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Identification of *Mycobacterium tuberculosis* Bacteria with TB Antigen MPT64 Rapid Test Against Patients with Suspect Pulmonary Tuberculosis in Lubuk Alung Pulmonary Hospital, Padang Pariaman

E Bahar[1*] and A E Putra[1]
Microbiology Department, Faculty of Medicine, Andalas University.
Jl. Perintitis Kemerdekaan No. 94 Padang 25127, Indonesia.

* elizabethbahar@yahoo.co.id

Abstract. Tuberculosis (TB) is old disease which is still the most killer among infectious diseases and the world is still not free from TB. According to the WHO 2017 report there are an estimated 1,020,000 cases in Indonesia, but 420,000 have just been reported. The extent of TB problems requires prevention and control as soon as possible for early diagnosis. AFB methods, culture and PCR diagnosis of TB have limitations. Culture on Lowenstein Jensen media is a standard diagnosis of TB gold, but growing colonies need identification of *M tuberculosis* bacteria. The aim of the study was to identify *M tuberculosis* bacteria using a rapid test (MPT64 Rapid SD TB Ag). As many as 100 samples of patients suspected of TB obtained 48 (48%) positive cultures. From 48 positive cultures with rapid test, 46 (95.8%) *M tuberculosis* bacteria and 2 (4.2%) non *M tuberculosis* were obtained. It can be concluded that identification with the rapid test (SD TB Ag MPT64) is *M tuberculosis* bacteria.

Keyword: Tuberculosis, Antigen, MPT64, *M tuberculosis*

1. Introduction

Tuberculosis (TB) is an old disease that is still the most killer among infectious diseases and the world is still not free from TB. Based on the WHO report 2017 [1], there are an estimated 1,020,000 cases in Indonesia, but only as many as 420,000 cases were reported. Therefore Indonesia is currently ranked second in the TB cases after India [2]. Patients with suspected TB who have not been examined and treated will be a source of transmission again to those around them. This is what causes the TB problem to end. Not to mention contributions from co-infection with TB-HIV, MDR-TB and diabetes mellitus (DM) [3,4].

The size and extent of the problems caused by TB requires all parties to be able to commit and cooperate in the prevention and control of TB. The resulting losses are very large, not only from the health aspect but also from the social and economic aspects. In essence, TB can be said to be a threat to the ideals of development in improving the welfare of the people as a whole, therefore the war on TB also means the fight against poverty, unproductive and weakness.

Rapid diagnosis is a top priority for handling TB cases using various methods, but each has limitations. Acid fast bacillus (AFB) staining methods for almost all health facilities can do so, because it is fast and economical so that it is the main pillar for TB diagnosis. However, the limitations of AFB cannot distinguish *M tuberculosis* from other *Mycobacterium* [5]. In addition, the limitations of AFB are very much influenced by many external factors, such as the quality of coloring and the expertise of examiners resulting in the accuracy of this examination being questioned [6].

Other diagnostic approaches such as nucleic acid amplification, antibody detection to use DNA formulation with PCR, still relate to funds that are not small and have high expertise [7]. Culture is a

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definite diagnosis of the discovery of *Mycobacterium tuberculosis* (*M tuberculosis*) cells, but it takes 4 - 8 weeks to wait for bacterial growth. The results of culture in the form of bacterial colonies need to be identified *M tuberculosis* first. The method of identifying *M tuberculosis* bacteria can use the rapid test method (SD TB Antigen / Ag MPT64 Rapid) and conventional biochemical reactions. At present the method of identifying *M tuberculosis* bacteria is more likely to be rapid test, because it is fast and accurate compared to conventional methods of biochemical reactions.

Examination using rapid test (SD TB Ag MPT64 Rapid) is using the principle of double antibody chromatographic lateral flow immunoassay. The sample will flow to the absorbent area and cellulose membrane. Proteins The antigens in the sample will bind to the conjugate antibodies that have been labeled to form an antigen-antibody complex. Then it will be captured by antibodies located in the test zone so that a pink line will appear (JD Biotech TB Procedure Manual Antigen Rapid Test, 2011).

This method was presented [8] who developed a protein from MPB64 *M tuberculosis* as a target for antigens in the form of rapid tests with sensitivity and specificity of 98.4% and 97.6% respectively. Furthermore [11] examined a sample of isolates from pulmonary and extra pulmonary with the rapid test method (SD TB Ag MPT64 Rapid) and obtained a sensitivity value of 100% and culture as gold standard.

