



## Difference Results between Delayed 24 Hours-room and Direct Examination of Microscopic acid Resistant Bacteria Sputum

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### Abstract

**Background:** *Sputum condition for laboratory examination of the patient with tuberculosis (TB) is essential. To ensure the excellent quality of the specimen, sputum should be immediately sent to the laboratory as soon as possible. Immediate examination in the small clinic is not possible due to limited personnel and the extent of the service area. Therefore, it is necessary to prove that there is a difference in the results of sputum examination after being delayed for 24 hours in room temperature storage and by direct observation.*

**The Objective:** *This study aimed to determine the difference between the results of microscopic examination of acid-resistant bacteria (ARB) in sputum which was directly examined and by delayed for 24 hours at room temperature. Consequently, the accuracy of each study would increase.*

**Method:** *This experiment used descriptive statistics. The difference of results between the two treatments was tested by difference test of two independent samples.*

**Result:** *Results showed that there was a difference through macroscopic observation. Sputum delayed for 24 hours in room temperature experienced changes in viscosity, odor, color and difficulty in preparations. Also, through microscopic observation, it was known that the examination results in sputum delayed for 24 hours at room temperature had a condition of mold-contaminated, difficulty in ARB observation, and high error rate.*

**Conclusions:** *There were macroscopic differences (viscosity, odor, color) and microscopic (presence of molds on the sample, high error rate) on sputum delayed for 24 hours at room temperature. It is strongly recommended that sputum TB testing is done immediately to avoid positive or negative results.*

**Keywords:** *acid resistant bacteria, microscopic examination, sputum, tuberculosis.*

### Introduction

Tuberculosis (TB) is a type of infectious disease that is directly caused by *Mycobacterium tuberculosis*. Tuberculosis is a contagious, chronic infectious disease that becomes a health problem in the world and the leading cause of death in

developing countries. In 2009, the results of the Household Health Survey in Indonesia showed that TB disease was the third leading cause of death after cardiovascular disease and respiratory diseases in all age groups (Kemenkes RI, 2010).

The diagnosis of TB is established by anamnesis, physical examination, and investigation of bacteriological examination and radiological examination (Luhur, 2009). Bacteriological examination aims to identify the bacteria *Mycobacterium tuberculosis* in patients with sputum. Sputum is the material released from the lungs and trachea through the mouth. Making sputum preparation, laboratory personnel should check the physical condition of sputum and choose the one with the following characteristics: viscous, yellowish-green purulent, and sometimes there are blood spots to make the preparations to be qualified.

Sputum specimens should be collected within two consecutive days of the visit. Thus, the primary priority in the TB control program is to find the microscopic acid-resistant bacteria (ARB) bacteria and treatment of the patient with the positive TB result from sputum. Sputum condition for laboratory examination is essential. Proper sputum contains some particles or slightly thick and slimy, sometimes even purulent and yellowish green (Bastian, Ivan, and Lumb, 2008). To guarantee a good quality sputum specimen, it should be immediately sent to the laboratory as soon as possible after taking. If sputum is stored at room temperature for one day, it can lead to sputum being dilute and the quality of the preparation to be unfavorable.

Laboratory of Suruh Clinic, Suruh District, Semarang Regency, Indonesia is part of health laboratory services which has a vital role in the control of Pulmonary TB related to early detection and monitor the successful treatment of TB. Microscopic sputum examination is the most efficient, easy, cheap and specific test in TB treatment. Early detection and sputum tests are essential activities in the Suruh Clinic.

The work area of Suruh Clinic is 31,40 km<sup>2</sup>. The number of laboratory staff is only one person with the comprehensive coverage as extensive as Suruh District. The distance from one village to another is quite far away, so the sputum taken from one

community to the Suruh Clinic Laboratory cannot be directly examined. Sometimes, sputum taken from the suspects of TB is stored for a day at room temperature. The delay of this examination can undoubtedly affect the quality of sputum. Sputum quality will help to determine the results of the microscopic study of ARB. This issue is the background of interest to examine the differences between direct and 24 hours-delayed room temperature storage microscopic BTA sputum inspection.

This study aimed to determine the difference between the results of microscopic examination of acid resistant bacteria (ARB) in sputum which was directly examined and by delayed for 24 hours at room temperature. Consequently, the accuracy of each examination would increase.

