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The Comparison of *Mycobacterium tuberculosis* Detection using Molecular Rapid Test and Immunochromatography in Patients Suspected of Having Tuberculosis in Pangkajene and Kepulauan Regencies

Faradillah Suhar[1]*, Muhammad Nasrum Massi[2], Rizalinda Sjahril[2]

[1]Biomedical Science Department, Postgraduate Program of Universitas Hasanuddin,
Jl. Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia
[2]Microbiology Department, Faculty of Medicine, Universitas Hasanuddin,
Jl. Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia

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ABSTRACT

Mycobacterium tuberculosis is an acid-resistant bacterium that causes tuberculosis and can be detected using a variety of methods. This study aimed to determine the comparison of the detection results of *Mycobacterium tuberculosis* using the molecular rapid test (MRT) and immunochromatographic method. This research was conducted at Batara Siang Hospital, Pangkajene, and Kepulauan Regencies, in July-September 2019 using 100 samples. This study is cross-sectional research, applying *the chi-square* test for analysis. The results of statistical tests revealed a significant difference between the results of tests using molecular rapid tests and immunochromatography (<0.001). The sensitivity, specificity, positive probability value, negative probability value, and accuracy of the immunochromatography method against the molecular rapid test (MRT) were 91.3%, 100%, 100%, 93.1%, and 96%, respectively.

Keywords: rapid molecular test; immunochromatography; tuberculosis

*Corresponding author:
Faradillah Suhar
Biomedical Science Department, Postgraduate Program of Universitas Hasanuddin
Email: faradillahsuhar@pasca.unhas.ac.id

INTRODUCTION

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*, and this disease has triggered an increase in mortality and morbidity (Shi, 2018). The clinical manifestations for each individual in dealing with *M. tuberculosis* are different. Some do not show any symptoms while the manifestations of infection in other individuals develop gradually so that these symptoms are not recognized until the disease has entered an advanced stage. Common manifestations demonstrated by patients with suspected tuberculosis are weight loss, night sweats, hemoptysis (coughing up blood), anxiety disorders, and anorexia (loss of appetite) (Yasmin Asih & Effendy, 2004).

In 2018, WHO reported around 570,289 TB cases in Indonesia making Indonesia the country with the third-largest TB sufferers after India and China (WHO, 2019). In the same year, in the South Sulawesi region, 23,427 TB cases were found, which were divided into 13,573 cases of male patients and 9,854 female patients. In addition, 7,958 new cases of pulmonary TB were confirmed by bacteriological examination (Kemenkes RI, 2018).

TB cases found in Pangkajene and Kepulauan (Pangkep) Regencies in 2017 were 814 and in 2018 decreased to 696 cases. However, when compared to the cases in 2016, which reached 379 cases, TB sufferers in 2018 in Pangkep Regency were still relatively high (Dinas Kesehatan Kabupaten Pangkep, 2019).

Early diagnosis of tuberculosis can be done by paying attention to clinical manifestations, as well as several supporting examinations, such as radiological examinations (Madjawati, 2010). Other common supporting examinations are laboratory examinations which include smear staining, molecular rapid tests, and cultures (Jeong, Lee, & Yim, 2017). Molecular Rapid Test (MRT) is the latest breakthrough for TB diagnosis based on molecular examination using the semi-quantitative Real-Time Polymerase Chain Reaction (RT-PCR) Assay method that targets the rpoB gene hotspot region in *M. tuberculosis*, which is integrated and automatically processes preparations by extraction of *deoxyribonucleic acid* (DNA) in disposable cartridges (Kurniawan, Raveinal, Fauzar, & Arsyad, 2016).

Research conducted by Zeka, Tasbakan, and Cavusoglu (2011) reported that the sensitivity of MRT was 86.2% and specificity was 99.4%, while for BTA staining, the sensitivity was 46.6% and specificity was 99.7%. Van Rie et al (2013) also examined suspected cases of TB with smear-negative and found that the sensitivity and specificity of smear staining were 27% and 99%, while the RT-PCR method on MRT showed a sensitivity of 67% and specificity of 99%, respectively. However, the drawback of MRT is that the device is not evenly distributed throughout Indonesia and special skills are needed to use the tool. Therefore, the immunochromatographic examination method has been developed since it is fast, easy, and practical, and does not require special skills to detect TB antigen. Research conducted by Gustiani, Parwati, Tjandrawaty, & Lismayanti (2014) noted that from 149 samples, the sensitivity of the tool was 95.9% and the specificity was 88.2%. Another study conducted by Aryati (2012) resulted in a sensitivity of 85% and a specificity of 90.9%.

