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Comparison of Examination Results of Acid-Fast Bacilli (AFB) Ziehl Neelsen (ZN) Method on BTA Preparations that are Directly Stained with Delayed Preparations

Asni Hasanuddin[1], Rony Setianto[2], Belinda Arbitya Dewi[3]

[1,2]Health Analysis Study Program Indonesia Timur University, Makassar, South Sulawesi, Indonesia
[2,3]Pharmacy Department, STIKES Rajekwesi Bojonegoro, East Java, Indonesia
Coressponding authors: asnihasanuddin87@gmail.com

Abstract

Tuberculosis is a direct infectious disease caused by the bacterium Mycobacterium tuberculosis. Most tuberculosis germs attack the lungs, which is known as Pulmonary Tuberculosis (pulmonary TB), but can also affect other organs, which is called extrapulmonary TB. Pulmonary TB germs in the form of rods, have special properties, namely acid resistance to staining. The purpose of this study was to determine the results of examination of acid fast bacilli (AFB) by the Ziehl Neelsen method on smear preparations which were immediately stained and left to stand for three days. The research was conducted using a descriptive approach with a sample of 10 people using a purposive sampling technique. In this study, staining was carried out twice, namely direct staining and delayed staining for 3 days and examined under a microscope. The results showed that there was no difference between the preparedness that was immediately colored and the one that was postponed for three days.

Abstrak

Tuberkulosis adalah penyakit menular langsung yang disebabkan oleh bakteri Mycobacterium tuberculosis. Sebagian besar kuman tuberkulosis menyerang paru-paru yang dikenal dengan Tuberkulosis Paru (TB Paru), tetapi dapat juga menyerang organ lain yang disebut TB ekstra paru. Kuman TBC Paru yang berbentuk batang, memiliki sifat khusus yaitu tahan asam terhadap pewarnaan. Tujuan penelitian ini adalah untuk mengetahui hasil pemeriksaan basil tahan asam (BTA) dengan metode Ziehl Neelsen pada sediaan apusan yang langsung diwarnai dan didiamkan selama tiga hari. Penelitian dilakukan dengan menggunakan pendekatan deskriptif dengan sampel sebanyak 10 orang dengan menggunakan teknik purposive sampling. Pada penelitian ini pewarnaan dilakukan dua kali yaitu pewarnaan langsung dan pewarnaan tertunda selama 3 hari dan diperiksa di bawah mikroskop. Hasil penelitian menunjukkan bahwa tidak ada perbedaan antara kesiapsiagaan yang langsung diwarnai dengan yang ditunda selama tiga hari.

Keywords: *Tuberculosis, Phlegm, BTA*

Kata kunci: *Tuberculosis, Dahak, BTA*

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INTRODUCTION

Tuberculosis is a direct infectious disease caused by the bacterium *Mycobacterium tuberculosis*. Most tuberculosis germs attack the lungs, which is known as pulmonary tuberculosis (pulmonary TB), but can also affect other organs of the body, called extrapulmonary TB. Pulmonary TB germs are rod-

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shaped, have special properties, which are resistant to acid on staining. Therefore it is also known as acid fast bacilli (BTA). Lung TB germs die quickly in direct sunlight, but can survive for several hours in a dark and damp place. In the body's tissues, these germs can be dormant, sleeping for a long time for several years[1]. In Indonesia, pulmonary TB is the fifth disease in the world (India, China, South Africa, Nigeria and Indonesia). Based on the TB Global Report in 2010, the prevalence of pulmonary TB cases in Indonesia nationally in 2010 was 285 per 100,000 population, the TB mortality rate has fallen to 27 per 100,000 population[2]. Every year there are 660,000 cases in Indonesia, of which 61,000 die. The majority of pulmonary TB sufferers are of productive age, so the economic burden is quite large. Another challenge is the increase in multi-drug resistant (MDR-TB) cases and pulmonary TB-HIV co-infection [3].

