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EXAMINATION FOR TYPHOID FEVER USING *IMMUNO MAGNETIC BEADS INHIBITION* (TUBEX TF) METHOD AT RSUD SITI FATIMAH SOUTH SUMATERA PROVINCE 2024

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Abstract

Background: Typhoid fever was a systemic infectious disease caused by the *Salmonella typhi* bacteria. The symptoms included increased body temperature, headache, nausea, loss of appetite, weakness, diarrhea, liver and spleen damege and death. Usually occurs within 1 to 3 weeks after exposured to *Salmonella typhi* bacteria. **Aims**: This research aimed to find out how to check for typhoid fever using the *Immuno Magnetic Beads Inhibition* (Tubex TF) method at RSUD Siti Fatimah South Sumatra Province in 2024. **Methods**: The methodology used in this research was quantitative descriptive with a research sample of 4 serum samples from patients with suspected typhoid fever collected by purposive sampling. **The results**: The results showed one possitve sample with a score of 4 on Tubex TF and three negative samples with a score 2 on Tubex TF. **Conclusion**: This showed that the Tubex TF method can differentiate the severity of thyphoid fever effectively.

Keywords : Typhoid fever, *Salmonella typhi*, Tubex TF

1. Introduction

Typhoid fever is a disease caused by *Salmonella typhi* bacteria. The disease occurs in the small intestine and can cause continuous symptoms. This condition is strongly influenced by personal and environmental hygiene, including the habit of keeping oneself, food and surroundings clean. Factors such as people's habits that do not contribute to healthy living and unclean environments also play a role in spread of this disease (1).

S. typhi or *Salmonella Paratyphi* bacteria is the bacteria that triggers typhoid fever which is classified into the genus of *Salmonella*. This bacterium is able to survive for several months to a year if attached in feces, butter, milk, cheese and frozen water. It is an intracellular parasite facultative parasite that can live in machrophages and causes gastrointestinal symptoms (2). These bacteria are rod-shaped (bacilli bacteria), and belong to the gram-negative bacteria group. These bacteria do not form spores, but have flagella that allow them to move (motile). *Salmonella typhi* and *Salmonella*

Paratyphi bacteria can survive in a free environment for several weeks. They can be found in contaminated water, ice, garbage and dust. Typhoid disease can be easily transmitted from one induvidual to another, especially individuals who do not keep themselves and their environment clean. Particularly in areas with poor sanitation, there is a higher risk of developing typhoid fever. This condition often occurs in the areas with dependency on access to clean water and inadequate enviromental sanitation.

The *Salmonella* genus consists of two main species, *Salmonella enterica* and *Salmonella bungori* (also known as subspecies V). *Salmonella enterica*, the most common species in the genus, is divided into several subspecies that are distinguished by their chemical and biological characteristics. Subspecies of *Salmonella enterica* include subsp. *Enterica*, subsp. *Salamae*, subsp. *Arizonae*, subsp. *Diarizonae*, subsp. *Houtenae*, and subsp. *Indica* (3).

According to the Kauffman-White Scheme, *Salmonella typhi* can be grouped into serovars based on differences in antigen formulas, including O antigen (somatic), Vi antigen (capsule), and H antigen (flagellum). The O antigen formula is determined by the composition and structure of the polysaccharide, and may change due to lysogenic infection by bacteriophages. Subdivision of *Salmonella typhi* serovars can also be done based on biovars, which are related to the ability of the bacteria to ferment xylose. This results in xylose positive and negative *S.typhi*, which can be used as epidemiological markers. In addition, subdivision of these serovars can also be based on resistance patterns to antibiotics (2).

Typhoid disease can be transmitted directly by contact with the feces (stool), urine, or vomit of an individual infected with *Salmonella typhi* or *Salmonella Paratyphi* bacteria. Contact can include not only direct contact with the sick person, but also with contaminated objects by their bodily fluids. The disease can also be transmitted indirectly through the consumption of contaminated food or drink with *S. typhi* bacteria. *Salmonella typhi* bacteria play an important role in causing localized inflammation in the tissues where they thrive, especially in human gut. This process generally occurs as these bacteria pass through the intestinal wall and into the blood circulation, leading to the systemic phase of infection (4).

The clinical manifestation of typhoid fever is generally fever. The symptomps vary from mild to severe indications, from asymptomatic to spesific disease exposure with many complication. It can even end in death. In the first week, symptoms of acute infection include fever, headache, dizziness, muscle pain, loss of appetite, nausea, vomitus, constipation or diarrhea, abdominal disconfort, caugh, and nosebleeds. The fever follows a straircase pattern, increasing day by day, decreasing in the morning and increasing in the afternoon. In the second week, fever, relative *bradycardia*, typhoid

tongue (dirty center, red edges and tip, with tremors), *hepatomegaly*, *splenomegaly*, *meteorismus*, impaired consiousness, and less commonly roseolae (5).

