Identification of Air Bacteria Using Gram Style Methods in The Integrated Laboratory of Bina Mandiri University Gorontalo

Erfan Lainjong

Universitas Bina Mandiri Gorontalo and erfan.laindjong@ubmg.ac.id

ABSTRACT

In the field of microbiology, the study of microorganisms that are not visible to the naked eye is conducted in order to investigate the components that are found within bacteria. Gram stain, which is one of the most extensively used processes to characterize a wide variety of bacteria, is required in order to see microorganisms. The purpose of this research is to get an understanding of the chemical and theoretical foundations of the differential staining method, as well as the procedure for distinguishing between the two primary categories of bacteria, which are gram positive and gram negative and gram negative bacteria. An experimental approach was taken in this study as the technique of investigation. In the context of laboratory research, the experimental technique is a method that is utilized. This experimental approach is being used with the intention of determining the outcomes of a treatment that was administered on purpose by the researcher. Additionally, the gram stain method was utilized in order to offer an overview of airborne bacteria. This was accomplished through the utilization of a qualitative descriptive methodology. In the gram staining experiment that was carried out, the findings revealed that samples of NA medium in airborne bacteria were carried out using a microscope at a magnification of 10x. The purpose of this experiment was to observe the color of the bacteria, and the results showed that the bacteria produced a purple hue. In addition to the results of the kind of bacteria acquired in the gram stain experiment, which indicated that the bacteria in question belong to the gram-positive bacteria group, this demonstrates that the bacteria in question are E. coli bacteria, which have a form known as monobacillus.

Keywords: Airborne Bacteria, Gram Stain

1. INTRODUCTION

The study of microorganisms that are not visible to the human eye is known as microbiology. The purpose of this research is to investigate the components that are found within bacteria. In the same way as bacteria have their own unique morphology, structure, and properties, microorganisms that are found in nature also have certain characteristics. The water, in which the bacterial cells are floating, provides a striking contrast to the nearly colorless live microorganisms of the environment. Bacteria may be found in virtually any environment, including the soil, water, and air; they can also be found in symbiosis with other species or as agents of parasites (pathogens), and they can even be found in the human body. The size of bacteria, in general, ranges from 0.5 to 5 micrometers. However, there are some bacteria that may reach a diameter of up to 700 micrometers, like thiomargarita.

Generally speaking, they have a cell wall, just like plant and fungal cells, but the building elements that make up their cell walls are extremely different (peptidoglycan). As a result of the presence of flagella, certain species of bacteria are able to move about, which is referred to as motility. The use of staining or staining is one method that may be utilized to study the form of a bacterial cell in order to make it as easy as possible to identify. Additionally, it serves the purpose of determining its physiological features, namely the reactivity of the bacterial cell wall by a series of stains. The objective of staining is to provide a color to the germs, which will ultimately allow for their identification with greater ease. There is also an endospore that may be dyed, which is an

additional feature. [6] Endospores are creatures that are created in stressful conditions due to a lack of nutrients. These organisms have the potential to continue existing in the environment until conditions become suitable.

Gram stain is one of the most widely used procedures for characterizing many bacteria. From gram staining, cell morphology can be seen, including characteristics of gram, cell shape, and cell arrangement. Gram staining or the gram method is an empirical method for differentiating bacterial species into two major groups, gram positive and gram negative, based on the chemical and physical properties of their cell walls, this method is named after its discoverer, scientist Denmar khans Christian gram. Gram staining is divided into two, namely compound coloring because it uses more than one kind of dye. And differential staining because this staining is able to differentiate or differentiate bacteria, so that bacteria can be classified into two, namely Gram negative and Gram positive [11].

The gram staining technique must be in accordance with the procedure because it can lead to misidentification of data whether it is gram positive or gram negative so that this experiment is needed in order to know the operation of the gram staining mechanism [7].

2. LITERATURE REVIEW

2.1 Gram

Stain Gram stain, also known as the Gram technique, is an empirical approach that is used to differentiate bacterial species into two primary categories, namely grampositive and gram-negative, based on the chemical and physical features of their cell walls. The Danish scientist Hans Christian Gram (1853–1938) is credited with developing this method in 1884 in order to identify between pneumococus and the bacterium Klebsiella pneumoniae. This method was named after the Danish scientist who created it. Because it is an essential step in the earliest stages of identification, the Gram stain method is also a highly helpful differential stain. It is the method that is utilized the most frequently in laboratories that specialize in microbiology. The existence of ionic connections between the cellular components of the bacterium and the active chemical of the dye, which is referred to as chromogen, is the fundamental basis of coloring. The quantity of fat layer on the bacterial cell membrane and the thickness or thinness of the peptidoglycan layer on the cell wall are the two factors that determine the staining intensity of this staining. There are two categories of bacteria that may be distinguished from one another based on the gram staining method: gram positive and gram negative [4].

