

## RAPID CULTURE METHOD USING BIPHASIC MEDIA ON BACTERIOLOGIC DIAGNOSIS TO DETECT *Mycobacterium tuberculosis* FOR DETERMINING PULMONARY TUBERCULOSIS

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### ABSTRAK

Diagnosis menentukan TB paru berdasarkan pada deteksi *Mycobacterium tuberculosis* secara cepat dan akurat penting untuk menentukan terapi yang adekuat. Metode kultur standar menggunakan media Lowenstein Jensen (L-J) untuk mendeteksi *Mycobacterium tuberculosis* rumit dan membutuhkan waktu yang lama untuk mendiagnosis. Kultur pada media padat dapat meningkatkan sensitivitas dalam memeriksa pertumbuhan koloni mikobakteri. Nutrisi yang dilengkapi media dengan serum dalam media agar serum-darah dalam media fase kaldu akan meningkatkan laju pertumbuhan koloni. Selektivitas media kultur dengan menambahkan malachite green dan penisilin. Tujuan dari penelitian observasional laboratorium ini adalah menentukan validitas metode kultur menggunakan media biphasic agar serum-darah yang membandingkan dengan metode kultur standar LJ untuk mendeteksi *Mycobacterium tuberculosis* dari spesimen sputum 100 pasien TB paru. Komposisi gizi dari media biphasic agar serum-darah untuk pertumbuhan mikobakteri koloni yang optimum adalah 3,3gram nutrisi agar (Oxoid) dalam 90ml akuades, 25mg malachite green, 3ml gliserol, 10ml serum, dan 100U/ml penisilin, fase kaldu mengandung nutrisi yang sama dengan menambahkan darah yang diencerkan; metode kultur biphasic ini memiliki sensitivitas laboratorik tinggi dengan deteksi limit 102cfu/ml dan spesifisitas tinggi untuk *Mycobacterium tuberculosis*. Laju pertumbuhan koloni mikobakteri dalam media biphasic 10-14 hari, metode biphasic memiliki validitas klinis yang tinggi, sensitivitas 100% dan spesifisitas 100% untuk mendeteksi *Mycobacterium tuberculosis* dalam sputum TB paru patients. Jadi, metode kultur cepat menggunakan Media biphasic darah-serum agar dalam diagnosis bakteriologis untuk mendeteksi *Mycobacterium tuberculosis* dalam dahak penderita TB paru tersangka memiliki sensitivitas dan spesifisitas tinggi. (FMI 2013;49:91-96)

**Kata kunci:** Metode kultur cepat, media bifasik, agar serum-darah, *Mycobacterium tuberculosis*, sputum TB paru

### ABSTRACT

Diagnosis to determine pulmonary TB based on detection of *Mycobacterium tuberculosis* rapidly and accurately is important to determine the adequate therapy. Standard culture method using Lowenstein Jensen (L-J) media to detect and diagnose *Mycobacterium tuberculosis* require long time. Culture on solid clear medium may increase the sensitivity in examining the growth of mycobacterial colonies. Supplemented nutrition of media with sera in agar media and sera plus blood in broth phase media will increase growth rate of colonies. Selectivity of culture media by added malachite green and penicillin. The objective was to determine the validity of culture method using a biphasic blood-sera agar medium comparing with the standard culture method L-J for the detection of *Mycobacterium tuberculosis* from sputum specimens of 100 suspect pulmonary TB. The nutrition composition of biphasic media blood-sera agar which optimum colony growth of mycobacteria are 3.3gram nutrient agar (Oxoid) in 90ml aquadest, 25µg malachite green, 3ml glycerol, 10ml sera, and 100U/ml penicillin, and broth phase contain similar nutrition by added blood diluted; these biphasic culture method has a high laboratory sensitivity with limit detection of 102cfu/ml and high specificity for *Mycobacterium tuberculosis*. Growth rate of mycobacterial colonies in biphasic media 10-14 days; biphasic method has high clinical validity, 100% sensitivity and spesificity 100% to detect *Mycobacterium tuberculosis* in sputum of suspect pulmonary TB. In conclusion, rapid culture method using biphasic media blood-sera agar for bacteriologic diagnosis to detect *Mycobacterium tuberculosis* in sputum of suspect pulmonary TB patients have high sensitivity and specificity. (FMI 2013;49:91-96)

**Keywords:** rapid culture method, biphasic media, blood-sera agar, *Mycobacterium tuberculosis*, sputum of pulmonary TB

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### INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by the bacteria *Mycobacterium tuberculosis*. This disease generally attacks human lung parenchymal tissue and contagious. Chronic disease progresses with

repeated healing phase and recurrence (Belisle et al 2005, Brooks et al 2004, Lodha & Kabra 2004). This disease affects nearly 75% of population in productive age, 15 to 50 years, so it is economically detrimental to a country (Brooks et al 2004, Lodha & Kabra 2004, Mirza et al 2003). In view of increasing TB problem, we

have to immediately address the problem globally to successfully eliminate the disease.