Based on the description above, it is crystal clear that no research has compared the accuracy of the examination using MRT and immunochromatography. The researchers also realize that the accuracy of MRT and the immunochromatography in detecting TB infection needs to be compared because both methods have high sensitivity and specificity but differ in terms of the practicality of the equipment, as well as the examination duration. In addition, it is necessary to determine the clinical symptoms of TB and their radiological features to support an accurate diagnosis of TB. This study aimed to compare the results of *M. tuberculosis* detection using MRT and

immunochromatography.

MATERIALS AND METHODS

The independent variable in this study was a patient suspected of having tuberculosis, who underwent a sputum examination in the laboratory at the request of a physician. The dependent variables were MRT and immunochromatography, two methods commonly applied for detecting *M. tuberculosis*. MRT can detect *M. tuberculosis* through genes while immunochromatography completes the identification through antigen-antibody binding. The research applied cross-sectional analysis to compare the results of *Mycobacterium tuberculosis* detection between MRT and immunochromatography in suspected tuberculosis patients. The samples were taken with consecutive sampling technique.

The research was carried out in the Microbiology Laboratory of Batara Siang Hospital, Pangkajene and Kepulauan (Pangkep) Regencies, South Sulawesi. The study was conducted from July to September 2019. The inclusion criterion was patients diagnosed with pulmonary tuberculosis who had never received treatment. The exclusion criterion was sputum sample contained too much saliva. During the study, a total of 116 samples were collected but only 100 samples met the criteria, while 16 samples did not meet the criteria. The research ethics permit was issued by the research ethics committee of Universitas Hasanuddin, with Number: 507/UN4.6.4.5.31/PP36/2019.

Materials and Equipment

The instrument used in this study was an MRT consisting of GenXpert® Cephalid, cartridge, immunochromatographic test cassette (JD Biotech), dropper pipette, and patient medical records. The materials were buffer (JD Biotech) and reagent samples (GeneXpert®Cephalid). The research sample was phlegm/sputum.

Method

The research on the inhibition test of clove flower ethanol extract (*Syzygium aromaticum*) on *Trychopyton rubrum* fungus was experimental, with samples were taken from pure cultures that had been cultured on *Saboroud Dextrose Agar* media and given ethanol extract of clove flower (*Syzygium aromaticum*) then incubated at 37°C for 24 hours. The culture results were seen and the size of the inhibition zone formed was observed. The concentration used for this study was the concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, with the diffusion method with samples using SDA media, the fungi *Trichophyton rubrum*, clove flower ethanol extract. For positive control using SDA media, 2% ketoconazole, *Trichophyton rubrum* fungi and negative control using SDA media, *Trichophyton rubrum* fungi and sterile aquadest.

Procedure

Molecular Rapid Test (MRT)

The samples for MRT were prepared by adding reagent samples to sputum already available two times the volume of the specimen. The samples were homogenized and left for 10 minutes at room temperature. After that, the samples were put into a cartridge and the cartridge was then put into GeneXpert®Cephalid. The results of the examination were released in approximately two hours. The examination with MRT is only performed once but it can be repeated if an error happened in the first examination.

Immunochromatographic method

In the immunochromatographic method (JD Biotech), 200µL of buffer solution was put into a pot containing sputum and homogenized using a plastic pipette for 30–60 seconds. The samples were then incubated for at least 30 minutes. The supernatant formed was taken 200µL and mixed with 100µL of buffer, homogenized, and dripped 3-4 drops into the sample holes on the test cassette. Interpretation of the results was carried

out after 15 minutes. Examination using the immunochromatographic method is only done once, but it can be repeated if the first examination is invalid.

Data Analysis

The data obtained were analyzed in the SPSS 24.0 program using the Chi-square test. If the value of Sig. <0.05 (p<0.05), there is a significant relationship or difference between variables X and Y, but if Sig. > 0.05 (p> 0.05), there is no significant relationship or difference between variables X and Y. In the detection of *M. tuberculosis* by the immunochromatographic method, the sensitivity, specificity, positive probability value, negative probability value, and accuracy were identified by comparing the results of the MRT using a 2x2 table (Table 1).

Table 1. The 2x2 Table

Immunochromatography	**TCM**		**Total**
	Positive	Negative	
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	N

Sensitivity: a/(a+c) x 100%
Specifity : d/(b+d) x 100%
Positive probability value: a/(a+b) x 100%
Negative probability value: d/(c+d) x 100%
Accuracy: (a+d)/N x 100%

RESULTS AND DISCUSSION

Table 2 demonstrates the results of the research that the majority of respondents were male, aged 36-45 years. Most respondents were smokers and the most dominating occupation of the respondents was labor. Table 3 presents the results of the examination using a molecular rapid test. A total of 46 samples were found positive and 54 samples were negative. Meanwhile, in the immunochromatographic method, 42 samples were identified as positive and 58 samples were negative. The results of the immunochromatographic examination are summarized in Figure 1.