From the 2010 WHO Global Report, obtained data on pulmonary TB in Indonesia, the total of all pulmonary TB cases in 2009 was 294,731 cases, of which 169,213 were cases of new smear-positive pulmonary TB, 108,616 cases of smear-negative pulmonary TB, 11,215 extra pulmonary TB cases, 3,709 pulmonary TB cases Relapse and 1,978 cases of re-treatment outside of relapse cases. Pulmonary TB is not a new disease in Indonesia, but until now it is still a major health problem. It is estimated that the number of pulmonary TB cases in Indonesia accounts for around 5.8 percent of the total number of pulmonary TB in the world. The prevalence of tuberculosis in Indonesia is 281 cases per 100,000 population with a treatment success rate of 90.3%. This number decreased compared to 2010 of 289 per 100,000 population[4]. On March 3 2014, the Stop TB Partnership Forum for the Southeast Asia, West Pacific and East Mediterranean Regions was held in Jakarta. The forum involved 100 participants from 13 countries consisting of national TB program managers, national stop TB partnerships, and related NGOs [5].

The forum stated that although the prevalence has decreased significantly in recent years, the number of tuberculosis sufferers in Indonesia is still relatively high. In fact, currently the number of TB sufferers in Indonesia is ranked fourth in the world. Indonesia has the fourth highest number of TB sufferers after China, India and South Africa. The prevalence of TB in Indonesia in 2013 was 297 per 100,000 population with new cases reaching 460,000 cases every year. Thus, the total cases until 2013 reached around 800,000 - 900,000 cases. In South Sulawesi, the number of tuberculosis (TB) sufferers is still

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high. Based on data from the Provincial Health Office, in 2011, there were 8,939 cases of this infectious disease. This figure has increased significantly compared to the previous year which only had 7,783 cases. In the city of Makassar, the number of suspected TB cases is around 13,701, BTA(+) is around 1,737 cases, relapses are around 92 cases[6]. Takalar Regency was ranked first in the number of cases with a growth in TB sufferers above 109%, followed by Pare-pare 79%, Pinrang 75%, followed by Makassar 70% and the lowest was Luwu Regency 33% and Jeneponto 36%.

Until now, the tuberculosis revention program using the DOTS (Directly Observed Treatment shortcourse) strategy has not been able to support all health centers. In the prevention of tuberculosis, examination of microscopic preparations of Acid Resistant Basil (BTA) from sputum specimens is a key component for establishing a diagnosis as well as evaluating and following up treatment. Therefore the results of smear microscopic examination must be precise and thorough. In order to guarantee the quality of the results of microscopic examination of AFB microscopic preparations, quality assurance activities must be carried out in various aspects regularly and continuously. Specimens were collected in wide-mouthed sputum pots of 6 cm or more in cross-section.

METHODOLOGY

The research that will be carried out is research with a descriptive approach to find out the results of microscopic examination of BTA (acid fast bacilli) between preparations which after fixation are immediately stained with preparations that are delayed for several days. The location has been carried out at the Laboratory of Bhayangkara Hospital, where the research was carried out in June 2022. The first thing to do, namely: 1. Sample preparation. Samples in the form of sputum from all patients who came to check at Bhayangkara Hospital were then collected in a wide-mouthed pot with a transparent lid, colorless, not easily broken and leaky. 2. The tools used in this research are: Microscope, staining rack, object glass, spirit lamp, and one or stick 3. The materials used in this research are The patient's phlegm, Carbol fuchsin, Methylene Blue, Aquadest, 3% HCl-Alcohol, and Oil immersion. 4. How it works. 1. Manufacture of smear preparations. The patient's identification number is written on the glass object using a permanent marker or on a sticker placed on the

