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Solid Media (Lowenstein Jensen) and Liquid Media (Mycobacteria Growth Indicator Tube) Usage Against *Mycobacterium tuberculosis* Culture in Sputum Suspect Tuberculosis

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ABSTRACT

Tuberculosis examination using Lowenstein Jensen (LJ) media, requires an incubation time of 8 weeks after inoculation. The objective of this studi to compare result media Mycobacterium tuberculosis proliferation on solid media (LJ) and liquid media (MGIT) on sputum of suspected TB. The liquid culture method was Mycobacteria Growth Indicator Tube (MGIT). This method was fully automatic and non-radiometric. MGIT contains 7.0 ml of modified Middlebrook 7H9 broth. Culture results will come out in 42 days if there was no bacterial growth. Comparative research on 30 sputum suspects of TB were examined at the Surabaya Health Laboratory Center on 03 January to 20 March 2020. The sputum samples were examined through the stages of decontamination, homogenization, concentration and inoculated on LJ media and MGIT media and incubated at 37^0C. Identification of the colonies that grew with ZN staining and MPT 64 immunochromatography test. The results of the Wilcoxon Signed Rank Test statistical test obtained p-value of 0.157 ($p > 0.05$). It can be concluded that there is no difference in the culture results of *Mycobacterium tuberculosis* on solid media (Lowenstein Jensen) and liquid media (Mycobacteria Growth Indicator Tube).

Keywords: *Mycobacterium tuberculosis;* Lowenstein Jensen (LJ);Mycobacteria Growth Indicator Tube (MGIT)

INTRODUCTION

Tuberculosis (TB) is a contagious infectious disease by *Mycobacterium tuberculosis*. Based on the 2019 Global Tuberculosis Report released by the WHO it is known that there were predicted 10 million TB cases by 2018. The majority cases for increasing notification of TB cases occurred in India and Indonesia; two countries ranked first and third worldwide [(1)]. The problem of TB in Indonesia is not only the high TB sufferers but also the low case finding and the length of time a TB diagnosis [(2, 3)]. Culture examination, identification and sensitivity test of *Mycobacterium tuberculosis* complex are needed to diagnose TB cases. Examination of culture can increase sensitivity for diagnosis while differentiating *Mycobacterium tuberculosis* complex and NTM [(4)]. Solid media that usually used is the Lowenstein Jensen (LJ) media, which is an egg-based selective media used for Mycobacterium culture and isolation. However, this method requires a prolonged incubation of about eight weeks after the time of inoculation. In order to minimize the risk of broader transmission and a more severe disease course, TB diagnostics require a faster culture medium [(5)].

The LJ media is often considered the primary medium of Mycobacterium Growth. The media is later used for both verification and validation during our comparative studies. Even validation of liquid media, in combination with a semi-automatic culture system, is usually compared with LJ culture as a diagnostic gold standard [(6)]. The liquid culture method, Mycobacteria Growth Indicator Tube (MGIT), has been introduced for the discovery of better Mycobacterium and faster Mycobacterium growth. This is because the media is liquid (MGIT) is more sensitive than solid media (LJ) [(7)].

Furthermore, we use a fully automatic, non-radiometric the BACTEC MGIT 960 system to incubate and examine 960 samples at once with an automated result. MGIT contains 7.0 ml of modified 7H9 Middlebrook broth. Growth supplements or OADC are added to the medium before inoculation. This supplement is essential for the culture of *Mycobacterium tuberculosis* complex [(8)]. Preliminary studies that have been conducted in Tripoli-Libya show that the sensitivity of direct microscopic examination of sputum smears for initial TB