The Gram stain technique is used to identify bacteria, and Gram-negative bacteria are bacteria that do not retain the methyl purple dye. After being washed with alcohol, gram-positive bacteria will keep the dark purple methyl dye, but gram-negative bacteria will not keep the color. After the addition of methyl purple, a counterstain dye is introduced in the Gram stain test. This dye causes all gram-negative bacteria to become red or pink. This test is helpful for distinguishing these two species of bacteria based on variations in the structure of their cell walls, which these bacteria have in common. The distinctions between gram-positive and gram-negative bacteria are as follows: 1. Bacteria Gram-negative

Nacteria Gram-negative bacteria are bacteria that do not retain the methyl purple dye in the Gram stain method. Gram-positive bacteria will retain their dark purple color after washing with alcohol, while gram-negative bacteria will not.

2. Gram Positive

Bacteria Gram positive bacteria are bacteria that retain the methyl purple dye during the Gram stain process. These types of bacteria will stain blue or purple under a microscope, while gram-negative bacteria will appear pink. The difference in classification between the two types of bacteria is mainly based on differences in the structure of the bacterial cell wall [1].

Bacteria have various forms of morphology, namely, round, rod and spiral.

- 1. Rod-shaped bacteria The rod-shaped bacteria are known as bacilli. The word basil comes from bacillus which means stem. The form of bacillus can also be divided into:
 - a. Single bacillus, which is a bacterium that is only in the form of a single stem, for example salmonella typhi, which causes typhus.
 - b. Diplobasil is a bacteria in the form of a rod that holds two or two.
 - c. Streptobacil is a rod-shaped bacterium that extends together to form a chain, for example, Bacillus anthracis, which causes anthrax disease.
- 2. Ball-Spherical bacteria are known as Coccus. These bacteria can also be divided into:
 - a. shaped bacteriaMonococcus, which is a single spherical bacterium, for example Neisseria gonorrhoeae, which causes gonorrhea.
 - b. Diplococcus, which is a ball-shaped bacteria that works together with two or two, for example, Diplococcus pneumonia, which causes pneumonia or pneumonia.
 - c. Sarkina, which is a spherical bacterium in groups of four so that it looks like a cube.
 - d. Streptococci, which are spherical bacteria that cluster lengthwise to form chains.
 - e. Staphylococci, which are ball-shaped bacteria that colonize to form a group of irregular cells so that they look like a bunch of grapes.
- 3. There are three kinds of spiral forms:
 - a. Spiral-shaped bacteriaSpiral, which is a group of bacteria that looks like a spiral, such as Spirillum.
 - b. Vibrio, this is considered as an imperfect spiral form, for example, Vibrio Cholera, which causes cholera.
 - c. Spirochaetes, which are spiral-shaped bacteria that are flexible.

When moving, the body can lengthen and contract [6].



Figure 1: The Shape of Bacteria

Table 1. Relative Personality Differences Gram Positive Bacteria and Gram negative

properties	bacterialgram (+)	bacteria Gram (-)
The composition of the cell wall	lipid content is low (1-4%)	of high lipid content
Resistance to penicillin	More sensitive	Less susceptible to
inhibition by the dye bases (VK)	More inhibited	Less inhibited
Nutritional requirements	Most species are relatively complex	Relatively simple
Resistance to physical treatment	More resistant	Less resistant

Gram-negative bacteria have 3 cell wall layers. The outermost layer, namely the lipo posaccharide (lipid), may be washed by alcohol, so that when it is stained with safranin it will turn red. Gram-positive bacteria have a thick layer of peptidoglycan cell wall. After staining with crystal violet, the pores of the cell walls are narrowed due to decolorization by alcohol so that the cell walls retain their blue color [6].

Gram positive bacterial cells may appear red if the decolorization time is too long. Meanwhile, gram-negative bacteria will appear purple if the decolorization time is too short [6].

