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Comparison of Carbol Fuchsin Concentration on the Results of Acid-Fast Bacteria Staining (AFB)

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ABSTRACT

Background & Objective: Infections caused by the bacterium *Mycobacterium tuberculosis* are known as tuberculosis. (TB). The treatment process takes a long time because this bacillus bacteria is very strong. Compared to other parts of the human body, this bacteria more frequently attacks the lungs. Patients who test positive for acid-fast bacilli (AFB) are the cause of pulmonary tuberculosis (TB). The aim of this research is to determine how the concentration of Carbol Fuchsin differs in the results of Acid-Fast staining. (BTA).
Method: This research was conducted in a laboratory and used purposive sampling techniques with the Ziehl-Neelsen staining method, resulting in 5 positive acid-fast bacilli (AFB) samples.
Result: The results of this study indicate that the best concentration is 1.5% carbol fuchsin, so it can be concluded that the optimal staining concentration is 1.5% carbol fuchsin.
Conclusion: Based on the research conducted, it was concluded that the higher the concentration of carbol fuchsin, the stronger it binds to the cell walls that have a lipid layer, thus being able to bind the red colour.

Keywords: Tuberculosis; Carbol Fuchsin; Acid-Fast Bacilli

Introduction

The infectious disease known as tuberculosis (TB) attacks the lungs, which are one of the organs of the body. The bacterium *Mycobacterium tuberculosis*, also known as acid-fast bacillus (AFB), is the cause of tuberculosis (Pralambang & Setiawan, 2021). The report from the Ministry of Health of the Republic of Indonesia indicates that in 2021, 443,235 cases of TB were identified and treated in Indonesia (kemenkes, 2022). Tuberculosis is a major problem worldwide, especially in developing countries like Indonesia. Every day, 5,000 people die from tuberculosis. (TB). This is very concerning and must be addressed because individuals

suffering from pulmonary tuberculosis can transmit it to those around them (Nizar, 2017) The discovery of cases through microscopic examination of Acid-Fast Bacilli (AFB) from respiratory tract specimens or sputum is crucial in the early diagnosis and monitoring of pulmonary tuberculosis, as part of the DOTS strategy (Kesehatan et al., 2016) . The staining technique used is Ziehl-Neelsen (ZN), which can detect Acid-Fast Bacilli (AFB) using a microscope (Purba & Manurung2, 2017).

Acid-Fast Bacilli (AFB) is a group of bacteria with thick cell walls and high lipid content, which makes traditional staining difficult. The standard method for identifying acid-fast bacilli (AFB) is the Ziehl-Neelsen staining, which uses carbol fuchsin as the primary dye, followed by decolorization with acid-alcohol, and counterstaining with methylene blue or malachite green. Carbol fuchsin is crucial because it can penetrate the lipid cell wall and impart a red color to AFB, making it easily identifiable under the microscope (Kalma & Adrika, 2019).

The concentration of carbol fuchsin used can affect the intensity of staining and the clarity of the results. A concentration that is too low may not provide adequate staining, while a concentration that is too high can lead to excessive staining that obscures the observation results. Therefore, this research was conducted to evaluate the effect of various concentrations of carbol fuchsin on the quality of BTA staining.

Objective

This research determined that the concentration of Carbol Fuchsin differs in the results of Acid-Fast staining (AFB).

Method

Describe This research was conducted at the Bacteriology Laboratory of Muhammadiyah Health Polytechnic Makassar. This study employed an experimental design with several treatment groups, each using different concentrations of carbol fuchsin. The independent variable in this study is carbol fuchsin, while the dependent variable is the staining results of Acid-Fast Bacilli (AFB) at a magnification of 100x using an objective lens.

The Acid-Fast Bacilli (AFB) samples were obtained from sputum cultures, which involve examining sputum to detect the presence of bacteria that cause respiratory tract infections, particularly lung infections. (pneumonia). Phlegm is a fluid produced by the respiratory tract that is expelled from the respiratory system when coughing. The three concentrations of carbol fuchsin tested in this study are 0.5%, 1%, and 1.5%. Staining was performed according to the modified Ziehl-Neelsen protocol for each concentration of carbol fuchsin.

Carbol Fuchsin at concentrations of 0.5%, 1%, and 1.5% was added to test tubes containing sputum. Then, the tube was heated over a water bath at temperatures of 60°C and 80°C. The results of this treatment consist of the preparation of the specimen and BTA staining. The observations were compared with the control using the Ziehl-Neelsen method. The data obtained were processed by examining the microscopic images of the BTA staining. Next, the study explored how heating temperature and the concentration of Carbol Fuchsin affect the results of AFB staining. This research employed purposive sampling techniques with criteria for samples

of patients with positive AFB examination results. The data in this study will be processed descriptively and presented in the form of tables.