diagnosis is still low. Meanwhile, LJ is considered a superior diagnostic tool in developing countries [9]. MGIT is also the one of the fastest diagnoses to detect resistance to *Mycobacterium tuberculosis* [10]. Previous research at the Ethiopian National Tuberculosis Reference Laboratory, Addis Ababa, Ethiopia in 2017 concluded that the BACTEC MGIT 960 system had a positive smear result with a positive culture result for *Mycobacterium tuberculosis* which was better with shorter completion times in comparison with the traditional LJ methods [11]. Research in Beijing Chest Hospital Laboratory shown MGIT was more outperforms than LJ medium from abscess sample in skeleton tuberculosis cases [12]. Research that has been conducted by the Faculty of Medicine, University of Indonesia at the Cipto Mangunkusumo Hospital Laboratory Jakarta shown that the level of contamination by fungi with LJ and MGIT media is likely to have the same level of contamination [13]. Research at the Pasteur Institute of Algeria has shown that BACTEC MGIT 960 can shorten the growth time to an average of 7 days. However, LJ still has a role in completing a reliable diagnosis [14]. Diagnosis using culture and microscopy is the most superior conventional diagnostic tool for detecting bacterial infection [15]. Nonetheless efforts must be created to decrease the level of contamination that is higher in the BACTEC MGIT system than the LJ method [11]. Therefore, the authors conducted research on the use of solid media (Lowenstein Jensen) and liquid media (Mycobacteria Growth Indicator Tube) whether they show different results on the culture of *Mycobacterium tuberculosis* against TB suspected sputum which is examined at *Balai Besar Laboratorium Kesehatan* (BBLK) Surabaya.

The objective of this studi to compare result media Mycobacterium tuberculosis proliferation on solid media (LJ) and liquid media (MGIT) on sputum of suspected TB. To achieve that goal, the first step is breed Mycobacterium tuberculosis on MGIT and LJ medium. After obtaining the culture results, a comparison of bacterial growth was carried out to determine whether there were differences in bacterial colonies into LJ medium and MGIT medium in sputum samples.

METHODS

Type of research used was comparative research that compares the results of culture *Mycobacterium tuberculosis* on solid media (LJ) and liquid media (MGIT) [16]. The subjects in this study were TB suspects sputum examined at BBLK in January 2020, with the following criteria: (1) Purulent, (2) Minimum volume of 3 ml, totaling 30 samples [17]. The study was conducted at the National Tuberculosis Reference Laboratory, BBLK Surabaya, on January 3 - March 20, 2020. Data collection technique in this study was to observe observations of culture *Mycobacterium tuberculosis* on solid media (LJ) and liquid media (MGIT). Data obtained, processed and tabulated in tables and graphs, and presented descriptively to determine the comparison of the number of positive smear diagnoses with negative culture results and the positive culture of *Mycobacterium tuberculosis* on solid media (LJ) and liquid media (MGIT) as well as the number of negative smear diagnoses with negative culture results and positive culture of *Mycobacterium tuberculosis* on solid media (LJ) and liquid media (MGIT). Data were examined by the Wilcoxon Signed Rank Test to analyze differences in the results of *Mycobacterium tuberculosis* culture on solid-solid media (LJ) and liquid media (MGIT).

Mycobacterium tuberculosis culture with NALC-NaOH method. Sputum samples were cultured through the stages of decontamination, homogenization, concentration and then inoculated on solid media (LJ) and liquid media (MGIT). Sputum thawing, decontamination and concentration procedures were achieved by the method of N-acetyl-L-cysteine NaOH (NALC-NaOH), the same volume of of 4% NaOH and 2,9% $Na_3C_6H_5O_7$ mixed into the mixture added N-acetyl- L-cysteine makes a final concentration of 0.5% [18]. Pour the test sample into a 50 ml centrifuge tube. Append NALC-NaOH just as much. Whisk until homogeneous, no more than 30 seconds and let stand for another 15 minutes at room temperature. Append PBS to a volume of 45 ml. Go back and forth several times. Centrifuge for 15 minutes, speed 3000 g, temperature 10ºC. Discard the supernatant. Afterwards, add 1 ml of PBS. Inoculation on LJ media was 100µl and MGIT media was 500 µl. Incubation at 37°C [4].

Lowenstein-Jensen (LJ) is a selective medium to isolate and culture *Mycobacterium* [19]. This egg-based media contains solid eggs, potato starch, salt and compacted glycerol. Initially, this media was formulated by Lowenstein, which included congo red and malachite green to inhibit unwanted bacteria. Jensen modified Lowenstein's media by changing citrate and phosphate, removing congo red and increasing the concentration of malachite green. This action is to preclude the growth of the majority of contaminants that survived specimen decontamination. This formulation also promotes the growth of *Mycobacterium* as early as possible [8].

