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## **Rapid Plasma Reagin (RPR) Inspection in Pulmonary Tuberculosis Patients**

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### **Abstract**

Rapid Plasma Reagin (RPR) is one method of non-treponemal examination for the diagnosis of syphilis by detecting non-specific antibodies (reagin) in syphilis suspect blood. Rapid Plasma Reagin examination was performed using immunoassay method. In this immunoassay method is done with agglutination, namely antigen and antibody ties in the patient serum. In patients with tuberculosis is predicted to have non-specific antibodies (reagin) in their serum. This is because the immune response is a hypersensitivity reaction type IV or slow type. This study aims to determine the results of Rapid Plasma Reagin examination in patients with pulmonary tuberculosis. This research is a laboratory observation conducted on 30 samples of patients with pulmonary tuberculosis. Samples were obtained from several hospitals and health centers in Makassar. The specimens test were conducted at the Laboratory of Health Analyst Department of Health Polytechnic of Ministry of Health Makassar in April 2017. The results were obtained from Rapid Plasma Reagin examination, strong reactive of 5 specimens (16.67%), weak reactive of 25 specimens (83.33%) of 30 serum samples of patients with pulmonary tuberculosis. Conclusion, Rapid Plasma Reagin is reactive in patients with pulmonary tuberculosis. This occurs because of the presence of non-specific antibody (reagin) in the serum of patients with pulmonary tuberculosis with cardiolipin antigen used. Accordingly, in order to establish the diagnosis of syphilis in patients, it is necessary to conduct more specific confirmatory examinations to reduce the likelihood of biologically false positive results.

**Keywords:** Syphilis; Tuberculosis; Rapid Plasma Reagin.

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## 1. Introduction

Immunoassay for syphilis plays an important role in the laboratory diagnosis of syphilis, since the course of this disease has been long and until today *Treponema pallidum* has not been successfully cultured on a hatchery medium, whereas direct examination (microscopic) can only be done on a specific material or specimen for example, taken from *ulcus ulcer lesions durum* that often appear only in a relatively short time and often give false negative results [1,2]. *Treponema pallidum* cannot be grown on an artificial culture medium, so laboratory tests that can help diagnose syphilis are by looking at organisms with a dark field microscope or by detecting antibodies in serum and cerebrospinal fluid. Two categories of detectable antibodies, which are *non-treponema antibodies* (reagin), shown to antigens containing fats from damaged patient cells, and *treponemal antibodies*, shown to the surface antigen of *Treponema pallidum* [1,2,3]. The widely used treponemal antibody tests are currently *fluorescent treponemal antibody-absorbed double stain test* (FTA-ABS DS), *microhemagglutination T. pallidum* test (MHA-TP), and treponemal hemagglutination test for syphilis. The use of these tests is usually limited to confirm the positive results of the non-treponema antibody test i.e. Rapid Plasma Reagin or *Venereal Disease Research Laboratory* (VDRL) [1,2]. Non-treponemal antibody tests are used primarily to filter or screen patients for syphilis and to monitor responses to syphilis treatment. A positive Reagin Rapid Plasma test in a patient not treated for syphilis should be confirmed by testing for treponemal antibodies due to many conditions that may lead to a “*biologically false positive*” nontreponema antibody test result (Sacher, McPherson, 2004). One that can cause *false positives* is *Mycobacterium tuberculosis* (*M. tuberculosis*) infection [4,5]. Immune responses to bacteria that cause tuberculosis and syphilis are different. *Treponema pallidum* forms cardiolipin antibodies that can damage endothelial cell tissue, which can be detected as **reagins**. While *M. tuberculosis* can trigger a slow type of hypersensitivity reaction, which can damage the tissue. The result of tissue damage caused by immune response to *M. tuberculosis* bacteria is suspected to be also detected as **reagin** antibodies [6]. *Treponema pallidum* is one of spirochaeta bacteria. These bacteria are spiral-shaped. There are four subspecies that have been found, namely *Treponema pallidum pertenue*, *Treponema pallidum carateum*, and *Treponema pallidum endemicum*. *Treponema pallidum* is a motile spirochaeta that commonly infects through direct sexual contact, entering into the host's body through a gap between epithelial cells. These bacteria can also be transmitted to the fetus via transplacental pathways during late pregnancy. The helical body structure allows *Treponema pallidum* to move with a distinctive motion pattern to move in a thick medium like a lender (mucus). Thus, these bacteria can access up to the circulatory system and the host's lymph via tissue and mucous membranes [7,8].

