faradilla, 2023), there was no difference in the quality of slides in Giemsa staining using phosphate buffer and AC waste. This finding sparked the researchers' interest in continuing the study to explore whether leukocyte type morphology can be clearly observed using Giemsa staining with AC waste diluent as an alternative. This research can open up opportunities to utilize AC waste as an effective diluent solution in the staining process and contribute to efficiency and sustainability in laboratory practice. In the previous study, the clarity of the field of view and the contrast of erythrocyte cells were observed, whereas this study focuses on observing the morphology of leukocyte types based on their color quality. his research was conducted to determine whether there is a difference in the quality of slides from the examination of leukocyte type morphology using Air Conditioner (AC) waste as a buffer substitute in Giemsa staining.

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Materials and Method

This research used an experimental method, focusing on observing the differences in leukocyte types in PBS (Peripheral Blood Smear) that had been stained with Giemsa, which was previously dissolved using both phosphate buffer and AC waste water.

Description of Materials or Research Subjects:

a. Procedure for Preparing 5% Giemsa Solution with Phosphate Buffer Diluent

First Prepare the tools and materials, second Pipetted 19 mL of neutral pH buffer into a tube. Third dissolve 1 mL of Giemsa stock into the buffer in the tube. Afterthat the liquid are stired until homogeneous. Finnaly the reagent is ready for use.

b. Procedure for Preparing 5% Giemsa Solution with AC Waste Water Diluent

First prepare the tools and materials, Sechond pipetted 19 mLof AC waste water into a tube. Third dissolved 1 mL of Giemsa stock into the AC waste water in the tube. Aftrethat stired until homogeneous. Finnaly the reagent is ready for use.

c. Giemsa Quality Testing Procedure

First place a filter paper on a Petri dish so that the center of the paper does not touch anything. Sechond drop 1-2 drops of Giemsa stock solution onto the filter paper. Allow it to soak and spread evenly. Next, slowly drop 3-4 drops of methanol onto the center of the Giemsa circle with a time interval of a few seconds, until the diameter of the Giemsa circle reaches a length of 5-7 cm. The following will be formed: a) A blue ring forms in the center of the circle.; b) A purple ring forms on the outer part.; c) A thin red ring is present on the edge. If the Giemsa is damaged, it cannot be used, indicated by the absence of the purple or red color formation (Benusu, 2019).

Research Design: In this study, the static group comparison method was applied. In this method, one group will be subjected to a specific treatment. The sampling method for this study employed Simple Random Sampling, where the existing population was selected randomly without considering any potential stratification within the population. The samples will be tested using 1 variation and 1 control. The number of repetitions found in this study was determined using the Federer formula. Therefore, the PBS staining using phosphate buffer will be prepared into 16 slides, and the staining using AC waste water will also be prepared into 16 slides. The stages used in the research began with the collection of air conditioner waste, followed by the preparation of 5% GIEMSA solution, GIEMSA quality testing, PBS preparation, GIEMSA staining, and finally, the calculation of staining scores for the slides using phosphate buffer and air conditioner waste.

Research Procedure:

The data type utilized in this research is primary data, obtained from the results of the examination of leukocyte type morphology on PBS (Peripheral Blood Smear) stained with Giemsa, which was diluted using phosphate buffer and AC waste water. The following is the procedure for research data collection: Pre-Analytical Phase (Collection of Air Conditioner waste water, Preparation of 5% Giemsa Solution with Phosphate Buffer Diluent, Preparation of 5% Giemsa Solution with AC Waste Water Diluent and Giemsa Quality Test).

Analytical Phase include PBS Preparation and Giemsa staining. The procedure Peripheral Blood Smear (PBS) Preparation firist Clean and dry the microscope slide (object glass). Sechond drop the blood sample about 2 cm from one edge or about 0.5 cm from the area designated for the identity label. Third place the pusher slide in front of the drop at an angle of 30 -40, then gently push the pusher slide backward until it touches the drop. Next, The blood will spread along the edge of the pusher slide. Afterthat immediately push the pusher slide forward rapidly and with sufficient pressure. Last Allow the blood smear to air dry (Kiswari, 2014). Sechond, Giemsa Staining the basic principle of Giemsa staining is that acidic substances will only stain basic cells. Conversely, basic substances will stain acidic cells. The cytoplasm stains pink due to eosin, while the acidic nuclei of leukocytes stain purplish-blue due to methylene blue (Khasanah et al., 2023). The following are the steps for performing Giemsa staining: First, store the PBS slides on the staining rack, with the smeared side facing up. Sechond, Flood the entire blood smear with methanol, and let the methanol stand for 5 minutes or until it dries. Third, If there is still residual methanol, it can be discarded, and the slide should be allowed to air dry. Fourth, drop the Giemsa solution until it completely covers the entire surface of the PBS. Last, let it stand for 20 minutes (Nugraha and Badrawi, 2018).