Potato Flour and L-Asparagine are two sources of nitrogen and vitamins. Magnesium Sulfate & Monopotassium Phosphate increase the development of organisms and function as a buffer. Malachite green prevents the development of most of the contaminants that survived from decontamination of the specimen while supporting the growth of Mycobacterium. When heated, egg albumin freezes, thus allowing a solid surface to inoculate. Glycerol functions as a carbon supplier. It is beneficial for the development of human-type tubercle bacilli [19]. Samples on solid media (LJ) were inoculated and incubated at 37º C, every seven days, there was a look of bacterial growth. Furthermore, identification is carried out on the growing colonies (yellowish-white, dry and brittle surfaces with irregular edges such as cauliflower) with ZN staining and MPT 64 test [20].

On *Mycobacterium tuberculosis* bacteria will show positive smear ZN staining results (clustered in a characteristic shape) namely serpentine cord, typical cord) and MPT 64 positive test. If until the 8th week there is no growth in the LJ media, it can be concluded as negative (21). Liquid Media (Mycobacteria Growth Indicator Tube) MGIT media consists of 7.0 ml of modified Middlebrook 7H9 broth. This media requires MGIT BACTEC MGIT instrument called a growth or OADC 960. Supplements (Oleic Acid, Albumin, Dekstrosa and Catalase) was added to the medium prior to inoculation. This supplement is essential for the development of various forms of mycobacteria, especially those included in the *Mycobacterium tuberculosis* complex.

Moreover, a mixture antimicroba called PANTA (Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim and Azlocillin) also needs to be added to restrain bacterial contaminants (22). The MGIT tube includes fluorochrome (tris 4,7-diphenyl-1, 10-phenanthroline ruthenium chloride pentahydrate) which is entrenched in silicon at the bottommost of the tube. These fluorescent compounds are sensitive to the existence of dissolved oxygen in the media. During the development of bacteria in the tube, free oxygen is used and substituted by CO. Free oxygen reduction produces a fluorescence sensor in the MGIT tube once we visualize underneath the ultraviolet (UV) light. The fluorescence concentration is straightforwardly proportional to the level of oxygen reduction. In this case, the BACTEC MGIT 960 instruments could discover this fluorescence (8).

On liquid media (MGIT), we injected each MGIT tube with 0.5 ml of the processed specimen. Afterwards, the tube was incubated at 37° C in the MGIT 960 BACTEC instrument. Also, it was observed automatically every 60 minutes for increased fluorescence. The culture tube was continued until it turns into positive or a maximum of 42 days for negative samples. Later, all positive tubes were confirmed by the ZN staining method and by an immunochromatographic test, namely MPT 64 (11).

RESULTS

Our research that has been conducted on the comparison of the use of solid media (Lowenstein Jensen) and liquid media (Mycobacteria Growth Indicator Tube) on culture *Mycobacterium tuberculosis* on 30 samples of suspected sputum TB at the BBLK Surabaya carried out a microscopic examination of AFB (Acid Fast Bacilli), the culture of *Mycobacterium tuberculosis* on solid media (Lowenstein Jensen) and liquid media (Mycobacteria Growth Indicator Tube). Cultures are considered negative when the colonies weren't visible after the 8 week incubation period (4). Microscopic smear-positive results with positive cultures each of 23 samples in LJ media and MGIT media, microscopic smear-negative with negative cultures 4 samples in LJ media and 2 samples in MGIT media, negative smear microscopic with positive culture 3 samples in media LJ and 5 samples on MGIT media. Percentage of positive smear microscopic results with positive culture yields of 100% in LJ media and MGIT media, microscopic smear-negative results with negative culture results of 57% in LJ media and 29% in MGIT media, negative smear microscopic results with culture results positive by 43% in LJ media and 71% in MGIT media.