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back of the preparation. Next, select and take part of the purulent phlegm using an ose or stick with the tip flattened. To flatten the preparation, small spirals were made during the semi-dry smear using a sharp stick so that the distribution of leukocytes was more even and the reading area was more homogeneous. The use that has been used is dipped in a bottle of disinfectant sand, then burned until the ose is smoldering. When using a stick, immediately throw it into a bottle filled with disinfectant. Furthermore, the preparations were dried in air and then fixed by passing 3 times through the flame of a spirit lamp. The preparations were then dried on the shelf and avoided being exposed to direct sunlight. 2. Ziehl-Neelsen method of staining. The preparation is placed on the staining rack with the smear facing upwards, each preparation is approximately 1 finger apart. The entire surface of the preparation was flooded with carbol fuchsin. The preparation is heated from below using a spirit flame on the preparation until steam comes out (do not boil) then allowed to stand for 5 minutes. After that, it was rinsed carefully with running water and then flooded with alcoholic acid until no red carbol fuchsin color was visible. Furthermore, the surface of the preparation was flooded with methylene blue for 10-20 seconds, then rinsed with running water, and then dried on a drying rack. 3. Smear reading. One drop of immersion oil is placed on the surface of the preparation, then the 100 x objective lens is carefully rotated over the smear. The focus is adjusted carefully until the cells are visible. BTA is visible in the form of rods, bright red on a blue background without any traces of fuchsin dye. BTA found established the diagnosis of TB and the number of BTA found indicated the number of germs in the TB patient. The results of this report refer to the scale of the International Union Against Tuberculosis and Lung Diseases (IUATLD) and the World Health Organization. The research results are presented in tabular form and then presented.[6]

RESULT AND DISCUSSION

AFB sputum examination was carried out by collecting 3 sputum samples which were taken sequentially in two days of visits. The sample is called the SPS sample (in the morning-in time). Sputum sampling for TB examination is carried out by: S (When): sputum is collected into a sterile container (sputum pot) when the patient comes for the first visit. On

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discharge, the patient will be given an empty sputum pot to fill in a second sputum sample the following day. F (Morning): on the second day, you must collect the sputum in the morning, at home after waking up. The pot must be brought and handed over to the officer at the health facility. S (when): You will be asked to collect the third phlegm in the last pot when you come to hand over the morning phlegm at the health facility. The phlegm collected should be thick, cloudy, and sticky mucus that is expelled from the lungs. Not from the nose or saliva from the mouth[7]. There are several methods of collecting phlegm that you can do: Coughing hard: the patient will be asked to take a deep breath, hold it for 5 seconds, and exhale slowly. After that, take a few deep breaths and try to cough hard. This step will draw phlegm from the lungs to collect in the mouth. Sputum induction: for patients who cannot cough up phlegm, sputum production can be stimulated by administering saline solution via a nebulizer. Bronchoscopy: another way to collect sputum is by bronchoscopy. You will usually be given an anesthetic. later. The bronchoscope will be inserted through the nose or mouth into the throat until it reaches the lungs. Sputum contained in the lungs will be aspirated into a bronchoscope tube and collected in a sterile pot. Gastric aspiration: this method is used on sputum swallowed by the patient. Usually this action is performed on patients who are unconscious or children. Examination of sputum samples The samples that have been collected will then be analyzed in the laboratory. There are two types of sputum examination in the AFB test, including: AFB staining test In this sputum test, the sample will be smeared on a glass object to be colored, heated, and doused with an acid solution. Under a microscope, laboratory workers will observe the color changes in the sample. Test results are usually available after 1-2 days. Sputum culture in this sputum test for tuberculosis, the sample will be put into a special medium that supports the cultivation of acid-fast bacteria. A positive result from this culture of acid-fast bacteria can confirm a diagnosis of TB or another infection. However, it takes 6-8 weeks for bacteria to grow in sufficient numbers to detect infection. Diagnosis of TB disease by examination of Basil Acid Resistant (AFB). In principle, this examination is done by looking at germs *Mycobacterium tuberculosis* as a cause of tuberculosis directly under the microscope. In in this examination sputum is used as a specimen because sputum is a a good medium for the growth and life of TB germs,

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so that the germs TB germs will collect in the phlegm[8].