Immuno Magnetic Beads Inhibition (IMBI), also known as Tubex TF, is a test used specifically to detect IgM antibodies to *S. typhi* O9 lipopolysaccharide antigen. This antigen is specific for *Salmonella typhi* and other *Salmonella* serogeroup D bacteria that have rare sugar (*a-Dyvelose*). Tubex TF is specifically designed to detect antibodies in the acute phase of infection (IgM), but also can capture the presence of antibodies in convalecent phase (IgG) which are present in small amounts, especially at the beginning of infection when the immune response has not been fully stimulated.

The Tubex TF test is a serologic test used to diagnose *S. typhi* bacterial infection, using the principle of semi-quantitative colorimetric competitive agglutination. This test is used to determine the presence of anti-*Salmonella typhi* O9 antibodies in the patient's serum. These antibodies are produced in response to *Salmonella typhi* infection (6).

Tubex TF is a test used to find the presence of anti-O9 antibodies in patient serum by assessing the ability of antibodies to inhibit the reaction between antigens coated with brown dye and antibodies coated with blue dye. The degree of inhibition in this test parallel to the concentration of anti-O9 antibodies in the sample. The antigen-antibody separation process is activated by read visually using a color scale. Interpretation of the results is done by comparing the color of the supernatant (the solution remaining after separation) through the color scale (7).

The purpose of this study was to determine how to test for typhoid fever with the *Immuno Magnetic Beads Inhibition* (Tubex TF) method, determine the results of Tubex TF examination of the serum of patients suspected of typhoid fever and determine the Tubex TF testing score of the serum of patients diagnosed with typhoid fever at RSUD Siti Fatimah, South Sumatra Province.

2. Research Methods

Widal examination is a serologic examination supporting the diagnosis of typhoid fever that is still often proposed by clinicians today. The Widal examination procedure is relatively easy so that it can be done in various health facilities, the results are quickly obtained, with relatively economical costs (8).

In addition to the widal test, there are several other serological examination methods that can be done quickly and easily and have better sensitivity and specificity than Salmonella IgM/IgG serology examination, among others. The sensitivity and specificity of *S. typhi*-specific antigen detection depends on the type of antigen, the type of specimen, the technique used, the type of

antibody used in the test (polyclonal or monoclonal and the time of specimen collection (early or advanced stage in the course of the disease) (5).

The type of research used in this study is descriptive quantitative. The population in this study consisted of inpatients and outpatiens suffering from typhoid fever at RSUD Siti Fatimah South Sumatra Province from January to May 2024, totaling 178 people. The sampling technique used was purposive sampling method. The sample used in this study was serum from four patients diagnosed with typhoid fever in June 2024 at RSUD Siti Fatimah South Sumatra Province which was calculated based on Slovin formula.

$$\mathbf{n = \frac{N}{1 + N\,(e)^2}}$$

$$n = \frac{178}{1 + 178\,(0{,}5)^2}$$

$$n = \frac{178}{1 + 178\,(0{,}25)}$$

$$n = \frac{178}{1 + 44{,}5}$$

$$n = \frac{178}{45{,}5}$$

= 3,91 = 4 samples.

Description:

n : Number of samples

N : Total population

e : Allowable error rate, e = 0.5

The Slovin formula used in this study, because the Slovin formula can save time and resources, produce a representative sample and the calculation method of the slovin formula is simple so it is easy to apply. Due to the price of Tubex TF equipment which tends to be expensive, the error rate used by researcher in this study is 50% (e = 0.5) and only 4 samples are used from a total of 178 existing populations. The samples used are based on criteria determined by the researcher to be able

to represent the characteristics of the population, namely the serum of patients diagnosed with typhoid fever at RSUD Siti Fatimah, South Sumatra Province in 2024. With two types of serum samples used, namely normal serum which is clear yellow in color and abnormal serum that has undergone lysis or referred to as serum lysis.

The examination technique in this study uses the *Immuno Magnetic Beads Inhibition* method with the measuring instrument used in the form of the Tubex TF Test and the material is the serum of patients diagnosed with typhoid fever at RSUD Siti Fatimah, South Sumatra Province in 2024. Data analysis using primary data obtained directly from research in the laboratory of RSUD Siti Fatimah, South Sumatra Province in 2024.

3. Results and Discussions

The results of blood serum examination in patients diagnosed with typhoid fever at RSUD Siti Fatimah, South Sumatra Province in June using the *Tubex TF* method can be seen in the table below :

Table 1. Tubex TF Test Results on Serum of Typhoid Fever Patients at RSUD Siti Fatimah Laboratory South Sumatra Province

No.	Inspection Result	Total	Tubex Skor
1.	Positive	1	4
2.	Negative	3	2

Source : Primary Data (2024)

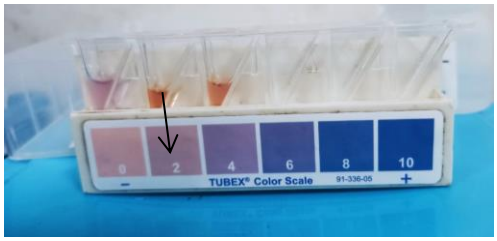

Figure 1. Tubex TF sample. Result: Negative (Tubex TF Score = 2)