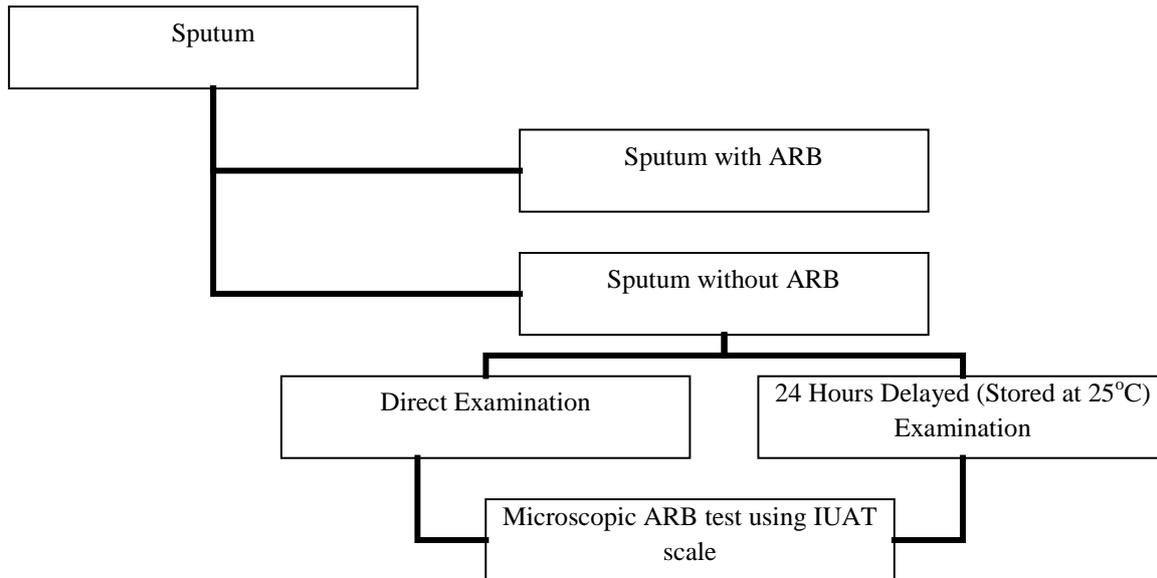
### Materials and Methods

Type of research used was descriptive-analytic with the aim to know the difference between the results of the examination of sputum performed directly with the results obtained after 24 hours of storage.

The sample population was from all suspect TB patients who had sputum examination in Suruh Clinic from August to September 2015. Samples were obtained by using sputum slide glass and examined under a microscope using an International Union Against Tuberculosis (IUAT) scale. Samples used in this research were sputum of the patients with ARB-positives that have been colored by Ziehl Neelsen staining method. Then, samples were ready to be examined microscopically.

The data analysis of the differences results between direct and 24 hours-delayed room temperature storage microscopic ARB sputum inspection was described using the table. The difference between the two treatments was tested by difference test of two independent samples with 95% confidence degree ( $\alpha = 0,05$ ) using statistical software.

**Conceptual Framework**



**Results**

Results obtained as follows

1. Macroscopic Observation of Sputum

**Table 1.** Macroscopic Examination of Sputum

<b>Direct Examination</b>	<b>24 Hours-Delayed (Stored at 25°C) Examination</b>
<ul style="list-style-type: none"> <li>• Purulent sputum with deep green-yellowish color</li> <li>• Can be distinguished between sputum and saliva</li> <li>• Distinctive smell</li> <li>• The turbid color of sputum and clear saliva</li> <li>• Easy to make microscope slide preparation</li> <li>• Challe</li> </ul>	<ul style="list-style-type: none"> <li>• Watery sputum with dull green-yellowish color</li> <li>• Can't be detected between sputum and saliva</li> <li>• Stingy smell</li> <li>• Sputum and saliva are mixed, turbid color</li> <li>• nging to make microscope slide preparation</li> </ul>

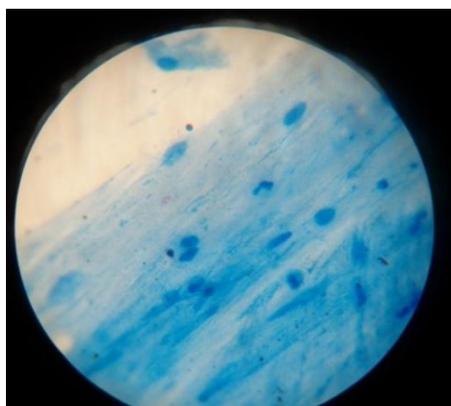
2. Microscopic Observation of Sputum

**Table 2** Microscopic Observation of Sputum

<b>Direct Examination</b>	<b>24 Hours-Delayed (Stored at 25°C) Examination</b>
<ul style="list-style-type: none"> <li>• Contrast background</li> <li>• The counting of ARB positive is easy</li> <li>• Error level in the counting of ARB is much lower</li> </ul>	<ul style="list-style-type: none"> <li>• There were molds in the context</li> <li>• The counting of ARB positive is difficult because of the existence of molds</li> <li>• Error level is higher</li> </ul>

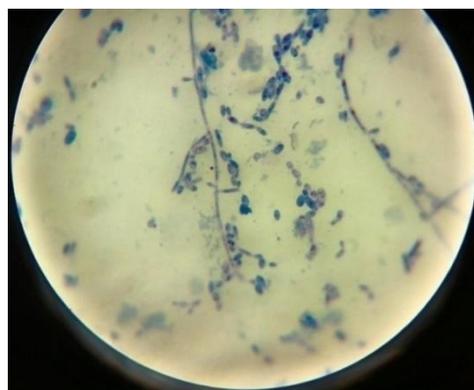
3. Figures of Microscopic Observation of Sputum