The *chi-square* test performed on the *M. tuberculosis* detection between the molecular rapid test and the immunochromatographic methods was <0.001, signifying a significant difference between the result of the molecular rapid test and that of the immunochromatographic test. However, Table 4 presents that the sensitivity, specificity, positive probability value, negative probability value, and accuracy of immunochromatographic method to molecular rapid tests were good, indicated by the values of 91.3%, 100%, 100%, 93.1%, and 96%, respectively.

TB disease can infect all age groups, but the most affected are those in the reproductive age (25-50 years old) because, at reproductive age, people have a high level of activity and mobility to meet the demands of living needs, causing them to have close contact with the outside world and increase the risk of exposure. One of the behaviors that can trigger a decrease in the immune system of the body is smoking, especially if it is not balanced by adequate nutrition (Fransiska & Hartati, 2019).

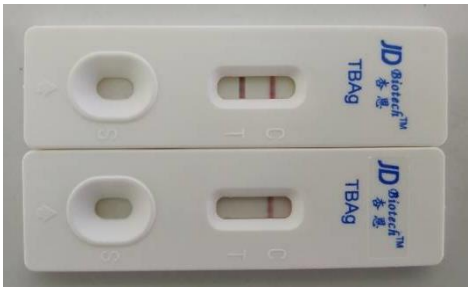
Active smokers who consume at least seven cigarettes per day can trigger the proliferation of *M. tuberculosis* in the lungs. Gender and occupation are also considered to be the supporting factors for pulmonary TB because men are often associated with smoking habits and menial jobs such as labor, increasing the risk to be infected by tuberculosis (Pangaribuan, Kristina, Perwitsari, Tajayanti, & Lolong, 2020).

Table 2. Frequency distribution of respondents based on their characteristics

Characteristic	Group Variation	Total
Gender	Male	65
	Female	35
Age	25-35	20
	36-45	44
	46-60	36
Smoking status	Non-smoker	12
	Smoker	88
Occupation	Labor	35
	Farmer	16
	Fisherman	12
	Employee in private sector	10
	Housewife	11
	Entrepreneur	3
	Taxi bike driver	4
	Driver	5
	Unemployed	4

Table 3. The comparison of the results of M. tuberculosis detection using MRT and immunochromatography

Immunochromatography	Molecular Rapid Test (MRT)			*p-value*
	Positive	Negative	Total	
Positive	42	0	42	<0.001
Negative	4	54	58	
Total	46	54	100	



(a)

(b)

Figure 1. The results of immunochromatography: (a) positive; (b) negative

Table 4. Diagnostic values of immunochromatography with sputum samples

Diagnostic Value	Immunochromatography
Sensitivity	91.3 %
Specificity	100 %
Positive probability value	100 %
Negative probability value	93.1 %
Accuracy	96 %

This study revealed a significant difference between the results of *M. tuberculosis* detection using molecular rapid test and immunochromatography. During the research process, researchers followed the standard operating procedures (SOP) that had been set, but discrepancies in the results between molecular rapid test and immunochromatography were still found. This is because the rapid molecular test was still considered better in the

early detection of tuberculosis. After all, it could detect the rpoB gene, making *M. tuberculosis* detected even in a small quantity. This is different from the examination tool that adopts the working principle of immunochromatography that only detects three specific antigens produced by *M. tuberculosis* during its active period, namely 85A, 85B, and 85C complexes, or better known as 30-32 kDa proteins, which are found in the bacterial cell walls. In addition, a minimum concentration of *M. tuberculosis* of $3x10^4$ to $3x10^5$ colony forming units (CFU)/mL is required in the sputum to be detected using the immunochromatographic method (Ayati, 2012).

Another factor contributing to the discrepancy in the results between the molecular rapid test examination and the immunochromatographic method is the damage to the epitope of the recombinant antibody contained in the examination tool, making it unable to capture the *Mycobacterium tuberculosis* antigen and produces a false negative (Sodiqah, Massi, & Sjahril, 2018). A similar study was also carried out by Singh & Grover (2011), which concluded that the acid-fast bacillus (AFB) and PCR staining methods are better than the immunochromatographic method. However, a study conducted by Shenoy & Mukhopadhyay in India (2014) revealed that the immunochromatographic method can be used as a screening test, but if the results are negative, it can be confirmed using culture.

CONCLUSION

This research concludes that although a statistically significant difference between the results of molecular rapid test and immunochromatography test occurs, immunochromatography is considered as an alternative early examination for tuberculosis detection, especially in peripheral areas that do not have adequate health workers.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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