Post-Analytical Phase are Assessment of leukocyte type morphology on PBS stained using phosphate buffer diluent and AC waste water diluent was performed. The results of the staining were read by 3 panelists who provided a predetermined score. The criteria for assessing leukocyte type morphology on PBS were given a score of 1 and 2 with the following criteria: **(**a**)**. A score of 1 is

given when the clarity and color quality are poor. **(**b**)**. A score of 2 is given when the clarity and color quality are good.

Instruments and Equipment:

The Instrument used in the observation are object glass (microscope slide), staining rack, dropping pipette, microscope, beaker glass, stirring rod, filter paper, and pH meter.

Data Analysis:

The data were obtained through the observation of leukocyte type morphology using a microscope. This data was compiled in the form of scores based on evaluation criteria, namely clarity, color quality, and the distinctness of the boundary between the nucleus and the cytoplasm of the leukocyte types. SPSS was used to process the data in this study, which involved hypothesis testing preceded by a normality test. The data will analytic use Wilcoxon test to analyze of difference each group.

Results:

This research was conducted at the Hematology Laboratory of the Medical Laboratory Technology Department, Poltekkes Kemenkes Banten in March 2024. In this study, AC waste water was collected at the Tangerang campus around at 10:00 AM, which was subsequently filtered and its pH measured before being used as an alternative buffer in Giemsa staining.

a. (b)

Picture 1. (a) Results of the Giemsa quality test for the Giemsa solution using phosphate buffer diluent, (b) Results of the Giemsa quality test for the Giemsa solution using AC waste water diluent.

The results of the Giemsa quality test showed the same results for the Giemsa diluted with phosphate buffer and AC waste water: a blue ring formed in the center, followed by a purple ring on the outside, and a thin red ring at the outermost edge.

Observation of Leukocyte Cells on PBS (Peripheral Blood Smear). The assessment of Giemsa staining slides was performed using microscopic evaluation. A total of 16 slides were prepared using Giemsa dissolved with phosphate buffer, and 16 slides were prepared using AC waste water as the diluent. The microscopic assessment was conducted by the researcher and 2 other verifiers, one of whom is an expert in Hematology. The assessment criteria focused on the nucleus, cytoplasm, and granules of each cell, specifically checking for appropriate color, shape, and number of lobes in each cell.

a. (b)

Picture 2. (a) Eosinofil cell using phosphate buffer diluent;(b) Eosinofil cell using AC waste water diluent.

The research results shown in the table above involved 16 repetitions for slides using both AC waste water and phosphate buffer. Slides using phosphate buffer mostly received a score of 2, except for the cytoplasm and granules, where 3 repetitions received a score of 1. In contrast, slides using AC waste water received a score of 1 in 2 repetitions for the cell nuclei, 9 repetitions for the cytoplasm, and 10 repetitions for the granules.

a. (b)

Picture 3. (a) Neutofil cell using phosphate buffer diluent; (b) Neutrofil cell using AC waste water diluent

In neutrophil cells, the majority received a score of 2 in every cell category. In slides using AC waste water, only 2 repetitions for the cell nuclei, 5 repetitions for the cytoplasm, and 6 repetitions for the granules received a score of 1.

a. (b)

Picture 3. (a) Lymphocyte cell using phosphate buffer diluent; (b) Lymphocyte cell using AC waste water diluent

In lymphocyte cells, 2 repetitions for the cell nuclei and 4 repetitions for the cytoplasm received a score of 1 in slides using AC waste water.

a. (b)

Picture 3. (a) Monocyte cell using phosphate buffer diluent; (b) Monocyte cell using AC waste water diluent

The following is an image of monocyte cell observation results using phosphate buffer and AC waste water diluents: In monocyte cells stained with AC waste water diluent, 81% of the cell nuclei and 88% of the cytoplasm achieved a score.

The data obtained from microscopic observation were first statistically processed using a normality test to determine if the data were normally distributed. Based on normality test for the nuclei, cytoplasm, and granules of each cell with p value (0.000) < (0.05). Therefore, the data were not normally distributed, and the Wilcoxon test was subsequently performed.

From the statistical test table, an Asymp. Sig. value > 0.05 was obtained for the eosinophil nuclei, neutrophil nuclei, lymphocyte nuclei, monocyte nuclei, and monocyte cytoplasm. Therefore there is no significant difference in the leukocyte type morphology on PBS using phosphate buffer diluent and AC waste water.

Table 1. The data of statictic test based nuclei, cytoplasm and Granules cell with * mark is Asymp. Sig. value < 0.05

	p-value
Nuclei Eosinofil Cell	0.157
Cytoplasm Eosinofil Cell	0.034*
Granules Eosinofil Cell	0.020*
Nuclei Neutrofil Cell	0.157
Cytoplasm Neutrofil Cell	0.025*
Granules Limphocyte Cell	0.014*
Nuclei Limphocyte Cell	0.157
Cytoplasm Limphocyte Cell	0.046*
Nuclei Monocyte	0.083
Cytoplasm Monocyte	0.157

Conversely, an Asymp. Sig. value < 0.05 was obtained for the eosinophil cytoplasm, eosinophil granules, neutrophil cytoplasm, neutrophil granules, and lymphocyte cytoplasm. This indicating there is a difference in the leukocyte type morphology on PBS between the use of phosphate buffer and AC waste water.