Based on the results of examination of acid fast bacilli (AFB) by the ziehl neelsen (ZN) method on smear preparations which were immediately stained and which were postponed for three days as many as 10 samples which were carried out at the Laboratory of Laburan Baji Hospital Makassar on 08 to 11 August 2022 obtained results as in the table below.

Tabel 1. Results of Examination of Acid Resistant Basil (AFB) Ziehl Neelsen (ZN) Method on AFB preparations which were immediately stained and which were delayed for three days.

No	Sample Code	Check up Result		Desription
		Direct Preparations	Delay 3 Days	
1	A1	+++	+++	Same
	B1	+++	+++	Same
	C1	+++	+++	Same
2	A2	-	-	Same
	B2	+	+	Same
	C2	+	+	Same
3	A3	+++	+++	Same
	B3	+++	+++	Same
	C3	+++	+++	Same
4	A4	+++	+++	Same
	B4	+++	+++	Same
	C4	+++	+++	Same
5	A5	-	-	Same
	B5	+	+	Same
	C5	+	+	Same
6	A6	-	-	Same
	B6	++	++	Same
	C6	++	++	Same
7	A7	+++	+++	Same
	B7	+++	+++	Same
	C7	+++	+++	Same
8	A8	-	-	Same
	B8	+	+	Same
	C8	+	+	Same
9	A9	-	-	Same
	B9	++	++	Same
	C9	++	++	Same
10	A10	+++	+++	Same
	B10	+++	+++	Same
	C10	+++	+++	Same

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Examination of Acid-Fast Bacteria or AFB or is one of the examinations carried out to establish a diagnosis of Tuberculosis (TB), this examination is carried out to detect the presence of bacteria that cause tuberculosis, especially pulmonary tuberculosis. Tuberculosis bacteria can survive in an acidic environment, so the examination is called acid-fast bacteria examination

In the prevention of tuberculosis, examination of microscopic preparations of Acid Resistant Basil (AFB) from sputum specimens is a key component for establishing a diagnosis as well as evaluating and following up treatment. Therefore the results of smear microscopic examination must be precise and thorough. In order to guarantee the quality of the results of microscopic examination of AFB microscopic preparations, quality assurance activities must be carried out in various aspects regularly and continuously. Specimens were collected in sputum pots with wide mouths with a cross section of 6 cm or more with screw caps that were not easily broken or leaked. The diagnosis of tuberculosis is established by examining three sputum specimens, namely every morning – every hour (SPS). This means that when a suspected tuberculosis comes to visit for the first time it is called when, when he comes home the suspect brings a pot to collect the second phlegm called morning phlegm which is taken immediately after waking up. When the suspect comes to bring the morning phlegm, the phlegm is taken again, which is called phlegm when. After the phlegm has been taken, preparations are made immediately. All preparations that have been fixed are immediately stored in the preparation box to avoid the risk of breaking. Then the preparations were sent to the Microscopic Referral Health Center (PRM) for a pulmonary TB laboratory for sputum examination[9].

Microscopic examination of sputum is efficient, easy, cheap, specific, sensitive and can be carried out in all laboratory units of health care facilities that have microscopes and trained TB microscopists. Good sputum is collected in a transparent pot, volume 3-5 ml, mucoid thickness and the color is yellowish green (purulent). The results of the macroscopic study of sputum samples for direct AFB examination and 24 hours delay there are some physical differences. 1. Viscosity, initially thick sputum (purulent, mukopurulent), after being stored 24 hours at room temperature space (25°C) becomes dilute. Watery sputum can occurs because the room temperature tends to warm (25°C) within 24 hours can make decreased

