Tropical Health and Medical Research
Vol. 7, No. 2, August 2025, pp. 67-76
ISSN (Online): 2684-740X
Journal homepage: https://tropicalhealthandmedicalresearch.com

The Effect of Sputum Delivery Time on the Sensitivity of Culture and AFB Microscopy in the Diagnosis of Drug-Resistant TB

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Abstract: Drug-resistant tuberculosis (DR-TB) is a major challenge in TB control in Indonesia. Early detection using liquid and solid media cultures is crucial due to the limited sensitivity of Acid-Fast Bacilli (AFB) microscopic examination. In addition, the time of sputum specimen delivery can also affect bacterial viability. This study aims to determine the relationship between liquid and solid media culture results with AFB microscopic results in immediate and delayed sputum in patients with DR-TB. This is an observational analytical study with a cross-sectional design, involving 40 sputum samples from DR-TB patients examined at the South Kalimantan Provincial Health Laboratory from January to April 2025. Samples were grouped based on the time of sputum delivery (<7 days = immediate; ≥7 days = delayed), and examined using AFB microscopic methods, Mycobacterium Growth Indicator Tube liquid culture, and Lowenstein-Jensen solid culture. Data analysis was performed using the Spearman correlation test. Of the 40 sputum samples examined, 2 (5%) were positive by AFB microscopy, 8 (20%) were positive by liquid culture (MGIT), and 6 (15%) were positive by solid culture (Lowenstein-Jensen). The results showed that in immediate sputum, *Mycobacterium tuberculosis* (MTB) was detected in 7 samples using liquid media and in 5 samples using solid media. In contrast, in delayed sputum, only one sample was positive in both media. Most samples with negative AFB microscopy still yielded MTB growth in culture, particularly in immediate sputum. There was no significant correlation between AFB and liquid culture results in immediate sputum (p=0.172), but there was a substantial correlation between AFB and solid culture (p=0.025). In delayed sputum, both liquid and solid cultures showed a significant correlation with AFB results (p=0.046). Liquid culture was proven to be more sensitive than AFB microscopic examination, especially for immediately examined sputum. MTB viability and accuracy of results decreased in late-delivered sputum, so optimal specimen delivery management is necessary to maintain diagnostic quality.
Keywords: Acid-fast bacilli (AFB) microscopic examination; delayed sputum; immediate sputum; liquid culture media; solid culture media.

INTRODUCTION

Tuberculosis (TB) remains a global health problem, and Indonesia is one of the countries with the highest burden. Global TB Report 2022: Indonesia ranks second after India with an estimated 969,000 cases[1]. Final data from the Tuberculosis Information System as of March 13, 2023, reported 724,309 TB cases in Indonesia in 2022[2]. In

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addition to facing challenges in controlling drug-sensitive TB, Indonesia is also burdened with an increase in cases of drug-resistant TB, which requires more complex treatment, especially in the diagnostic aspect.

Diagnosis of RO TB cannot be done through microscopic examination alone, but requires a sensitivity test. *Mycobacterium tuberculosis* uses phenotypic methods, such as liquid culture with Mycobacterium Growth Indicator Tube (MGIT) and solid culture[2]. Culture, identification, and sensitivity testing for *Mycobacterium tuberculosis* complex is *the gold standard* in the diagnosis of TB, because it has a higher sensitivity than microscopic methods. 5,000–10,000 bacteria/mL are required for AFB to appear positive microscopically, while culture only needs 10–100 bacteria/mL, thus increasing case detection by 20–30%[3].

In Indonesia, monitoring of the treatment of RO TB patients is carried out routinely through microscopy and culture in reference laboratories[4]. In South Kalimantan Province, TB culture implementation only began in August 2023, based on the official recommendation letter from the Ministry of Health No. PM.01.03/C.III/6660/2023. Establishing a diagnosis through culture is crucial because 50–60% of specimens with negative smear results still show positive AFB culture results. Therefore, since 1993, the CDC has recommended the use of liquid media in addition to solid media in culture examinations for mycobacteria[5].