Table 1. Comparative analysis of the microscopic results of AFB with the results of the *M. Tuberculosis* culture on LJ media and MGIT media

Media	AFB positive	AFB positive	AFB negative	AFB negative	Total sample
	Negative culture	Positive culture	Negative culture	Positive culture	
LJ	-	23	4	3	30
MGIT	-	23	2	5	30

Data obtained from this study with microscopic smear-positive results with positive cultures of 23 samples (100%) on solid media (LJ) and 23 samples (100%) on liquid media (MGIT). This data shows that the solid media (LJ) and liquid media (MGIT) have good performance in growing the bacteria *Mycobacterium tuberculosis* in smear positive microscopic samples. Microscopic smear-negative results with a positive culture of 3 samples (43%) on solid media (LJ) and five samples (71%) on liquid media (MGIT). The sensitivity of the culture examination is higher than that of smear microscopic examination. A positive smear examination requires a total of 5,000-10,000 germs per ml if examined 300 fields of view, whereas a positive culture check only requires the number of live bacteria between 10-100 germs per ml [11]. This has led to the discovery of negative smear microscopic results with positive culture results.

DISCUSSION

There are two different culture results on solid media (LJ) and liquid media (MGIT), namely sample codes A13 and A30. In the sample code, culture results on LJ media are negative, while culture results on MGIT media are positive. This action shows that liquid media (MGIT) is better in growing *Mycobacterium tuberculosis* in negative microscopic samples than in solid media (LJ). This situation happens because liquid media (MGIT) is

more sensitive than solid media (LJ). Previous studies have shown that MGIT media is specific (93.3%), accurate (96.3%), very sensitive (100%), and a faster method of detecting Mycobacterium compared to LJ cultures [7]. Other studies have shown the highest sensitivity and scored very well in detecting non-TB mycobacteria demonstrated by the BACTEC MGIT 960 System, both when used alone or in combination with LJ. The highest specificity is shown by the LJ method itself. Thus, MGIT performs best for sensitivity, while LJ sets the current standard for specificity [6].

Lowenstein-Jensen (LJ) is a selective media used for the culture and isolation of Mycobacterium. This egg-based media contains solid eggs, potato starch, salt and compacted glycerol. Potato Flour and L-Asparagine are two sources of nitrogen and vitamins. Magnesium Sulfate & Monopotassium Phosphate augment the development of organisms and function as a buffer. Malachite green prevents the growth of most of the contaminants that survived from decontamination of the specimen while encouraging the growth of Mycobacterium. Glycerol functions as a carbon source and is beneficial for the growth of human-type tubercle bacilli [8].

Growth supplements or OADC (Oleic Acid, Albumin, Dextrose and Catalase) added to MGIT media before inoculation. This supplement is especially important for the development of a multitude of mycobacteria, particularly those included in the *Mycobacterium tuberculosis* complex. Oleic Acid has an essential role in mycobacteria metabolism. Albumin serves to bind free fatty acids that may be poisonous to mycobacteria. Dextrose works as an energy source. Catalase works to obliterate toxic peroxides. Polyoxyethylene stearate (POES) works to increase the development of *Mycobacterium tuberculosis* and help in giving a uniform inoculum [11]. The effect of adding a growth supplement (OADC) causes a positive MGIT tube to contain one or more mycobacterial species. Mycobacteria that grow faster can be detected before mycobacteria that grow slower. Therefore, a positive MGIT tube must be properly identified. Because of the richness of MGIT broth and the non-selective nature of the MGIT indicator, it is crucial to follow the decontamination procedure. This step is vital to reduce the contamination possibility. Compliance with procedural instructions, which include the use of the recommended inoculum volume of 0.5 ml; particularly crucial for optimal mycobacterial recovery [24].

Our study results showed that there were two sample codes with different culture results, but after analysis with the statistic, Wilcoxon Signed Rank Test there were no differences in the results of culture *Mycobacterium tuberculosis* on solid media (Lowenstein Jensen) and liquid media (Mycobacteria Growth Indicator Tube). Thus, it can be seen that solid media (LJ) and liquid media (MGIT) can be used as culture media for *Mycobacterium tuberculosis* in diagnosing TB cases. Solid media (LJ) and liquid media (MGIT) have advantages and disadvantages of each. Culture on solid media (LJ) requires a prolonged incubation of about 8 weeks after the time of inoculation [25]. To minimize the risk of broader transmission and a more severe disease course, TB diagnostic requires a faster culture medium. Culture on solid media (LJ) is cheaper than liquid media (MGIT). Culture on liquid media (MGIT) has been introduced for faster Mycobacterium growth, and culture results will come out in 42 days if there is no bacterial growth. The disadvantage of culture on liquid media (MGIT) is that the price is higher than the culture of solid media (LJ).