Various studies to find out more about the pathogenesis of tuberculosis have grown rapidly at molecular level. At this level there is a very complex mechanism that includes the interaction between the molecules of the various cells that make up regulated response network to control *Mycobacterium tuberculosis* infection. Based on the developing knowledge of the pathogenesis, developed also increase the accuracy of diagnosis, prevention and treatment methods are extremely significant in the development of TB control governance.

Tuberculosis is known to be a preventable (preventable) and reversible (curable) infectious disease if proper diagnosis is done immediately and followed immediately with appropriate treatment as well, so that we can expect imminent recovery, reduced morbidity, prevent complications or progression, not allowing the latency of infection (dormant stage), and negate the risk of transmission (Belisle et al 2005, Cousins et al 1996). Global tuberculosis problem is more complex and serious with increasing prevalence, increasing drug resistance of multidrug resistant tuberculosis (MDR-TB), the emergence of extensively drug resistant tuberculosis (XDR-TB), and increasing TB-HIV co-infection (Mirza et al 2003, Perkins 2000).

An important first step in the control of tuberculosis is the establishment of tuberculosis diagnosis based on the discovery of *Mycobacterium tuberculosis* from specimens of patients with suspected tuberculosis. Proper diagnosis and quick determination of adequate immediate treatment can improve healing and prevent infection. Until now, the conventional standard method for the detection of *Mycobacterium tuberculosis*, such as the method of media culture on Lowenstein Jensen (L-J), has a constraint of lengthy diagnosis (Belisle et al 2005, Cousins et al 1996). Methods of culturing on solid-clear and transparent media allows easy observation of colony growth, thus increasing the sensitivity of detection and subsequently the easiness to identify *Mycobacterium tuberculosis*. The addition of serum in the solid agar as a nutritional supplement can increase the speed of growth of mycobacteria colony. The combination of solid slant agar media and liquid media, nutritive liquid media plus serum and blood on the surface of solid media can increase colony growth rate faster than that in solid or in liquid media only. The addition of green malachite and penicillin in the solid agar or liquid media may improve the selectivity or specificity, because green malachite and penicillin selectively kill other bacteria instead of the mycobacteria. This research aims to study the validity

of culture method in biphasic media for the detection of *Mycobacterium tuberculosis* in sputum of patients with suspected pulmonary tuberculosis, as well as the speed of test results.

## MATERIALS AND METHODS

The study design was observational laboratory to test the validity (sensitivity and specificity) rapid culture method for the diagnosis of *Mycobacterium tuberculosis* by using biphasic agar-blood serum compared with conventional standard culture method of Lowenstein Jensen medium (the gold standard). Sample consisted of sputum from patients with suspected pulmonary tuberculosis in Pulmonary Disease Division, Karang Tembok Hospital, Surabaya. Sample size was 100 patients, enrolled with consecutive random sampling.

Patients with suspected pulmonary TB were adult patients (age 15-50 years) with chronic cough and phlegm productive for more than 2 weeks, which may be accompanied by blood or blood spots (Brooks et al 2004). In this study sputum was obtained from the sputum of patients with suspected pulmonary tuberculosis by coughing, purulent phlegm, yellow-green, and could be accompanied by blood or blood spots. Sputum volume was 3-5 ml (2). *Mycobacterium tuberculosis* colony was grown on solid agar surfaces on biphasic medium and L-J, with characteristics of dry-rough, cream, positive AFB (Ziehl Neelsen), positive niacin accumulation test, and positive nitrite reduction test (Cousins et al 1996).