**Direct Examination**



*Contrast background*

**24 Hours-Delayed (Stored at 25°C) Examination**



*Molds in the background*

**Table 3.** Microscopic Observation of Sputum with Direct Examination Method and 24-Hours Delayed (Stored at 25°C) Examination Method

	Sample Pos 1 (1+)	Sample Pos 2 (2+)	Sample Pos 3 (3+)	Total
Direct Examination	3	8	14	25
24 Hours Delayed (Stored at 25°C) Examination	7	6	12	25

**Table 4.** Difference Test

		N	Mean Rank	Sum of Ranks
24 Hours- Delayed (Stored at 25°C) Examination - Direct Examination	Negative Ranks	6 <sup>0</sup>	3.50	21.00
	Positive Ranks	0 <sup>0</sup>	.00	.00
	Ties	19 <sup>0</sup>		
	Total	25		

It can be seen that from Table 4 that 25 sputum samples between 24 hours delayed (stored at 25°C) examination and direct examination that there were as much as 6 sputa with tendency of decreasing result of ARB (negative error), no trend of increasing effect (positive failure), and 19 same results in both method. This result indicates that 24-hours delayed (stored at 25°C) sputum can lead to false negative effects or even false positive results.

### Discussion

Microscopic sputum examination is still considered as the efficient, easy, cheap, specific, and sensitive way to detect the presence of TB-causing bacteria. The successful of microscopic sputum test depends on the quality of sputum. Thus the excellent condition of sputum is needed to prevent negative results in the detection of ARB. Proper sputum is viscous with deep green-yellowish color (purulent) (Kemenkes RI, 2012). The results of the research by macroscopic observation showed that there were some differences between direct examination and delayed for 24 hours at 25 °C storage examination. The difference occurred in the viscosity of sputum. Sputum was viscous initially, but after being kept at room temperature, it became watery. Exposure to the warm room temperature (25°C) in a long time leads to the decline in the viscosity of

sputum. The consistency of a colloid may decrease due to warm or hot temperatures. Sputum dilution can be caused by warm temperatures because warm temperatures break the granules in the sputum compound so that the liquid will come out of the granules and sputum appears more watery (Imaningsih, 2013).

The condition of watery sputum is a sign of decreased quality. Watery sputum will be difficult at the time of preparation of ARB microscope slide preparation because the preparation becomes thin and uneven. This condition makes the preparation so difficult to be observed under the microscope. Excellent preparation of sputum is thick sputum with the size about 3 cm long and 2 cm wide, oval-shaped, and flat (Kemenkes RI, 2012).

The smell of sputum stored for 24 hours at the temperature smelled stung, different with the distinctive fresh sputum smell. Sputum odor changes caused by the growth of microbial decay and the possibility of molds, so the scent becomes stingy. Sputum is the material secreted by *tract tracheobronchial* which is come out by coughing (Kemenkes RI, 2012). Sputum is also the source of nutrients for microbes other than *Mycobacterium tuberculosis*, so it is possible if sputum is left at room temperature, it can be overgrown by other microbes such as molds and other decomposer bacteria. Other decomposer

bacteria and fungi that grow on sputum cause the putrid smell of sputum. The stinky smell can disrupt the preparation process because the analyst is disturbed by the stinky smell (Nurhidayah, 2014).

The appearance of molds and other bacteria could interfere the microscopic observation of sputum, mainly on the reading results of ARB. Fungi and other microbes might cover the ARB on the preparation. Thus the readings were interrupted, leading to false positive or negative effects on ARB reading. The quality of sputum dramatically determines the accuracy of detection of TB cases in the society. Incorrect results of sputum test are hazardous because they may cause inappropriate treatment and eventually become a source of transmission and spread of TB disease (Purnomo W, 2014).

In 25 samples of direct examination and delayed for 24 hours at 25°C storage examination, six samples obtained different results. In addition to causing differences in the results of ARB reading, samples stored for 24 hours or more at a temperature of 25°C also required a longer time in reading due to many factors; such as molds, yeasts, and backgrounds which did not contrast.

The result of difference test analysis between sputum check using direct examination and delayed for 24 hours at 25°C storage examination was done by Z test. Based on the analysis test, there was a real difference between the result of direct and delayed microscopic observation of ARB, where  $p$  was  $<0,05$ .

### Conclusion

The results of this study can be summarized as follows:

1. Macroscopic examination of directly examined sputum resulted in purulent sputum with deep green-yellowish color and distinctive odor.
2. Macroscopic examination of 24 hours delayed at 25°C storage sputum examination resulted in watery sputum

with dull green-yellowish color, stinky odor, and there was an appearance of molds.

3. There was a difference of results between sputum which was directly examined with late 24 hours at 25 °C storage examination.

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