In a study by Jha et al. (2025), analysis of 778 samples showed that MGIT liquid media successfully detected *Mycobacterium tuberculosis* (MTB) in 223 samples (28.7%), higher than solid culture, which detected only 190 samples (24.4%), with an additional seven samples being contaminated. Detection by microscopy was even lower, at only 85 isolates (10.9%). These findings confirm that MGIT, as an automated method, has higher sensitivity and is able to provide faster results than conventional methods such as microscopic staining and solid culture[6]. Mishra et al. (2021) also reported that of all positive specimens, 94% were successfully detected by MGIT, while solid culture only detected 89%, indicating the superiority of MGIT in detecting TB cases, especially in specimens with low bacillus counts[7].

Study Silviani et al. (2023) showed that delaying microscopic and TCM examination for 5 hours and 24 hours caused a decrease in the number of AFB+, but a decrease in grade did not occur in all samples[8]. Research Sugireng et al. (2023) also showed that of the 20 samples examined, 9 of them experienced a decrease in grade AFB positive after being delayed for 12 hours at room temperature[9].

At the South Kalimantan Provincial Health Laboratory, sputum specimens for RO TB patients are often delayed due to the collection system (pooling) and inter-facility delivery. Although *Mycobacterium tuberculosis* can survive in sputum for up to one week, a delay of more than 2 hours without refrigeration will reduce bacterial viability, especially in samples with few germs. Therefore, delivery using a cold chain is very important, and delaying the examination for more than 7 days is not recommended[3].

This research was conducted in the context of limited local studies that directly compare the viability *of Mycobacterium tuberculosis* in sputum examined immediately and that which was delayed, especially in relation to culture results on liquid media (MGIT) and solid media (Lowenstein-Jensen), as well as the results of AFB microscopy. To date, there has been little research examining the impact of delayed specimen delivery on culture effectiveness in a referral laboratory service system, particularly in resource-

limited areas such as South Kalimantan Province. Therefore, this study aimed to evaluate the relationship between liquid and solid media culture results and AFB microscopy results in both immediate and delayed sputum.

MATERIALS AND METHODS

This study is an observational analytical study with a cross-sectional design, which aims to determine the relationship between culture results *of Mycobacterium tuberculosis complex* on liquid and solid media with direct AFB microscopic examination results, both on sputum sent immediately and sputum that experienced delays in sending to the laboratory. The study was conducted from January to April 2025 at the Tuberculosis Laboratory, South Kalimantan Provincial Health Laboratory. The study population was all sputum specimens of suspected patients or patients undergoing RO TB therapy sent from referral hospitals during that period, with a total sample of 40 specimens, taken using the total sampling method.

The independent variables in this study were the time of sputum delivery (immediate and delayed) and the detection methods, including direct AFB microscopic results, culture results using liquid media (MGIT 960), and culture results on solid media (Lowenstein-Jensen). The dependent variable was the final result of each examination method, namely, whether it showed growth or not *M. tuberculosis*. Microscopic examination of AFB is declared positive if there are red acid-fast bacilli on a blue background according to the IUATLD (International Union Against Tuberculosis and Lung Disease) scale, with a negative category of up to 3+.

Culture examination using MGIT 960 liquid media is declared positive if the tool detects bacterial growth within 7–30 days, confirmed by Ziehl-Neelsen staining and identification *of M. tuberculosis complex* using the MPT64 rapid test, and no contamination is detected on BHI agar. Conversely, a negative result was determined if no growth was found after a maximum of 42 days of incubation. Culture on LJ solid media was declared positive if typical colony growth was observed for *M. tuberculosis* within 2–8 weeks, with buff-colored colonies, a dry, brittle surface, and an irregular shape, confirmed by AFB staining, niacin testing, and PNB. Results are assessed quantitatively based on the number of colonies (1–19 colonies to >500 colonies). If there is no growth by week 8, the result is considered negative.

The study procedure began with the identification of immediate sputum specimens (sent to the laboratory <7 days after collection) and delayed sputum (≥7 days). Grouping was based on the delivery data on the TB-05 form of the SITB application. Specimens were then processed with a decontamination procedure using the NaOH–Na-citrate–NALC method to remove contaminants and homogenize before inoculation. For liquid culture, 0.5 mL of sputum sediment was placed into an MGIT tube that had been supplemented with PANTA supplements and antibiotics, then incubated automatically in the MGIT 960 system. Positive tubes were further identified by AFB staining and subculture on BHI agar, while negative tubes were visually re-examined after 42 days.