The preparation of reference strains of *Mycobacterium tuberculosis* H 37 Rv ATCC 27294, *Mycobacterium fortuitum* ATCC 6841, clinical isolates of *Mycobacterium tuberculosis* strains was carried out according to the standard (Cousins et al 1996). Nutritive agar media (Oxoid) of 3.3 g was dissolved in 90 ml of distilled water, then 3 ml glycerol was added, 25 µg green malachite, thoroughly mixed, and sterilized using an autoclave of 121 degree C for 10 minutes. Once out of the autoclave, media temperature was allowed to cool at a temperature of 40-50 degrees, and added with 10 ml of serum (bovine serum), added with 100 U/ml penicillin (Merck), shaken evenly and quickly poured into a 7 ml MacCartney bottle, then bottle was tilted 30 degree until the agar solidified to become slant agar.

Slant agar media was tested for sterility by incubation of 37 degrees C for 2x24. If there is no growth of bacterial colonies, Agar media slant is ready for the preparation of sterile biphasic media. In 90 ml of liquid media nutritive (Oxoid) with green malachite 25 µg and 1 ml glycerol were sterilized, then added with 10 ml of

serum, and 1 ml of diluted blood (bovine blood with 5% dilution in sterile distilled water) was added with 100 U/ml penicillin (Merck). Each bottle of slant agar media was filled with 5 ml of liquid, slant agar media phase and blood media phase became biphasic blood-serum agar (BBSA).

BBSA media was also subjected to sterility test. Sterile BBSA media was subjected to media quality test by culturing *Mycobacterium fortuitum* ATCC 6841 culture, incubated at 37 degrees C in CO<sub>2</sub> incubator (5% CO<sub>2</sub>), for 7 days, good quality when optimal colony growth on the surface of the slant appears (visible) on day 3 - 4. The observations were done by tilting the tube until to the surface of the slant is not covered with blood. Media quality testing was done three times. Sterility test and test media quality test was also performed on L-J medium.

In BBSA media optimization, *Mycobacterium fortuitum* suspension of 103 and 105 CFU/ml dilution of a suspension of 107 CFU/ml (equivalent to 1.0 Mc Farland turbidity) in a liquid medium Middlebrook 7 H9, cultured twice at 100 ul on the surface of BBSA media. The culture was incubated in the CO<sub>2</sub> incubator in 37 degrees C, 5% CO<sub>2</sub>, for 7 days. Observations were made every day, observing colony growth characteristics and identification of positive AFB (Cousins et al 1996). In testing BBSA media quality stability, BBSA media was proven sterile and in a good quality, stored at 4 degrees C; every 1 week we carried out media quality testing up to 1 month (Cousins et al 1996). Laboratory sensitivity testing (internal), suspension of reference strain *Mycobacterium tuberculosis* H 37 Rv and 3 clinical isolate strains, dilution 10<sup>2</sup> CFU/ml, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup> CFU/ml, 100 uL (twice) was cultured on BBSA, incubated in 37 degrees C, 5% CO<sub>2</sub>, for 3 weeks. Every day we observed typical colony growth, the presence of contamination, and at 3 weeks of culture we noted typical clustered optimal colony growth. The same treatment was performed on L-J medium (Cousins et al 1996).

In laboratory specificity test (internal), the suspension of 105 CFU/ml reference strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29 213 were cultured 100 uL, twice on BBSA media. At the same time, the sensitivity test was compared to the culture of *Mycobacterium tuberculosis* H37Rv ATCC 27 294. The external validity (sensitivity and specificity) test of culture method in biphasic media for detecting *Mycobacterium tuberculosis* in sputum of patients with suspected pulmonary tuberculosis was compared to gold standard of culture method on L-J medium. Data resulting from biphasic methods culture laboratory and L-J was tabulated in 2 x 2 validity test table.

Culture laboratory procedures were performed according to WHO standard. Sputum (3-5 ml) of patients with suspected pulmonary tuberculosis was decontaminated and concentration (alkali Petroff method, WHO) : 1 sputum volume + 2 volume 4% NaOH was be vortex, and then incubated in 37 degrees C for 15 min, then centrifuged at 3000 × g for 15 min, the supernatant was removed, the sediment was washed 10 ml sterile PBS (vortex - centrifuge 3000 xg, 15 min), the supernatant was removed, sediment was be resuspention, cultured 100 ul, inoculated twice on L-J media, also in biphasic media, incubated at 37 degrees C, 5% CO<sub>2</sub>, observation of typical colony growth of each week for 3-8 weeks. If there was no colony growth, typically dry, coarse, creamy white, we made the identification. The results was designated as *Mycobacterium tuberculosis* if microscopically the Ziehl Neelsen AFB was positive, niacin accumulation test was positive and test on positive nitrite reduction (Cousins et al 1996, Hellyer et al 1999). The study was conducted at the Laboratory of Tuberculosis TDC Airlangga University Surabaya.