Bacillus subtilis is a gram-positive rod-shaped bacteria, and is often found naturally in soil and vegetation. *Bacillus subtilis* grown in various mesophilic temperatures ranging from 25-35°C. *Bacillus subtilis* has also evolved to be able to live even though under harsh conditions and faster to obtain protection against stress situations such as conditions of low pH (acid), alkaline, osmotic, or oxidative conditions, and heat or ethanol. These bacteria have only one DNA molecule which contains a set of chromosome sets. Its DNA size is BP 4214814 (4.2 Mbp) (TIGR CMR). 4,100 protein gene codes. Some of the advantages of this bacterium is that it is able to secrete large amounts of antibiotics out of cells [9].

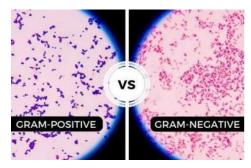


Figure 2: Gram Negative-Positive Bacteria

Methods for gram stain:

The application of an alkaline dye known as crystal violet is the first step in the gram stain method. In the subsequent step, the iodine solution is introduced, and during

this phase, all of the bacteria will be dyed blue. After then, alcohol is passed into the cells. Cells that are color negative are destroyed by alcohol, but gram-positive cells will continue to bind crystal violet-iodine molecules and will continue to be blue. A countersain, such as safranin red dye, is applied as the final stage. This causes gram-negative cells, which are colorless, to take on a color that contrasts with the hue of the gram-positive cells, which look blue. The structure of the cell wall is the primary factor that contributes to the variation in the gram reaction [13].

Gram stain gives good results, if used fresh cultures aged 24-48 hours. When old cultures are used, there is a possibility of deviations from the results of the gram stain. In old cultures, many cells have damage to their cell walls. This damage to the cell walls causes the dye to come out when washed with a bleaching solution. This means that gram-positive bacteria with damaged walls can no longer maintain the crystal violet-iodine color complex so that they appear as gram-negative bacteria [8].

The following points to consider in gram staining are as follows:

- The most critical phase of gram staining is the decolorization stage which results in iodine being released from cells. Do not give excessive alcohol which will cause excessive decolorization so that gram-positive cells look like gram-negative. But also, don't put too little in the drop of alcohol that won't dissolve iodine completely so that gram-negative cells are like gram-positive.
- 2. When preparing gram stain, it is advisable to utilize young cultures that have not been in existence for more than twenty-four hours. There is a correlation between the age of the culture and the capacity of cells to absorb the primary color, particularly in gram-positive bacteria. Due to the impact of age, it is possible that the gram variable would display a single kind of cell, with some cells colored purple and others colored red.

Classification of Bacteria

Classification of Bacillus subtilis [9].

Kingdom	: Bacteria
Phylum	: Firmicutes
Class	: Bacilli
Order	: Bacillales
Family	: Bacillaceae
Genus	: Bacillus
Species	: Bacillus subtilis

The rod-shaped bacteria known as Bacillus subtilis is a Gram-positive bacterium. Peptidoglycan, which is a polymer consisting of sugars and amino acids, is the material that makes up these microorganisms. Peptidoglycan, which is characteristic of bacteria and is referred to as murein. They are helpful for preserving cell shape and with standing cells that have a high internal turgor pressure [13]. Cells establish a barrier between the environment and bacterial cells, which is beneficial for maintaining cell composition.

In addition to being gram-negative and rod-shaped, Escherichia coli is a member of the family Enterobacteraceae, which is composed of fermentative bacteria. The presence of a significant number of E. coli bacteria in the human colon is beneficial to the digestive system of humans and serves to protect it from the presence of harmful bacteria [7]. On the other hand, the newly discovered strain of E. coli is a harmful bacterium that is responsible for symptoms of diarrheal sickness, advanced diarrhea syndrome, and hemolytic uremic (hus). It is possible to utilize it as an experimental waste in water, as an indication of the amount of water pollution, and for the detection of pathogens in human feces that are generated by Salmonella typhi [19]. These are all useful roles that it may play. *Escherichia coli* is also a type of bacteria that normally lives in the digestive tract of both humans and healthy animals. The name of this bacterium is taken from the name of a bacteriologist who comes from Germani, namely Theodor Von Escherich, who succeeded in isolating this bacteria for the first time in 1885. Dr. Escherich also succeeded in proving that diarrhea and gastroenteritis that occurs in infants is caused by the Escherichia coli bacteria.

classification E. coli [19].