For solid culture, 100 µL of processed specimens were inoculated into two LJ medium tubes. Incubation was performed at 35–37°C in a tilted position for 24 hours and then in an upright position for up to 8 weeks. Growth was checked weekly, and confirmation of emerging colonies was performed using Ziehl-Neelsen staining, niacin testing, and PNB testing. Data from both microscopic and culture examinations were

recorded and analyzed to determine the relationship between culture method, shipping time, and AFB results.

All laboratory examination procedures are carried out by trained personnel in accordance with applicable operational standards and have obtained ethical permission from the Health Research Ethics Commission of the Banjarmasin Ministry of Health Polytechnic, with certificate number: 1205/KEPK-PKB/2025, and implementation permits from the South Kalimantan Provincial Health Laboratory and participating TB-RO referral hospitals.

RESULTS AND DISCUSSION

In a study conducted from January 18 to April 24, 2025, on the relationship between the results of liquid and solid media cultures and the microscopic results of AFB in immediate and delayed sputum, the results of the examination were obtained on 40 research samples. Microscopic examination of Acid-Fast Bacteria (AFB) for sputum examination was carried out directly before the samples were cultured in liquid and solid media.

Table 1. Microscopic Examination Results of AFB from Sputum Samples

Results Inspection	Types of Sputum	Direct AFB Microscopy	Percentage (%)
Negative	Immediate	32	80.0%
	Delayed	6	15.0%
Scanty	Immediate	0	0.0%
	Delayed	0	0.0%
1+	Immediate	0	0.0%
	Delayed	1	2.5%
2+	Immediate	1	2.5%
	Delayed	0	0.0%
Total Samples		40	100%
Total Negative Samples		38	95.0%
Total Positive Samples		2	5.0%

The results of the AFB microscopic examination of sputum (Table 1) showed that out of 40 samples examined, 38 (95.0%) were AFB negative, while only two samples (5.0%) were AFB positive. Among the immediate sputum samples, 32 were negative (80.0%) and one was positive with a 2+ grading (2.5%). From the delayed sputum, six were negative (15.0%) and one was positive with a 1+ grading (2.5%). No samples were found in the “scanty” category. These results indicate that the majority of sputum samples were microscopically negative for acid-fast bacilli, and that positive findings were rare in both immediate and delayed conditions.

This indicates that most delayed sputum does not show the presence of acid-fast bacilli microscopically, which may indicate a decrease in the number of bacilli due to the delay in examination time or specimen degradation. These results confirm that the sensitivity of AFB microscopic examination of delayed sputum is lower than that of immediate sputum.

Sputum culture using liquid media was carried out using the MGIT tool, and for solid media using Lowenstein-Jensen media, with the following results in Table 2.

Table 2. Growth Results of *Mycobacterium tuberculosis* on Liquid and Solid Culture Media

Results Inspection	Types of Sputum	Liquid Culture (MGIT)	%	Solid Culture (LJ)	%
MTB Positive	Immediate	7	17.5%	5	12.5%
	Delayed	1	2.5%	1	2.5%
Total		8	20.0%	6	15.0%
MTB Negative	Immediate	22	55.0%	19	47.5%
	Delayed	6	15.0%	5	12.5%
Non-Tuberculous Mycobacteria (NTM)	Immediate	4	10.0%	3	7.5%
Contaminants	Immediate	0	0.0%	6	15.0%
	Delayed	0	0.0%	1	2.5%
Total Samples		40	100%	40	100%

Table 2 summarizes the results of MTB isolation based on sputum type (immediate and delayed) and culture media type (liquid/MGIT and solid/LJ). MTB was found to be more frequently detected in immediate sputum than in delayed sputum. In MGIT culture, MTB was found in 12 immediate sputum samples and 6 in delayed sputum. Meanwhile, on LJ solid media, MTB was detected in 11 immediate sputum samples and only three delayed sputum samples.

Table 3 describes the relationship between AFB examination results and culture results in immediate sputum. From liquid media (MGIT), 10 of 16 MTB-positive samples showed AFB-negative results (62.5%), while the other six samples showed AFB-positive category 1+. For solid media (LJ), 8 of 14 MTB-positive samples also showed AFB-negative results (57.1%), while the other six were AFB-positive. Thus, AFB sensitivity appears low for samples proven positive for MTB on culture, especially in immediate sputum. Meanwhile, Table 4 shows a similar pattern in delayed sputum. In the MGIT culture, of the 6 MTB-positive samples, five samples were AFB-negative (83.3%), and 1 sample was AFB-2+. Meanwhile, on LJ solid media, 3 of the 4 MTB-positive samples were AFB-negative, and only one was AFB-1+ positive. These results confirm that culture is more sensitive in detecting MTB than AFB microscopy, in both immediate and delayed sputum.