## RESULTS

Biphasic media of blood serum agar is optimal for colony growth of *Mycobacterium tuberculosis* in slant agar phase with the composition of 3.3 grams of nutritive agar (Oxoid), 3 ml glycerol, 25 µg green malachite, in 90 ml autoclave sterilized distilled water in 121 degrees C for 10 minutes. At a temperature of 40-50 degrees C 10 ml of serum and penicillin 100 U/ml were added. In liquid phase, in addition to composition similar to solid phase, we also added 1 ml of 5% blood diluted in distilled water. Liquid media phase increased colony growth. Solid media surface provided attachment for easy colony clustering. Selectivity or specificity was guaranteed by the non-anti-bacterial effect of mycobacteria from green malachite and penicillin.

The result of laboratory sensitivity test showed high sensitivity in a detection limit of 102 cfu/ml, and laboratory specificity revealed optimal growth of *Mycobacterium tuberculosis* and inhibited the growth of Gram positive and negative bacteria non mycobacteria. On sputum sediments, decontamination and concentration results were cultured on BBSA media and L-J media (gold standard). We detected and identified *Mycobacterium tuberculosis* sputum sediments 33 per 100 (33%) and it was detected as that the one on BBSA medium was equally positive on with that on L-J media (Table 1). The sensitivity and specificity were all 100%.

Table 1. Detection and identification of *Mycobacterium tuberculosis* in sputum specimens.

BBSA Culture Method	Culture method in L-J medium (gold standard)		Total
	+	-	
+	33	0	33
-	0	67	67
Total	33	67	100

## DISCUSSION

Data analysis with validity test revealed sensitivity of 100% and a specificity of 100%. In BBSA media we detected typical rough and dry colony and identified *Mycobacterium tuberculosis* with a mean time of growth 14 days. In L-J media we detected typical dry rough colonies in averagely 21 days. Observation of typical colony growth on BBSA media was easier due to transparent media. Typical colonies showed that rapid growth phase had been passed over. BBSA media was also rich in essential proteins nutrients from serum and blood, and also contained glycerol to meet the needs of carbohydrates metabolism of as the source of energy. Consequently, in averagely 14 days we had detected typical colonies with detection sensitivity of *Mycobacterium tuberculosis* from 100% sputum sediments with comparison to L-J media as gold standard. To test the sensitivity of anti-TB drugs the use of BBSA media is more easily and more quickly to detect the presence of typical colony growth in exposure to anti-TB drugs. The composition of the nutrient content in BBSA media ensures optimal growth of *Mycobacterium tuberculosis* and nutritional content does not inhibit the action of anti-TB drugs.

### Development of Tuberculosis Diagnosis

In order to eliminate TB in the U.S. since the 1990, public health laboratories have developed TB Test Performance (high quality and cost effective) to reduce the incidence of tuberculosis in the community, the development of laboratory services that support prevention, and treatment and control of TB. Critical components include the development of integrated systems that ensure speed and confidence (reliability) laboratory test results, the smooth flow of information among laboratories, clinicians, TB control services; establishment of communication lines, expedition of laboratory results reports, avoiding delays and inaccuracies that result in treatment errors, lost opportunities of transmission prevention, development of evidence based recommendations for the development and use of accurate laboratory techniques, maintaining staff proficiency, and improvement of

laboratory information system that includes specific actions and performance measures.

Similarly, CDC also developed standard guidelines with the purpose of establishing integrated systems between laboratories, clinicians, and public health agencies so that they can work together to ensure health care providers. TB control programs have the information necessary for treatment of TB patients, to prevent the transmission of TB, with the ultimate goal of TB elimination; CDC also recommends to optimize the use of laboratory services, quality assurance testing TB, effective reporting (complete and prompt) as well as smooth traffic information to ensure functions of prevention, treatment and prevention at the same time. Until now the accuracy of TB diagnosis is still debated. In systematic cases, accurate and rapid diagnosis is needed to achieve the targets of TB control strategies. However, we cannot ignore the case of latent infection and early disease as a time bomb that can explode any time as a source of disease transmission, so we also need early detection to reduce the chance of transmission to close contact.