Kingdom	: Bacteria
Phylum	: Proteobacteria
Class	: Gamma Proteobacteria
Order	: Enterobacteriales
Family	: Enterobacteriaceae
Genus	: Escherichia
Species	: E. coli

In general, Escherichia coli bacteria only recognize one type of culture, namely by sexual or vegetative means. This breeding takes place quickly, if external factors are favorable to him. If external factors are favorable, then after division, the new cells will grow until each of them becomes the size of the parent cell [10].

According to literature, *E. coli* belongs to the Enterobacteraceae family, which includes gram-negative and fermentative rod-shaped bacteria. *E. Coli* lives in large numbers in the human intestine, which helps the human digestive system and protects it from pathogenic bacteria. However, the new strains of *E. coli* are dangerous pathogens that cause diarrheal disease, advanced diarrhea syndrome, vomiting, hemolyticuremic (hus), intestinal infections, urinary tract infections, and neonatal meningitis. Its beneficial role is that it can be used as an experimental waste in water, an indicator of the level of water pollution and detecting pathogens in human feces caused by *Salmonella typhi*. In addition, *E. coli* is widely used intechnology genetic engineering and is commonly used as a vector to insert genes which is desired to be developed because these bacteria have a very fast growth and are easy to handle [5].

The introduction of microbial forms (morphology), except for microalgae, must be stained so that they can be observed clearly [11]. Bacterial life is not only influenced by external factors but on the contrary, bacteria are able to influence the state of the environment, for example, it can cause fever (heat) due to being infected by the Escherichia coli bacteria in the digestive tract and causing prolonged diarrhea. If Escherichia coli is in a medium containing a carbon source (glucose, lactose, etc.) it will change the degree of acid (pH) in the medium to become acidic and will form gas as a result of the process of breaking down glucose into other compounds.

3. METHODS

Approach In the context of laboratory research, the experimental technique is a method that is utilized. This experimental approach is being used with the intention of determining the outcomes of a treatment that was administered on purpose by the researcher. Through the use of controlled settings, the experimental technique is a study approach that is utilized to investigate the impact that particular therapies have on other individuals [14]. It is possible to conclude, on the basis of the definitions provided by a number of specialists, that experimental research is research that is carried out with the purpose of determining the effect that administering a treatment or therapy has on the topic of the research. The purpose of experimental research is to investigate the impact of a certain therapy on the symptoms encountered by a specific group in comparison to the symptoms experienced by other groups that were treated with a different treatment [20].

Description Materials

1.

•	Methylene Blue [3]
	Official name	: Methylthionini Chloridum
	another name	: Methylene blue
	RM/BM	: C ₁₆ H ₁₈ CIN ₃ S.3H ₂ O / 373.90
	Critical	: crystals or crystalline powder dark green, bronze berkilauan
	apparatus	seperti odorless
	Solubility	: Soluble in water and in chloroform
	Storage	: In a well closed container
	Use	: As the main paint in simple painting

2. Carbolic fuksin [3]

Name	: Magenta
Other names	: Karbolfuksin
Critical apparatus	: Powder crimson
Solubility	: Water soluble
Storage	: In a tightly closed container
Use	: As dye bases

3.	Crystal Violet [3]	
	Official name	: Crystal violet
	Critical apparatus	: crystals green old
	Solubility	: Difficult to dissolve in water
	Storage	: In a well-closed container
	Uses	: As a main paint or gram

Tools and Materials

Tools

The tools used at the time of the experiment are: microscopes, bunsen burners, coloring trays, glass objects, ose / inoculation needles.

Materials

Materials used at the time of the experiment, namely; air bacteria cultures, tool bacteria cultures, crystal violet, iodine grams, ethyl alcohol, and safranin.

Work Procedure

- 1. Prepared clean
- 2. slides Prepare each smear of three organisms and to one slide. This stage is carried out by placing a drop of water on a slide, and then moving each organism separately from the water drop with a sterile loop and cooling it.
- 3. Allow the smear to dry in air and then heat fixation as
- 4. usual.Flood the smear slowly with crystal violet and leave it for 1 minute
- 5. Rinsed the smear with tap water slowly
- 6. Soak the smear slowly with iodine bleach and leave it for 1 minute
- 7. Rinse with tap water is slowly
- 8. Dipucatkan color with 95% ethyl alcohol
- 9. Shampoo with tap water slowly
- 10. Given dye counter with safranin for 45 seconds
- 11. rinsed with water flowing slowly
- 12. dried with paper biboluous and observed under the objective lens