Based on the comparison (Table 5) between the P value of the culture results with liquid media in fresh sputum, the α (p) value was obtained, $p = 0.172$, the α (p) value > 0.05, which means there is no significant relationship between the Liquid Media Culture Results and the AFB Microscopic Results in immediate sputum. The P value of the culture results with solid media in fresh sputum was obtained, the α (p) value, $p = 0.025$, the α (p) value < 0.05, which means there is a significant relationship between the Solid Media Culture and the AFB Microscopic Results in immediate sputum.

Table 3. Microscopic Examination Results of AFB in Immediate Sputum Using Liquid and Solid Culture Media

Media Name	AFB Negative	Scanty	1+	2+
Liquid Culture (MGIT)				
Positive MTB	6 (18%)	0	0	1 (3%)
Negative MTB	22 (67%)	0	0	0
NTM	4 (12%)	0	0	0
Contaminants	0	0	0	0
Total Samples	32	0	0	1
Solid Culture (LJ)				
Positive MTB (1–19 colonies)	2 (6%)	0	0	0
Positive MTB (1+)	2 (6%)	0	0	1 (3%)
Negative MTB	25 (76%)	0	0	0
NTM	1 (3%)	0	0	0
Contaminants	2 (6%)	0	0	0
Total Samples	32	0	0	1

Table 4. Microscopic Examination Results of AFB in Delayed Sputum Using Liquid and Solid Culture Media

Media Name	AFB Negative	Scanty	1+	2+
Liquid Culture (MGIT)				
Positive MTB	0	0	1 (14%)	0
Negative MTB	6 (86%)	0	0	0
NTM	0	0	0	0
Contaminants	0	0	0	0
Total Samples	6	0	1	0
Solid Culture (LJ)				
Positive MTB (1+)	0	0	1 (14%)	0
Negative MTB	6 (86%)	0	0	0
NTM	0	0	0	0
Contaminants	0	0	0	0
Total Samples	6	0	1	0

Meanwhile, the P value for the culture results with liquid and solid media on delayed sputum (Table %) obtained an α (p) value, p = 0.046 and p = 0.046, the α (p) value < 0.05, which means there is a significant relationship between the Liquid Media Culture Results and Solid Media Culture Results with the AFB Microscopic Results on delayed sputum.

Table 5. Spearman Correlation Results Between Liquid and Solid Culture Media and AFB Microscopic Examination in Immediate and Delayed Sputum

Description	P-Value	Correlation Coefficient	Interpretation
Liquid Culture (MGIT) vs AFB on Immediate Sputum	0.172	–	No meaningful correlation
Solid Culture (LJ) vs AFB on Immediate Sputum	0.025	0.390	Significant correlation
Liquid Culture (MGIT) vs AFB on Delayed Sputum	0.046	0.764	Significant correlation
Solid Culture (LJ) vs AFB on Delayed Sputum	0.046	0.764	Significant correlation

This study evaluated the relationship between AFB microscopic results and culture *of Mycobacterium tuberculosis* (MTB) in liquid (MGIT) and solid (LJ) media, based on two sputum delivery conditions, namely immediate (<7 days) and delayed (≥7 days), with standard cold-chain storage. The majority of specimens came from patients in the 1st to 5th month of follow-up, indicating patients in the early stages of TB RO treatment according to guidelines Kementerian Kesehatan (2024) (10).

MTB culture results in liquid media showed a positive MTB rate of 20%, higher than that in solid media (15%). In immediate sputum, AFB microscopic results were negative, but positive MTB cultures were found in 18% of liquid media and 12% of solid media. This underscores the importance of culture as a method for detecting MTB even when microscopic results are negative, as stated by Siddiqi and Gerdes (2006) that 50-60% of specimens with negative AFB can give positive culture results (5). In addition, Pramana et al. (2015) reported a microscopic sensitivity of only 68%, much lower than that of culture (11).