Rapid Plasma Reagin Test is one of the fastest screening tests for syphilis. Rapid Plasma Test Reagin detects reagin antibodies in the serum, this method is quite sensitive, but there is a tendency to be less specific than other methods. Often this test is applied to blood donor as a syphilis detection test [9]. False positives may occur in certain viral and bacterial infections, connective tissue diseases and autoimmune diseases. In this regard, this screening test should ideally be followed or confirmed with more specific treponemal tests. Commonly used tests include Treponemal pallidum hemagglutination (TPHA) and more specific but relatively more expensive *fluorescent treponemal antibody absorption* (FTA-Abs). Tests based on enzyme-linked immunoassays or ELISA were also used to confirm the results of the syphilis screening test [13].

## **2. Materials and Methods**

### **2.1 Material**

Research materials include serum of pulmonary tuberculosis patients, KIT Rapid Plasma Reagin test PLASMATEC.

**Instruments used:** slide, stirrer pipette, well, test tube, micropipette, tube rack, centrifuge, timer.

#### **Laboratory Procedures:**

Reagents, positive serum control, negative serum control and serum specimens are adjusted to room temperature and homogenized. Then serum specimens of patients with pulmonary tuberculosis, positive control, and negative control dripped 50 µl each on the slide / plate circle.

The homogeneous reagents are then dropped by 1 drop on each slide circle filled with serum specimens, positive serum control, and negative serum control using the dropper drops available on the RPR KIT. Each mixture is homogenized with a stirrer pipe and the slide is tilted gradually for 8 minutes.

Furthermore the reaction product is observed and recorded.

#### **Reading Results:**

1. Strong reactive, if medium or large agglutination appears in the middle and at the edge of the circle.
2. Weak reactive, if it appears small subtle agglutination at the edge of the circle.
3. Non-reactive, if no agglutination or lumps appears.

### **2.2 Methode**

This research is included in laboratory observation research that is descriptive i.e. do Rapid Plasma Reagin examination in patient of pulmonary tuberculosis.

Pulmonary tuberculosis patient is a person infected with *Mycobacterium tuberculosis* as evidenced by the results of microscopic examination of acid-fast bacilli (AFB) sputum test is positive.

## **3. Results and Discussion**

### **3.1 Results**

From the results of research that has been conducted to determine the results of Rapid Plasma Reagin examination in patients with pulmonary tuberculosis, then from 30 patients with tuberculosis results obtained as listed in the following table.

**Table 1:** Results of Microscopic Examination of acid-fast bacilli (AFB) Sputum Specimen and Rapid Plasma Reagin Serum Specimen of Patients with Tuberculosis Lung

<b>Number</b>	<b>Sample Code</b>	<b>Microscopic of acid-fast bacilli Sputum</b>	<b>Rapid Plasma Reagin Serum</b>
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
1	A	Positive (+)	Weak Reactive
2	B	Positive (+)	Weak Reactive
3	C	Positive (++)	Strong Reactive
<i>Continued Table 1</i>			
4	D	Positive (+)	Weak Reactive
5	E	Positive (+)	Weak Reactive
6	F	Positive (+)	Weak Reactive
7	G	Positive (+)	Weak Reactive
8	H	Positive (+)	Weak Reactive
9	I	Positive (+++)	Strong Reactive
10	J	Positive (+)	Weak Reactive
11	K	Positive (+)	Weak Reactive
12	L	Positive (+)	Weak Reactive
13	M	Positive (+)	Weak Reactive
14	N	Positive (+)	Weak Reactive
15	O	Positive (+)	Weak Reactive
16	P	Positive (+)	Weak Reactive
17	Q	Positive (+)	Weak Reactive
18	R	Positive (++)	Weak Reactive
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
19	S	Positive (+)	Weak Reactive
20	T	Positive (++)	Weak Reactive
21	U	Positive (++)	Weak Reactive
22	V	Positive (+)	Weak Reactive
23	W	Positive (+)	Weak Reactive
24	X	Positive (+)	Weak Reactive
25	Y	Positive (+++)	Strong Reactive
26	Z	Positive (++)	Weak Reactive
27	A1	Positive (+)	Strong Reactive
28	A2	Positive (++)	Strong Reactive
29	A3	Positive (+)	Weak Reactive
30	A4	Positive (+)	Weak Reactive

Source: Primary Data, April 2017

Table 1 shows the results of Rapid Plasma Reagin examination of 30 serum specimens of pulmonary tuberculosis patients, all of which result in reactive reactions.

**Table 2:** Results of Rapid Plasma Reagin Examination in Serum Specimens of Patients with Pulmonary Tuberculosis

<b>Rapid Plasma Reagin Examination Results</b>					
<b>Strong Reactive</b>		<b>Weak Reactive</b>		<b>Amount</b>	
n	%	n	%	n	%
5	16,67	25	83,33	30	100

Table 2 shows 100% serum specimens of pulmonary tuberculosis positive patients Rapid Plasma Reagin with details: 16.67% strong reactive and 83.33% weak reactive. These data suggest that Rapid Plasma Reagin is not appropriate for screening for syphilis in patients suspected of tuberculosis because it is not specific.