4. RESULTS AND DISCUSSION

Based on research conducted observational data obtained are:

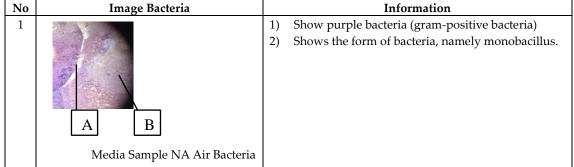


Table 2. The results of gram staining observations

A purple hue was produced by the gram staining experiment, which was carried out in accordance with the findings of the gram staining research that was conducted under a microscope with a magnification of ten times. The purpose of the study was to observe the color of the bacteria. At the gram staining stage, after being given a solution of Crystal violet, lugol, and safranin, and washing with bacterial alcohol, which appears purple, as well as the results of the type of bacteria obtained in the experiment, this demonstrates that these bacteria are included in the group of grampositive bacteria. This is because of the fact that the gram staining stage also includes the results of the experiment. In the Gram stain, E. coli bacteria were found [21]. During the subsequent operation, which was carried out under a microscope with a magnification of one hundred times in order to examine the form of the bacteria that were encountered, the findings revealed that these bacteria had a monobacillus shape [12].

Discussion

Gram stain is a stain that is utilized for the purpose of delineating gram-positive and gramnegative microorganisms. Crystal violet dye will be retained by Gram-positive bacteria, which will result in the bacteria appearing dark purple when seen via a microscope. While this is going on, gram-negative bacteria will lose their crystal violet dye after being washed with alcohol, and when they are given water dye fucsin or safranin, they will look red [22]. The difference in dye is caused by differences in the chemical structure of the cell walls. Dyes used in gram stain include crystal violet, alcohol, safranin, and iodine [8]. The purpose of this Gram stain is to make it easier to see bacteria microscopically, to clarify the size and shape of the bacteria, to see the structures in bacteria such as cell walls and vacuoles, and to produce physical and chemical properties typical of bacteria with dye. In staining, Gram positive bacteria stain purple while Gram negative bacteria stain red [14].

The purpose of gram staining is to identify microorganisms, which types of bacteria and bacteria can be identified according to their categories, namely gram-positive and gram-negative, one of the bacteria has several forms, namely bacillus (rod), coccus (round), and spirilum (curved) [12]. Bacillus-shaped bacteria are divided into *diplobacillus* and *tripobacillus*. In the form of *coccus* divided into *monococcus*, *diplococcus*, to *staphylococcus* (looks like grapes). Specifically, spirilum is only divided into two, namely half curved and not curved [10].

In this study only the Gram stain method was used to identify bacteria with their advantages and disadvantages. The advantage is that Gram stain is one of the simplest and inexpensive methods for rapid diagnosis of bacterial infection [16]. This method is much faster than bacterial culture, and serves as an initial guideline for deciding on antibiotic therapy before definitive evidence is available of specific infection-causing bacteria [16]. The drawback of this method is that it can only determine the size and shape of the bacteria and see the structure in the bacteria using dyes only. The condition of Gram stain and bacterial morphology sometimes changes due to antimicrobial therapy [6]. Gram negative rod species can become filamentous and pleomorphic whereas Gram positive bacteria can become variable after antimicrobial therapy [19].

In the first research we did was to prepare tools and materials. The tools used are petri dishes, glass objects, bunsen, ose, dropper, and microscope. While the materials used are NA, purple crystal, iodine, 70% ethanol, safranin, and aquadest.

The next step is to take bacteria from the isolation media using ose. Ose is also heated on top of the bunsen so that the condition is aseptic. After that, it is touched on a medium that has no bacteria, because if the conditions are too hot the bacteria can die. After that, bacteria are taken and scratched on the glass object. Then a glass object was fixed near the bunsen to condition the aseptic and to expand the internal and external structures of cells and microorganisms. Then drop with purple crystals using a dropper and let stand for 1 minute. This is because at 1 minute it is assumed that the purple crystal has locked and the function of the purple crystal itself is as a primary dye [22].

Note that crystal violet or purple is a triarylmethane dye. This stain is used as a histological stain in the gram method of bacterial classification. Crystal violet has antibacterial, fungal and deworming properties, and was previously important as a topical antiseptic [15].