In delayed sputum, positive MTB results were found in only 14% of samples (both liquid and solid media), all with 1+ microscopic results. No cases of MTB positive with negative microscopy were found in this group, indicating decreased culture sensitivity due to delayed delivery. Kementerian kesehatan (2022). M. tuberculosis can survive for a week, but viability will decrease without proper refrigeration, especially for paucibacillary specimens (12).

One of the challenges in this study was the high contamination rate in liquid media (23%) compared to solid media (5%). This exceeds the standards set by Siddiqi and Gerdes (2006), which are 5–8% for MGIT and 3–5% for LJ (5). Presialia and Kiranasari (2017) recorded a contamination rate of 9.3% for both, indicating that decontamination, storage, and media quality significantly influence culture results (13). The high contamination rate in our study is likely related to field conditions, such as the length of

time specimens were stored before processing and the lack of temperature control during shipping due to limitations in the optimal cold chain. Furthermore, the possible presence of normal flora or environmental contaminants that were not eliminated during the decontamination process is also a factor to consider. This presents a challenge, especially with liquid media, which are more susceptible to the growth of microorganisms other than Mycobacterium tuberculosis, thus increasing the risk of contamination.

The results of the correlation test between AFB microscopy and liquid culture (MGIT) in sputum immediately showed no significant correlation ($p = 0.172$). One of the main reasons for this finding is the more sensitive ability of MGIT liquid media in detecting viability *of Mycobacterium tuberculosis*, even in samples with low or undetectable bacilli counts microscopically. This is reflected in the data distribution, where the majority of immediate sputum samples came from the AFB-negative group (22 of 32 samples). However, a large proportion of this group still produced positive MGIT cultures (15 of 22 or 68.2%). This phenomenon indicates that even though microscopic examination does not detect bacilli, MGIT is still able to detect live bacterial growth, thus causing a lack of strong correlation between the two methods in immediate sputum.

In contrast, in delayed sputum, a significant correlation was found between microscopy and liquid culture (MGIT) results ($p = 0.026$). This is likely due to decreased bacterial viability due to delayed delivery. Because some *Mycobacterium tuberculosis* is no longer viable, the ability of MGIT culture to detect growth decreases, especially in samples with negative microscopic results. Therefore, in delayed sputum, positive MGIT results are more common in the group with positive AFB results, making the relationship between the two more statistically significant.

The results of the study showed that MTB cultures in liquid media showed a positive MTB rate of 20%, higher than the 15% in solid media. This finding is in line with the results of the previous study, Diriba et al. (2017), who reported that positive cultures were found in 87.4% of samples using MGIT compared to 66.7% on LJ solid media. This difference may be due to various technical factors such as decontamination method, media quality, and patient treatment status (14). Additional factors, such as good laboratory practices to follow and checking quality control methods for each batch of culture media and specimens, may also contribute to differences [15].

Overall, this confirms that liquid media have higher sensitivity than solid media, especially for sputum specimens examined immediately. However, the success of culture is highly dependent on specimen quality and speed of delivery to the laboratory. Therefore, referral hospitals need to ensure that specimen collection and delivery are carried out promptly and correctly to maintain bacterial viability and improve the accuracy of culture results.

Some limitations of this study include the relatively small sample size, particularly in the delayed sputum group (n=7), which may affect the power of the statistical analysis. Furthermore, factors such as sputum sampling technique, storage duration before shipment, and adherence to decontamination SOPs were not fully controlled. The high rate of contamination in liquid media also hampered the interpretation of the results. Further studies with prospective designs and close monitoring of the specimen logistics chain are highly recommended for further validation.

CONCLUSION

This study demonstrated that liquid culture (MGIT) has a higher sensitivity than solid culture (LJ) and AFB microscopy, especially in sputum examined immediately after collection. MTB culture can still detect bacterial growth in AFB-negative samples, demonstrating the importance of culture examination in establishing the diagnosis of DR-TB. However, delayed sputum delivery (delayed sputum) has been shown to reduce bacterial viability, which results in a decrease in positive culture results. It was found that in immediate sputum, only solid culture showed a significant correlation with microscopic results, while in delayed sputum, both liquid and solid cultures showed a significant correlation. This emphasizes the importance of the timing and method of specimen delivery in maintaining laboratory diagnostic quality. Further studies with larger sample sizes and control for confounding variables are needed to strengthen these findings.

CONFLICT OF INTEREST

In this study there is no conflict of interest

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