The Mannose Binding Protein 64 (MPB64) gene or Mycobacterium Protein Tuberculosis (MPT64) itself is a protein that has a weight of 24 kDa, which is one of the genomes of the cell wall. This protein is produced during the growth of *M tuberculosis* [5] and is not present in other Atypical and Mycobacterium [9]. The genes encoding MPB64 / MPT64 are in the RD (regions of difference) region which is deleted, so RD1, RD2 and RD3 are strongly suspected as virulence genes possessed by *M tuberculosis* [10]. This gene found in *M tuberculosis* can be used to identify *M tuberculosis* bacteria through antigen-antibody bonds [5].

Indonesian with a high number of TB patients, needs to be diagnosed with this fast and accurate method for identifying *M tuberculosis* , so that immediate treatment can be carried out for TB patients. The use of MPT64 Rapid SD TB Ag is expected to be able to touch peripheral health services, as well as be easier in monitoring and monitoring the community towards TB.

Based on the background of the use of rapid test on the identification of *M tuberculosis* bacteria quickly and accurately researchers have conducted research using MPT64 Rapid SD SD with the aim of identifying *M tuberculosis* bacteria in patients with suspected pulmonary tuberculosis who came to check their disease at Lubuk Alung Padang pulmonary hospital (RSP) Pariaman.

2. Experimental Methods

The research was classified in the descriptive study with a cross sectional study approach to patients suspected of pulmonary TB for identification of *M tuberculosis* bacteria using a rapid test (SD TB Ag MPT64 Rapid).

2.1.Place and time of research

The study was conducted in the Mobiobiology laboratory of Lubuk Alung Hospital in Padang Pariaman from January to October 2017

2.2. Population and sample

The population was subjects with pulmonary TB suspects based on clinical complaints who came to Lubuk Alung Hospital in Padang Pariaman. The research sample is part of the population that meets the inclusion criteria, which is willing to be a participant in the study which is marked by the willingness to sign informed consent, can issue good quality sputum in sufficient quantities (3-5 ml). The sample size uses the formula (Lemeshow and David, 1997).

$$n = \frac{(Z\ \alpha)^2 PQ}{d^2}$$

$$= 92 \text{ sufficient } 100$$

2.3. Material Research

Sputum, dyestuff (carbolic fuchin, 5% H_2SO_4, 70% alcohol, Methylene blue, immersion oil, Lowenstein Jensen media, N-acetyl cysteine solution, NaOH (NAlc-NaOH), PBS solution, rapid test (SD TB Ag MPT64 Rapid), object glass, lamp spirits, tweezers, cotton sticks, microscopes, srewcup test tubes, sentifuse 40 C, fulcon tubes, autoclaves, incubators, Bio Savety Class II (BSC II) 1300 SERIES A.

2.4. Procedure

The research was carried out in the BSC II space (1300 SERIES A). Sputum was smeared with smear using the Ziehl Neelsen method using carbolic fuhxine, alcoholic acid (H2SO4 andalcohol 96%) and methylene blue. The staining results are seen with a 10 X 100 microscope using immersion oil to detect bacterial cells (positive or negative smear). Next sputum is prepared with a solution of N-acetyl cysteine, NaOH (NAlc-NaOH) is as much in the fulcon tube, vortexed for 15 minutes. Then centrifuged using centrifuge 40C at 3000 g for 20 minutes. After that the supernatant is removed and the pellet is added as much as 20 ml PBS solution as a bacterial suspension and stored at -20^{0}C which is ready to be processed for culture material into Lowenstein Jensen's tilting media bottle.

A total of 100 ul of the bacterial suspension was inserted and spread on the surface of Lowenstein Jensen's tilted media and ready to be incubated at 37^0 C in the incubator waiting 4-8 weeks. The bacterial colonies growing in the form of grated cheese were identified. Identification of bacterial colonies using a rapid test (SD TB Ag MPT64 Rapid) by: mixing 20 ul PBS and a small amount of bacterial colonies taken with a toothpick stick into the test zone, stirring until homogeneous and wait 2-4 minutes. If a pink line shows positive *M tuberculosis* bacteria.