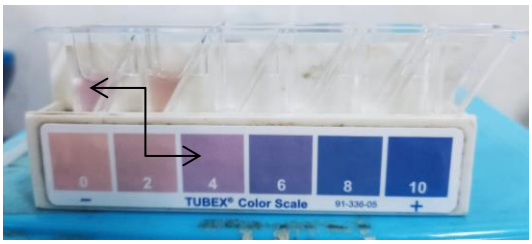

Figure 2. Tubex TF sample. Result: Positive (Tubex TF Score = 4)

Based on the figures 1 and 2 above, the results of the typhoid fever examination with Tubex TF method show a color change that occurs on the reaction well strip to a yellow color or referred to as a negative result (figure 1) and a purple color or referred to as a positve result (figure 2). A change in the color of the supernatant from blue to red (if red latex particles are added) or remaining blue (if anti-O9 antibodies are present) is an indication of the presence or absence of specific antibodies to *Salmonella typhi* O9 antigens in the tested sample (9).

The results of the study in Table 1 Tubex TF Test Results in the Serum of Typhoid Fever Patients at the RSUD Siti Fatimah Laboratory, South Sumatra Province show that in June 2024 there were four patients diagnosed with typoid fever, there was one person with a score of 4 indicating active typhoid fever infection from the measurement results with the Tubex TF method and three people showed negative results with a score of 2 indicating that there was no active typhoid fever infection. The Tubex test is a blood serology test to detect the presence of IgM antibodies that indicate active infection with typhoid fever. This is directly proportional to the severity of typhoid fever experienced. For values with Tubex TF with a score <2 with Negative interpretation indicates no active typhoid fever infection, score 3 with Borderline interpretation indicates measurement cannot be concluded. Repeat testing, if still in doubt, re-sample a few days later, score 4-5 with Positive interpretation indicates active typhoid fever infection, score >6 with Positive interpretation indicates strong indication of active typhoid fever infection (10). However, there are several reasons why a clear Tubex TF score is not obtained due to poor adherence to testing protocol or errors that can be caused by poor specimen quality, requiring specimen retrieval and analysis. Therefore, it is necessary to increase understanding and knowledge related to typhoid fever examination using the Immuno Magnetic Beads Inhibition (Tubex TF) method.

The Tubex TF test can be used as one of the routine and ideal examinations because it is easy and fast. The method used in the Tubex TF test is *Immuno Manetic Beads Inhibition* (IMBI). In this process, when no antibodies are present in the serum being tested, and the liquid suspension of the two reagents (blue reagent and brown reagent) are mixed, binding will occur between the monoclonal

antibody particles of the reagents and the antigen particles. Both types of particles will adhere to the bottom of a V-shaped tube placed on a magnetic stand, used to attract magnetically labeled particles (11).

The disadvantage of the *Immuno Magnetic Beads Inhibition* (Tubex TF) method is that the equipment tends to be more expensive compared to other tests such as the widal test. In addition, Tubex test results may be difficult to interpret accurately, especially in borderline (inconclusive) results. The Tubex TF method is recommended in typhoid fever screening because it has several advantages including relatively high sensitivity and specificity in detecting anti-O9 antibodies, the procedure is also very simple and easy to perform, allowing testing of many test samples simultaneously. The results can be obtained quickly, usually in <10 minutes, and use a small amount of blood as a test sample.

Two serum samples were used in this study, namely normal serum which is clear yellow in color and abnormal serum that has been lysed or referred to as lysed serum. Hemolysis serum is a condition where a blood serum sample contains hemoglobin derived from broken erythocytes. It can be caused by several factors, such as blood collection techniques, sample processing and serum storage time. So that it can interfere with many laboratory examination methods, especially those that are sensitive to changes in color or substance content in the sample (12).

Typhoid fever is an infectious disease caused by the bacterium *Salmonella enterica serovar typhi*. The bacteria are usually spread through food or water contaminated by faces of humans infected with typhoid fever. Humans are the only natural host and reservoir of this disease. Typhoid fever is particularly widespread in Low and Middle Income Countries (LMIC), especially areas with poor sanitation, limited access to clean water, and inadequate sanitation practices (13).

Overall, reducing the risk of typhoid fever transmission requires individuals to raise awareness of the importance of good hygine practices, avoiding food and beverages that are not guarenteed to be clean, and vaccinating when available and advised by medical personnel. In addition to reducing the burden of disease in endemic countries and saving lives, widespread use of typhoid conjugate vaccinates in affected countries is expected to reduce the need for antibiotics for the treatment of typhoid and slow the increase in antibiotic resistance in *Salmonella typhi* (14).

4. Conclusions

Based on the research that has been carried out, it can be concluded that the Tubex TF method is effective for diagnosing typhoid fever. Judging from the results of the typhoid fever examination using the Tubex TF test on the serum of patients with typhoid fever where there is 1 positive sample with a score of 4 on Tubex TF and 3 negative samples with a score of 2 on Tubex TF from a total of 4 serum samples examined. This shows that the Tubex TF method is effective in distinguishing the severity of typhoid fever that is being experienced with marked positive or negative results.

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