Results

Based on the results of laboratory examinations conducted microscopically on 5 samples of Acid-Fast Bacilli (AFB) using varying concentrations of carbol fuchsin at 0.5%, 1%, and 1.5%, a total of 15 preparations were obtained. The samples used were sputum from TB patients with positive Acid-Fast Bacilli (AFB) criteria.

TABLE 1. Results of the microscopic analyzing of the Acid-Fast Bacilli staining

No.	Code Sample		***Carbol Fuchsin***	
		1,5%	**1%**	**0,5%**
1	A	+2 (10/LP)	+1 (27/100LP)	*Scanty* (5/100LP)
2	B	+1 (59/100LP)	+1 (40/100LP)	+1 (12/100LP)
3	C	+2 (10/LP)	+2 (8/LP)	+1 (35/100LP)
4	D	+1 (14/100LP)	*Scanty* (6/100LP)	*Scanty* (2/100LP)
5	E	+1 (20/100LP)	+1 (12/100LP)	*Scanty* (5/100LP)

Discussion

The results of the research conducted after the preparation of carbol fuchsin concentrations of 0.5%, 1%, and 1.5% in Table 1 indicate that the staining with carbol fuchsin concentrations is as follows: the higher the concentration, the more concentrated and intense the solution will be, allowing the cell walls with lipid layers to bind the red colour to the stained cells. Conversely, the lower the concentration, the more diluted and pale the carbol fuchsin colour becomes, making it lighter and less visible. The control preparation shows a red BTA and a clear blue background.

Based on the results of the research conducted, it was found that the best staining results were achieved using carbol fuchsin at concentrations of 1.5%, 1%, and 0.5%, with the average best staining being with 1.5% carbol fuchsin. The majority of the quality was good due to the high concentration of the 1.5% carbol fuchsin reagent, which made the BTA clearly visible. The solution is concentrated and strong, allowing the cell walls with a lipid layer to bind the red colour to those cells.

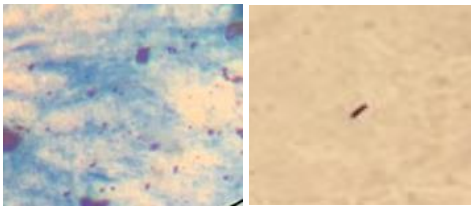

FIGURE 1. Results of BTA staining with 1.5% Carbol Fuchsin

1% carbol fuchsin yielded good results; the AFB shape was still clearly visible under the microscope, and the solution was concentrated and not too diluted. 1% carbol fuchsin is not much different from 1.5% carbol fuchsin, so the cell walls with lipid layers still clearly retained the red colour in those cells.

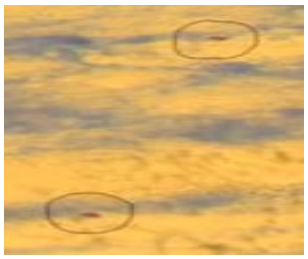

FIGURE 2. Results of AFB staining with 1% Carbol Fuchsin.

The concentration of 0.5% carbol fuchsin is not very good because the solution is diluted and paler, resulting in a lighter carbol fuchsin colour and a more faint red colour of the acid-fast bacilli (AFB) under the microscope.

The cell wall with a less distinct lipid layer binds less clearly.

FIGURE 3. Results of AFB staining with 0.5% Carbol Fuchsin.

Carbol fuchsin is used as the primary stain to identify acid-fast bacteria. Acid-fast bacteria have cell walls with very tight pores, making it difficult for pathogens to penetrate. According to the research by Selvakumar (Selvakumar et al., 2002), staining with 1% carbol fuchsin has an optimal concentration, a concentrated solution, and strength, allowing cell walls with a lipid layer to bind the red colour in the cells. The cell wall pores will close again after cooling, so the dye within the bacterial body does not wash out during the alcohol acid treatment. On the contrary, acid-sensitive bacteria release the first dye (Ariyani, 2019).

Conclusion

Conclusions Based on the research results regarding the comparison of carbol fuchsin concentrations and the outcomes of Acid-Fast Bacilli (AFB) staining, it shows that staining with 1.5% carbol fuchsin has the highest concentration. This is because the 1.5% carbol fuchsin reagent has a high concentration, a concentrated solution, and possesses the necessary strength to bind the red colour to the cell walls that have a lipid layer.

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Conflict of Interest

There is no conflict of interest in this research.

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