CONCLUSION

We have found that there is no difference in the results of culture of *Mycobacterium tuberculosis* in solid media (Lowenstein Jensen) and liquid media (Mycobacteria Growth Indicator Tube). Further researchers are expected to continue the research by using more samples to see the difference in culture results optimally and add other media as a comparison such as middle media brook (7H10) so that diagnostic tests can be performed to calculate both sensitivity and specificity of each media.

REFERENCES

1. WHO World Health Organization. TB Reports Singapore [Internet]. Available from: https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf
2. Kemenkes RI. Pedoman Nasional Pengendalian Tuberkulosis-Keputusan Menteri Kesehatan Republik Indonesia Nomor 364. Kementeri Kesehat Republik Indones [Internet]. 2011;(Pengendalian Tuberkulosis):110. Available from: http://rspau.ddns.net:8080/perpustakaan/property/uploads/d8c81b3affec9ce9d840d2a53d26475a.pdf
3. Lolong D, Simarmata O, Herawati M, Sihombing N, Tobing K, Nugraheini W, et al. Studi evaluasi deteksi kasus TBC dengan Tes Cepat Molekuler (TCM) di Indonesia. 2018;1(1):1–198.
4. Sjahrurachman A et al. Technical Instructions for Culture Examination, Identification and Sensitivity Test of Mycobacterium tuberculosis on Solid Media. 2012.
5. Munawaroh AL, Hidayati DYN, Utami YW. Comparative Study of Coco Blood Malachite Green Culture Media with Lowenstein Jensen (LJ) for Rapid Diagnostic, Specific, and Sensitive on Sputum of Tuberculosis Suspect Patient. Maj Kesehat FKUB. 2015;2(2):79–91.
6. Rageade F, Picot N, Blanc-Michaud A, Chatellier S, Mirande C, Fortin E, et al. Performance of solid and liquid culture media for the detection of Mycobacterium tuberculosis in clinical materials: Meta-analysis of