3. Results and Discussion

Table 1 and 2 shows that of the 100 pulmonary tuberculosis patients found varied characteristics and sputum examination using AFB staining method, culture and rapid test (SD TB Ag MPT64 Rapid) to bacterial colonies that grow.

Table 1. Characteristics of Patient Gender

No	Gender	Frequency (%)	Total (%)
1	Famale	63	63
2	Male	37	37

Table 2. Characteristics of Patient Age

No	Age (Years)	Frequency (%)	Total (%)
1	15 - 25	16	16
2	26 - 36	17	17
3	37 - 47	16	16
4	48 – 58	20	20
5	59 - 69	25	25
6	>70	6	6

From the characteristics of the sex and age of the patient can be more men than women 63% and 37%. The 59-69 year age group occupies the highest number of suspected pulmonary TB 59-69 with a frequency of 25 patients from 100 patients with pulmonary TB.

Table 3. Sputum examination with AFB, culture methods

No	Methods	Frequency		Total (%)
		Positive (%)	Negative (%)	
1	AFB	32	68	100
2	Culture	48	62	100

The description of sputum examination with several methods, in table 3, there are 48% positive culture results of growing colonies and 62% negative not growing colonies. Identification of *M tuberculosis* bacteria from bacterial colonies to 48 positive cultures using Ag MPT64 rapid test obtained the following results:

Table 4. Identification of *M tuberculosis* with Ag MPT64 rapid test

M tuberculosis	Non *M tuberculosis* (MOTT)	Total
46 (95.8%)	2 (4.2%)	48

This study carried out an examination of AFB, culture and Ag MPT64 rapid test against sputum and isolate colonies. All of these methods give different tuberculosis diagnosis results, because they have their own limitations. The exact diagnosis of someone infected with *M tuberculosis* bacteria requires culture as the gold standard. Colonies that grow in culture have not shown *M tuberculosis* bacteria and require conventional identification of biochemical reactions and MPT64 rapid test.

A total of 100 sputum of TB suspects in Lubuk Alung Hospital in Padang Pariaman who had been cultured found 48% positive colony growth. Identification with Ag MPT64 rapid test on 48 positive colony cultures found 46 (95.8%) *M tuberculosis* bacteria and 2 (4.2%) infected with *myocobacterium other than tuberculosis* (MOTT). Means of 100 patients suspected of TB obtained as much as 46% of *M tuberculosis* bacteria. The Ag MPT64 rapid test method has the disadvantage of having to wait for 4-8 weeks of culture results, but it is fast and accurate when compared to conventional methods in biochemical reactions.

In this study there was no diagnostic test of the methods used, in the form of sensitivity, specificity, positive predictive value and negative predictive value, because it only identified *M tuberculosis* for screening examinations. This is because the amount of *M tuberculosis* in one colony which is calculated by protein secretion is enough to be detected by MPT64 rapid test, so as to provide positive results. According to [12] research, that MPT64 rapid test gave a sensitivity value of 98.04%, a specificity of 98.68%.

The identification of MOTT bacteria from culture as much as 4.2% is probably due to many factors, including not enough protein secretions to be detected by MPT64 rapid test and MPT64 gene has undergone mutations or dilutions. Antibiotic therapy factors that may have been consumed by previous patients, such as the fluoroquinolone group which results in reduced *M tuberculosis* viability so that it cannot grow in culture. .

In addition the possibility of a mixed infection between MOTT and *M tuberculosis* or only a single infection MOTT, because MOTT can be found in the water environment, soil and can form colonization of the skin. MOTT bacteria grow faster and can get rid of *M tuberculosis* growth, so MOTT colonies grow more than *M tuberculosis* colonies [13, 15]. *Mycobacterium tuberculosis* bacteria are intracellular bacteria that grow slowly and have pathogenic properties that can survive in host macrophage cells. This

bacterium is acid resistant, because its cell wall consists of mycolic hydrophobic acid which is a specific component of the cell wall. This thick mycolic layer causes nutrients to enter the bacterial cells to be disrupted and result in slow growth of *M tuberculosis* bacteria, but can increase resistance to degradation through lysosomal enzymes [15]. The exact diagnosis of TB is found in *M tuberculosis* bacterial cells

4. Conclusion

Identification of *M tuberculosis* bacteria with rapid test (SD Ag TB MPT64 Rapid) fast and accurate. As many as 4.2% suffer from non-tuberculosis or MOTT and 95.8% *M tuberculosis* bacteria..

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