Discussion

In this study, microscopic observation of peripheral blood smears (PBS) was performed on the nuclei, cytoplasm, and granules of leukocytes. The leukocyte types observed were eosinophils, neutrophils, lymphocytes, and monocytes, but basophils were excluded. This was because basophils were not found in the blood smears prepared. Basophils are the least numerous cells, accounting for approximately < 2% of the total leukocyte count. Basophils are rarely found in normal peripheral blood. Basophils are white blood cells that contain (and can release) histamine and serotonin during

the immune response. Their numbers increase in allergic reactions and malignancies (Victoria et al., 2019). Furthermore, in the study by (Anwar et al., 2023), the staining method recommended for observing basophils is the combined Wright-Giemsa stain.

The nuclei of all observed leukocyte types showed identical staining results between the Giemsa staining performed with phosphate buffer and AC waste water. They received a score with a majority of 2 (good) and the Wilcoxon test Asymp. sig > 0.05. The staining result for the nuclei of every observed leukocyte type was a purplish-blue color, which is the same as the result obtained when diluted with phosphate buffer. The pH check of the AC waste water before it was used to dissolve the Giemsa showed a pH range similar to that of the phosphate buffer. If the pH is less than 6.8, the leukocyte nucleus, which should be purple, only forms partially and is partially red (Indriani, 2017).

Regarding the cytoplasm, only monocyte cells obtained sig > 0.05. meaning there was no significant difference between the cytoplasm of monocyte cells using phosphate buffer and AC waste water diluents.

However, for the granule section, all leukocyte types had Asymp. sig < 0.05. The research results showed a purplish-red color for the cytoplasm and granules in all leukocyte types, but in the leukocyte cells using the AC waste water diluent, there were several fields of view where the cytoplasm was poorly stained. In Giemsa staining, if the diluent pH is too low, basic cells tend to absorb the acid dye. If the pH is too high, the acidic components of the cells may excessively absorb the basic dye, causing the cytoplasm and granules to appear blue (Yati et al., 2019).

In Giemsa staining, the buffer solution is used to maintain a stable pH of the preparation, preventing pH changes caused by the addition of acids/bases from the substances contained in Giemsa and water during the staining process. The goal is to ensure the cells can absorb the resulting dyes. In Giemsa staining, phosphate buffer with a pH of 6.8-7.2 is typically used. However, other diluents can be used as an alternative, provided they have a pH within that range (Yati et al., 2019).

AC waste water, a product of the condensation process, may be utilized for certain uses. Based on the research by Indrawati T and Ningsih N.I.D. (2018), the properties of AC waste water are similar to aquadest (deionized water), making it a potential substitute for aquadest, which is commonly used as a diluent in the Giemsa staining process.

Several factors can affect how the blood smear preparation is stained using Giemsa, such as differences in diluent pH, poor quality of Giemsa stock, quality of the Giemsa diluent, thickness of the blood smear, dirty microscope slides, duration of methanol fixation, staining time being too short, and concentration of the staining solution (Yati et al., 2019). Based on the research findings, there are some weaknesses and shortcomings in this study. The weaknesses and shortcomings include: Not finding all types of leukocyte cells (specifically the basophil cell) because the blood sample used for PBS preparation was a non-pathological blood sample. The other limitation in this research is the lack of measurement of water quality parameters such as Total Dissolved Solids (TDS) and Electrical Conductivity (EC) on the AC waste water used as the Giemsa staining diluent. This measurement would have confirmed whether the AC waste content met the requirements to replace the standard diluent in Giemsa staining.

Conclusions

This experimental study compared the use of Air Conditioner (AC) waste water versus phosphate buffer as a diluent for Giemsa staining in preparing Peripheral Blood Smears (PBS) to examine leukocyte morphology.

1. Nuclei Staining Quality: There was no significant difference in the staining quality of the leukocyte nuclei across all types observed (eosinophils, neutrophils, lymphocytes, and monocytes), with most nuclei receiving a "good" score Asymp. Sig > 0.05. This is attributed to the AC waste water having a similar, suitable pH to the phosphate buffer.
2. Cytoplasm and Granule Quality: A significant difference was found in the staining quality of the cytoplasm and granules of most leukocytes Asymp. Sig < 0.05, including eosinophils, neutrophils, and lymphocytes. The AC waste water diluent resulted in less satisfactory

staining for these cellular components, suggesting that while the pH was appropriate for the nucleus, other properties (like lack of buffering capacity or trace impurities) likely affected the cytoplasmic and granular staining.

3. Monocyte Exception: The monocyte cytoplasm was the only component outside the nuclei to show no significant difference Asymp. Sig > 0.05.
4. Implication: AC waste water is a viable alternative for staining leukocyte nuclei, but its use is not recommended for achieving optimal staining quality of the cytoplasm and granules of all major leukocyte types.

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