recent studies. Vol. 33, European Journal of Clinical Microbiology and Infectious Diseases. 2014.
7. Hasan M, Munshi SK, Banu Momi MS, Rahman F, Noor R. Evaluation of the effectiveness of BACTEC MGIT 960 for the detection of mycobacteria in Bangladesh. Int J Mycobacteriology. 2013;2(4).
8. Nurjannah et al. TECHNICAL INSTRUCTIONS AND QUALITY CONSTRUCTION Culture Examination, Identification and Sensitivity Test of Mycobacterium tuberculosis complex to Anti-Tuberculosis Drugs on Solid Media. Jakarta; 2018.
9. Shoukrie A, Alameen A, Shaban D, Alamari W, Aboguttaia N, Askar N, et al. The Yield of Sputum Smear Direct Microscopy Using Ziehl-Neelsen Stain in Comparison with Lowenstein Jensen Culture on the Diagnosis of Pulmonary Tuberculosis in Tripoli-Libya. Mycobact Dis. 2018;08(01).
10. Adami AG, Gallo JF, Pinhata JMW, Martins MC, Giampaglia CMS, de Oliveira RS. Modified protocol for drug susceptibility testing of MGIT cultures of Mycobacterium tuberculosis by the MGIT 960. Diagn Microbiol Infect Dis. 2017;87(2).
11. Diriba G, Kebede A, Yaregal Z, Getahun M, Tadesse M, Meaza A, et al. Performance of Mycobacterium Growth Indicator Tube BACTEC 960 with Lowenstein-Jensen method for diagnosis of Mycobacterium tuberculosis at Ethiopian National Tuberculosis Reference Laboratory, Addis Ababa, Ethiopia. BMC Res Notes. 2017;10(1).
12. Presialia, Athira & Kiranasari A. Comparison of the Contamination Rate between Lowenstein-Jensen Media and BACTEC MGIT 960 as a Culture Method for Mycobacterium tuberculosis. J Kedokt Meditek. 2020;2(2):36–42.
13. Ma Y, Fan J, Li S, Dong L, Li Y, Wang F, et al. Comparison of Lowenstein-Jensen medium and MGIT culture system for recovery of Mycobacterium tuberculosis from abscess samples. Diagn Microbiol Infect Dis. 2020;96(4).
14. Djoudi, Ferhat & Benremila, Dalila & Kassa, Sabrina & Messaoudi, Massilva & Mezidi, Nadir & Khechida, Mounir & Touati, Rym & Yahi, Randa & Yala D. EC PULMONOLOGY AND RESPIRATORY MEDICINE Research Article Diagnosis and Drug Susceptibility of Mycobacterium tuberculosis from Pulmonary Specimens at Pasteur Institute of Algeria: Comparative Study between Classic Lowenstein-Jensen Culture and BACTEC MGIT. ECronicon Open Access. 2019;8(9).
15. Huang TS, Lee CC, Tu HZ, Lee SSJ. Rapid identification of mycobacteria from positive MGIT broths of primary cultures by MALDI-TOF mass spectrometry. PLoS One. 2018;13(2):1–15.
16. World Health Organization. Latent tuberculosis infection - Executive Summary. Who. 2018;6.
17. Widyastuti S et al. Standar Prosedur Operasional Mikroskopis TB Pemeriksaan Mikroskopis TB [Internet]. 2012. Available from: https://www.academia.edu/29530632/Standar_Prosedur_Operasional_Mikroskopis_TB_Pemeriksaan_Mikroskopis_TB
18. Che Engku Noramalina Che-Engku-Chik1, Nor Azah Yusof 2, 3, Jaafar Abdullah 2, 3, Siti Sarah Othman1, Mohd Hazani Mat Zaid 2, 3 HW. Detection of Tuberculosis (TB) using Gold Standard Method, Direct Sputum Smears Microscopy, PCR, qPCR and Electrochemical DNA Sensor: A Mini review. 2016;4(2). Available from: https://journal.hibiscuspublisher.com/index.php/JOBIMB/article/view/305/335
19. Hi Media L. Lowenstein Jensen Medium Base HiMedia. 2019;10–2.
20. Brooks, GF., Carroll KC, Butel JS M. Mikrobiologi Kedokteran Jawetz, Melnick, & Adelberg. 25th ed. Jakarta: EGC; 2013.
21. Mathewos B, Kebede N, Kassa T, Mihret A, Getahun M. Characterization of mycobacterium isolates from pulmomary tuberculosis suspected cases visiting Tuberculosis Reference Laboratory at Ethiopian Health and Nutrition Research Institute, Addis Ababa Ethiopia: A cross sectional study. Asian Pac J Trop Med. 2015;8(1).
22. Essawy TS, Saeed AM, Fouad NA. Comparative study between using Lowenstein Jensen, Bio-FM media and mycobacteria growth indicator tube (MGIT) system in identification of Mycobacterium tuberculosis. Egypt J Chest Dis Tuberc. 2014;63(2).
23. Chemeda A, Mihret A, Abebe T, Worku A, Ameni G. Genotyping of mycobacterium tuberculosis isolated from pulmonary tuberculosis patients among people living with HIV in Addis Ababa: Cross-sectional study. J Clin Tuberc Other Mycobact Dis [Internet]. 2018;12(June):34–7. Available from: https://doi.org/10.1016/j.jctube.2018.06.004
24. Salman HS, Rüsch-Gerdes S. MGIT Procedure Manual. Mycobact Growth Indic Tube Cult Drug Susceptibility Demonstr Proj [Internet]. 2006;(July):pp24-40. Available from: http://www.finddiagnostics.org/export/sites/default/resource-centre/find_documentation/pdfs/mgit_manual_nov_2007.pdf
25. Safithri F. Diagnosis of TB in Adults and Children by ISTC DIAGNOSIS OF ADULT AND CHILD TB BASED ON ISTC (International Standard for TB Care). J Heal Sci Fam Med [Internet]. 2011;7(2). Available from: https://ejournal.umm.ac.id/index.php/sainmed/article/view/4078