The principles of TB control are primarily to attempt to stop transmission of the disease. The disadvantage of AFB microscopic examination is that as time passes at the time of detection the AFB positive cases had already infected a lot of close contacts. Therefore, WHO currently recommends global TB control with immediate detection of the target by means of an accurate diagnosis (trustworthy, reliable) and fast (2-day targets for average laboratory turn around time), more favorable for the patient, easy to work with (feasible), recording and reporting management system in an efficient flow of information, and immediately give appropriate treatment to all TB cases resolved, thus giving a significant impact on TB control and decrease the transmission period.

Until then, TB-DOTS controlling strategy are still having problems in development, such as in the case of detection tools, equipment, training, quality control of AFB microscopic examination. The establishment of global laboratory network, which is highly needed for the detection of millions of new cases per year, has not been realized. Moreover, TB laboratory management is still below the standard, referral center laboratory is still limited, and the presence of anti tuberculosis drug resistance challenge resulting in increased cases of repeated failure treatment that extends the period of transmission.

Until recently, the diagnosis of TB according to WHO and Guidelines for National TB Control 2008 include an early stage based on the clinical manifestations of

tuberculosis suspects, later confirmed by laboratory diagnosis of microscopic AFB, which can be accompanied by data from X-rays, and in particular indication, it requires confirmation using culture methods for identification of *M. tuberculosis* and non-tuberculosis mycobacterium (NTM) as well as sensitivity to test Anti Tuberculosis Drugs (ATD). At this time and in the future, the difficulty in TB diagnosis has been visible, as in the case of initial infection, latent infection, extra pulmonary tuberculosis, NTM infection, monitoring the results of therapy healing, ATD resistance, and HIV co-infection. Pace with problem diagnosis in such cases, diagnostic tools have been developed, ranging from mycobacteriological laboratory diagnosis from culture up to molecular level, as well as the development of radiological laboratory, pathological-histological laboratory, pathological blood laboratory, and others.

### **Mycobacteriological Diagnosis**

The accuracy of mycobacteriological laboratory diagnosis is determined from clinical examination in suspected tuberculosis, representative specimens from the focus of infection and standard quality, and accurate, fast and easy (feasible technique) laboratory methods.

### **Microscopic Examination – AFB**

Microscopic examination of sputum smears is the most effective and efficient method of diagnosis of pulmonary tuberculosis to screen the cases of infectious pulmonary tuberculosis (infection reservoirs) in the community, especially in developing countries, because it is simple, fast in less than 1 hour and cheap, e.g Ziehl Neelsen or Kinyoun Gabet staining methods. On Tuberculosis Disease Prevention Program (Program Penanggulangan Penyakit Tuberculosis, P2TB), high sensitivity and specificity was found in microscopic examination of AFB three times in spot morning spot (SMS) in specimens of patients with symptoms of pulmonary tuberculosis accompanied by productive cough of more than 3 weeks. Three times AFB examination 3x (SMS) on the DOTS strategy is very useful for the achievement of case finding in P2TB national program if the quality control is properly implemented, ranging from sputum collection to microscopic examination, and trained as well as dedicated laboratory technician are needed.

The development to date is the use of ZN staining method, examination with binocular microscope and interpretation of results based on International Union Against Tuberculosis Lung Disease (IUATLD) standard. Results of 3x AFB examination as in IUATLD standard is very useful for the diagnosis of pulmonary

tuberculosis to determine the classification of treatment, assess the progress of treatment and determines the rate of transmission in P2TB. However, there are some drawbacks, such as in the early detection of cases of primary pulmonary tuberculosis, due to the low sensitivity of smear examination, which can detect mycobacteria only if the amount is at least 5000 bacilli per ml of sputum. Specificity has the disadvantage of not being able to distinguish between *M. tuberculosis* with other species of mycobacteria. Another difficulty is in cases of suspected pulmonary tuberculosis in children, cases of severe illness where it is difficult to obtain sputum, and cases of HIV-TB co-infection in which sputum smears are negative. To overcome these obstacles, the WHO recommends the implementation of the standard procedure of diagnosis flow of pulmonary tuberculosis in adults and detection flow of tuberculosis in children in the community. If the standard laboratory facilities allow, more accurate checks can be done to overcome obstacles in smear examination, such as acridine-orange staining method with fluorescence microscopy, which is more sensitive because it may have fluorescence that cover wider visual field.