### 3.2 Discussions

This study was conducted to determine the extent of Rapid Plasma Reagin examination results in patients with tuberculosis. Rapid Plasma Reagin Inspection is basically one of the examination methods for screening or diagnosing syphilis disease by detecting non-specific antibodies (reagin) in the patient's blood [10,11].

The term "non-specific" means that the test does not detect antibodies specific to the disease-causing bacteria, but rather to detect antibodies to the substances released by cells when damaged by certain bacteria, e.g. *Treponema pallidum* [12,14].

A qualitative Rapid Plasma Reagin Test reagin is a screening test with an undiluted patient serum and then mixed with carbonaceous particles of cardiolipin on the slide. After mechanical rotation for a certain time, the preparation is checked to see whether macroscopic agglutination of carbon particles (carbon) is present [8,9].

The principle of the Rapid Plasma Reagin test is based on the non treponemal antigen agglutination reaction used to detect reagin antibodies that arise in syphilis. Rapid Plasma Antigen Reagins used in this kit are modified from the Venereal Disease Research of Laboratory (VDRL) antigen which contains special carbon particles to enlarge the difference between positive and negative results visually [11,13].

Antibodies against syphilis begin to form at the end of the first stage, but levels are very low and often give negative results on serological tests. These antibody titers continue to rise and peak in the second stage to subsequently decrease gradually in the latent stage and show a rather low (but still positive) titer. Non treponemal or reagin antibodies as a result of syphilis or other infectious diseases. These antibodies are only formed after the disease spreads regional lymph nodes and causes tissue damage. These antibodies provide cross-reactions with several antigens from other tissues such as for example with lipid antigens from cardiac muscle extracts [4,5].

In addition to syphilis screening, Rapid Plasma Reagin levels (also called "titers") can be used to track disease progression over time and response to therapy. Rapid Plasma Reagin Test is an effective screening test, as it can detect syphilitic sufferers even without asymptomatic syphilis. But tests may produce false positives because many circumstances may lead to "biologically-positive" test results. Therefore a positive Reagin Rapid Plasma test in a patient not treated for syphilis should be confirmed with tests for treponemal antibodies. One positive result can be seen in the bacterial infection of *Mycobacterium tuberculosis* [6,9].

*Mycobacterium tuberculosis* is a bacterium that causes tuberculosis in humans. This bacterial infection can cause type IV (slow type of hypersensitivity) immune reactions that can cause damage to the infected tissue. The reaction can occur when the number of antigens that enter relatively more then interact between the antigens with receptors located on the surface of T. lymphocytes and activate it, activation of T. lymphocytes occurs when the antigen is captured by macrophages but cannot be removed or eliminated. This stimulates T. lymphocytes to produce cytokines that cause various inflammation reactions. Type IV hypersensitivity reactions (slow type) include cellular reactions [4,10].

Rapid Plasma Reagin Examination in tuberculosis patients often gives positive test results. This is due to the binding of reagin antibodies (which are unintentionally generated from type IV hypersensitivity reactions) with cardiolipin antigens (artificial antigens that resemble lipid antigens in *Treponema pallidum*.) The possibility of reagin formation of type IV hypersensitivity reactions in tuberculosis patients occurs because of lipid antigens in *Mycobacterium tuberculosis* cell wall that reacts with damaged host cells According to Sacher and McPherson, reagin antibodies are directed to fatty antigens formed from damaged host cells or possibly from the treponema itself [1,3]. Based on the research result data, Rapid Plasma Reagin examination in tuberculosis patients, from a total of 30 serum samples that showed all reactive or agglutination on examination. But among the 30 samples there are 5 of them are strong reactive and the rest are weak reactive. The samples encoded "C, I, Y, A1, and A2" are "Reactive Strong" where samples C and A2 are AFB positive ++, samples I and Y are AFB positive +++, and A1 AFB positive +. The rest of the 5 sample codes show weak reactivity is microscopic AFB positive +. From the data it can be said that the more AFB in the sputum, the higher (strong) also glob that occurs on Rapid Plasma Reagin test. Weak or strong agglutination shows the number of reagin antibodies contained in the serum of the patient. The stronger the agglutination that occurs, the more antibodies are contained in the serum of the patient [13,14].

#### **4. Conclusion**

The conclusions of this study are Rapid Plasma Reagin reactive in patients with pulmonary tuberculosis. In this regard, to the clinician or medical officer concerned for the diagnosis of syphilis in the patient, to conduct a more specific confirmatory examination to minimize the biologically false positive results. Further research is needed to determine the sensitivity and specificity of the diagnostic method or device for Rapid Plasma Reagin.

#### **Acknowledgement**

The authors would like to acknowledge to friends and family for supporting me during this study.

## **Competing Interest**

The authors declare that we have no competing interests.

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