After that, rinsed with aquadest, this is so that the remaining purple crystals that can fade and clarify the observation. After rinsing with aquadest, then dripping with iodine and left for 2 minutes, iodine functions as a purple crystal color amplifier, and is left to stand for 2 minutes because at that time it is assumed to have quite clearly given the bacteria a purple color. In contrast to the previous study, the gram staining procedure was carried out for only 1 minute after dripping with iodine and other materials, the results of this study found several types of bacteria, but the indwelling process for 1 minute in this study did not provide clear color results during identification [12].

After that, rinse again with aquadest, this aims to clean the remaining iodine in bacteria. Then washed with 70% ethanol serves to dissolve fat, then rinsed again with aquadest. This aims to clarify observations. After that drip with safranin. Safranin is a biological stain used in histology and cytology [20]. Safranin is used as a conterstain in several staining protocols. Safranin has a chemical structure [20]. In addition, Fafranin functions as a secondary dye and as a sign that the bacteria is

Gram negative [7]. Allow 10 minutes because this time it is assumed that the cell wall has locked onto the safranin. Then rinsed with aquadest, this aims to rinse the remaining safranin and to clarify observations [7]. Then the final step is to observe the preparation under a microscope. After observing it under a microscope atmagnification 10x, this 10x magnification is used to see the color of the bacteria, in the gram staining research that has been carried out it produces a purple color [7]. This shows that these bacteria are included in the gram-positive group of bacteria, as well as the results of the type of bacteria obtained in the gram stain experiment, namely *E. coli bacteria*. In the next procedure with a 100x magnification under a microscope which aims to see the shape of the observed bacteria, the result is that the bacteria has ashape *monobacillus* [7].

Escherichia coli is a normal bacterial flora in the human intestine, spread in the environment through water or equipment contaminated with human feces [4]. Water is the only vehicle for transmission of oral faecal pathogens such as *Escherichia coli*. Contaminated water, hand held hands, laboratory equipment, and laboratory clothing are risk factors that play a role in increasing the risk of bacterial contamination [19]. These bacteria will turn into pathogens and cause infection if they are outside their normal habitat (outside the intestine) such as the skin [4]. This contamination can occur directly from the laboratory environment or laboratory personnel [4].

The cell walls of Gram-positive bacteria are more straightforward, and they include a comparatively high proportion of peptidoglycan [5]. On the other hand, the cell wall of gram-negative bacteria is structurally more complicated and contains a relatively little amount of peptidoglycan to begin with. Lipopolysaccharides are carbohydrates that are bonded to lipids, and they are found in the outer membrane of the cell wall of gram-negative bacteria [5]. Gram-negative bacteria are typically considered to be more harmful than gram-positive bacteria when it comes to the pathogenic germs that cause disease [6].

The response to the inhibition of gram-positive microbes was stronger than that of gramnegative microbes. This is due to differences in the components of the cell wall between grampositive and gram-negative microbes. The cell wall of gram-positive microbes contains a lot of theikoronate and polysaccharide molecules [7]. These chemical components protect cells from enzyme lysis, while other substances determine the cell's reaction to gram staining and some attract and bind bacteria [7].

The results of this study are in line with previous research which stated that in his research there were bacteria genus / species of bacteria in the group *Micrococcus spon* NA media of air bacteria samples in class [10]. Judging from the results of these studies that in identifying gram-positive and gram-negative bacteria using gram staining, the results are faster and more effective. As for previous studies that used the gram staining method to identify gram-positive and gram-negative bacteria, the results of this study were that several types of bacteria were found in the pavilion inpatient room [2]. The bacterial variations are *Staphylococcus sp* and *Staphylococcus epidermis* [9].

The bacteria found are airborne contaminants [15]. The contamination is usually transmitted through the body / hands, the equipment used in the laboratory, and the clothes of the personnel used in the laboratory [15]. Therefore, it is very necessary to carry out periodic and regular checks for air sterilization, the environment of the laboratory room, the clothes of the staff and the tools used in the laboratory [14]. This can help minimize germs in the laboratory [14].

CONCLUSION

From the results of the study it can be concluded that in the airborne bacteria NA medium there are bacillus sp bacteria which are included in the gram-positive bacteria group which has a monobacillus form. The bacteria identified in the laboratory room air may be a type of contaminated bacteria from the body/hands and research clothes as well as some tools used in laboratory operations. The hope for future researchers is related to air bacteria using the gram staining method so that they can use the tools and materials to be used in the experiment in a sterile state.

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