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sputum consistency. Warm temperatures can causing the granules to rupture sputum compound, so the liquid will come out of granules, thereby appearing more watery[10]. The condition of watery sputum means that the quality is decreasing. Watery sputum will be difficult to make preparations AFB, because the results of the preparation will be thin, sometimes difficult average and in conclusion the preparation is not good. The Ministry of Health of the Republic of Indonesia, 2017 states that preparations that are called good must meet 6 criteria The standard is a purulent/mucopurulent specimen, good coloring, clean, good thickness, size 2x3 cm and evenness >80%. 2 Smell of sputum stored 3 days on room temperature smells sharp/ pungent, different with a characteristic fresh sputum odor. Changes in the smell of sputum caused by: growth of spoilage microbes and possibilities mold. Sputum is also a source of nutrition for other microbes besides *Mycobacterium tuberculosis*, so it is very possible if left on room temperature can be grown by other microbes such as mold and other spoilage bacteria. That smell stinging can interfere with the manufacturing process preparation, namely to the officer who makes it AFB preparations. The presence of fungi and bacteria / other microbes can interfere with microscopic examination especially in result reading. Mold or other microbes can cover it AFB contained in the preparation. Results of preparation reading can be negative or false positive. The quality of sputum determines the outcome smear preparation readings, false negative or positive results counterfeit can lead to incorrect treatment or untreated which eventually becomes a source of transmission in community [10]. Of the 10 direct sputum samples and sputum in store for 3 days at 25°C (temp room) by microscopic examination, was found sample with the same result. calculation results, preparations originating from Sputum samples stored for 3 days at 25°C require a longer time even in the manufacture of preparations, because of the quality of the sputum dilute and produce a thin preparation which means the quality of the test sample and the thickness is not good but the inspection results remain the same. Research conducted by Maria (2011) obtained result that the direct method and concentration method can be used for the diagnosis of pulmonary TB, but for concentration method it can increase the number of AFB because concentration collecting method AFB and damaging bacteria aside from AFB. Based on this on This research was

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conducted by method concentration. AFB examination method concentration can increase the number discovery of AFB, because on the method AFB concentrations were collected so that the AFB coverage figure will be increase. This is because AFB microscopic examination method concentration requires volume relatively large number of sputum specimens viz about 2-4 ml of phlegm so as to find AFB in more sputum easy, this is important for TB cases lungs with Mycobacterium counts little tuberculosis. But this thing becomes difficult to do when the amount small sputum specimens were obtained or less than 2 ml. If found thing like that, then inspection AFB microscopy direct method can be done.

There is a change in the consistency of sputum will complicate the laboratory personnel in make good preparations. which preparation must meet 6 standard criteria including purulent specimens or mucopurulent, good staining, clean, good thickness, appropriate size and evenness good (> 80%). Laboratory staff expected to understand the procedure good specimen management for support diagnostic accuracy. specimen sputum for AFB examination is recommended immediately after sampling. This is done to minimize error results are not as good as positive or false negatives it can cause error in decision making patient treatment [11].

In general, TB disease in Indonesia is now experiencing increase from year to year along with the increase in HIV cases [12] so that prevention efforts are needed and countermeasures. Prevention efforts that can be done include: provide health counseling about the importance of checking sputum early on if there are symptoms of tuberculosis, maintain personal hygiene and the environment. And for countermeasures can be done by providing medical treatment intensive.

CONCLUSION

Based on the results of research that has been carried out on 10 sputum samples examined immediately and which was suspended for 3 days from room temperature samples in the Bhayangkara Hospital, Makassar City. in June 2022, then got concluded that: Sputum samples examined directly macroscopically, the viscosity is mucoid (not watery), yellowish green color (purulent), sputum characteristic odor. Sputum samples suspended for 3 days at temperature room is the viscosity begins to decrease (melting), so it becomes runny. The color is dull yellowish, the smell is sharper than

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sputum immediately check and some of them have mold contamination. However, there was no difference in the results of the examination of the preparations that were immediately colored and those that were delayed for three days at room temperature. The presence of fungi and bacteria / other microbes can interfere with microscopic examination especially in result reading. Mold or other microbes can cover it BTA contained in the preparation. Results of preparation reading can be negative or false positive.

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