### **Culture Methods**

Standard culture method is still the gold standard method since from the source of the secretion, the bronchial infection, we can isolate viable mycobacteria as the cause of infection, according to Koch's postulates. In addition, the sensitivity is more than 95% and can detect mycobacteria 1-100 per ml, and the specificity is 100%, especially when the standard procedure is implemented. Solid medium is always recommended to use, because it can detect the typical colony morphology and reproducible. In addition, we should use commercial media products that have been a standard for quality control. For example, Lowenstein - Jensen (L-J) media with supplements of ribonucleic acid, and L-J medium with supplements of pyruvic acid, Middlebrook 7H10 and 7H11, Wallenstein, Ogawa media, and others. Most of the growth of typical colonies can be detected within 3 to 6 weeks, but some isolates can be detected after 8 weeks of incubation. These isolates should be identified for definitive diagnosis at species level using a series of biochemical tests, especially niacin accumulation test, nitrate reduction test, catalase test at 68 degree C, growth inhibition by 2-Thiophene Carboxylic acid Hydrazides (TCH), or using the method of DNA hybridization.

Constraints of conventional culture method are delay in diagnosis so the results cannot immediately determine the appropriate treatment. However, the methods are very useful for difficult cases such as pleural fluid, cerebrospinal, synovial, blood or urine, confirmation

after complete treatment and the surveillance. The development of culture methods at the moment and in the future on transparent solid and liquid media is to improve the accuracy and speed. The use of liquid media can shorten the time of mycobacterial growth to 10 days. Culture methods in liquid medium that is often used are MGIT (Becton Dickinson), BACTEC 460 TB System, BACTEC 900 TB (Becton Dickinson), Septi Check AFB system (Becton Dickinson), and ESP Culture System II (Accumed International Inc.).

Mycobacteria Growth Indicator Tube (MGIT). MGIT has been widely used for the detection of *M. tuberculosis* as well as for sensitivity test to OAT. MGIT is a method of culturing in liquid media. The procedure is faster and easier than conventional culture methods, and has reliable diagnosis sensitivity. Identification of mycobacteria species require confirmation using biochemical test series. The principle of the MGIT method is that the specimen (after decontamination-concentration process for sputum specimens or directly to pleural fluid specimens and cerebrospinal fluid) were cultured in tubes containing Middlebrook 7H9 liquid medium and the fluorescence that is embedded in a silicon sensor. The presence of mycobacteria growth is detected visually using ultraviolet light. The presence of oxygen lowers the fluorescence emission out of the sensor. When oxygen is used by mycobacteria growing in the medium, then we can detect an increase in fluorescence emission that shows there are mycobacteria in the specimen. The system can be made automatic.

BACTEC 460 TB (Becton Dickinson) is a method of culturing in liquid media which is highly sensitive and specific due to the use of selective media. The procedure is easy and provides fast diagnosis results (10 days), but it is expensive and has a radioactive waste. Then, BACTEC 900 TB was developed without the use of radioactivity. The working principle is that selective culture medium contains Middlebrook 12B 14C labeled palmitic acid. When mycobacteria grow 14C palmitic acid substrate metabolism occurs, so that radioactively labeled CO<sub>2</sub> (14CO<sub>2</sub>) is released in the atmosphere in the bottle and accumulate on the surface of the medium. The engine system draws the air containing 14CO<sub>2</sub> and measures the amount of radioactivity. Each bottle which produces radioactive is measured in the growth index. Growth is regarded as positive when the growth index is 310. Growth result in liquid medium must be confirmed using AFB staining and culture on media with serial biochemical tests for identification of mycobacteria species, or DNA probing. In Microcolony Culture method, the culture on transparent solid media, such as Middlebrook 7H11 or 7H10 and observation of typical

colony, are observed using a microscope or calibrated lens so that the results will be obtained within 10 days.

## CONCLUSION

BBSA media for detection of *Mycobacterium tuberculosis* from sputum specimens has a sensitivity of 100% and a specificity of 100%, as compared to culture method on L-J medium (the gold standard). Mean time of typical *Mycobacterium tuberculosis* colony growth on BBSA media is 14 days. This research should be followed-up to determine the validity at clinical level by evaluating clinical manifestations in patients with suspected pulmonary tuberculosis.

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