



## Comparison of Anti-Salmonella Typhi IgM Examination Results with SD Bioline and Tubex TF. Methods

Herlinawati<sup>1\*</sup>, Resna Hermawati<sup>2</sup>, I Gede Angga Adyana<sup>3</sup>

<sup>1</sup>Department of Immunology, Faculty of Medicine, Al-Azhar Islamic University, Indonesia

<sup>2</sup>Department of Clinical Pathology, Faculty of Medicine, Al-Azhar Islamic University, Indonesia

<sup>3</sup>Department of Immunology, Faculty of Medicine, Al-Azhar Islamic University, Indonesia

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#### \*) corresponding author

Herlinawati

Department of Immunology, Faculty of  
Medicine, Al-Azhar Islamic University,  
Indonesia

Email: [nindynovelial@yahoo.co.id](mailto:nindynovelial@yahoo.co.id)

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### ABSTRACT

*Salmonella typhi* is a gram-negative bacterium that is an intracellular facultative pathogen, which enters the human body and causes an acute systemic infectious disease called typhoid fever. Among the laboratory tests used to detect the presence of anti-*Salmonella typhi* IgM in serum are SD Bioline and Tubex TF. This study aims to determine comparison of the results of the anti-*Salmonella typhi* IgM examination with the SD Bioline and Tubex TF methods. Analytical observation with cross-sectional design. This research was conducted in RSUD DR. Soedjono Selong. The time of the study was January-April 2022. The sample of this study was patients who underwent typhoid fever examination who were hospitalized at RSUD DR. Soedjono Selong as many as 60 people. This research uses the method *consecutive sampling*. Anti-*Salmonella typhi* IgM was examined with the SD Bioline kit using the immunochromatography method and the TUBEX TF kit using the Inhibition Magnetic Binding Immunoassay (IMBI) method. Data analysis was performed using SPSS, namely univariate test, and bivariate test with Chi-square. There were 26 (43.3%) male and 34 (56.7%) female patients. The youngest is 2 years old and the oldest is 64 years old with an average age of 25 years  $\pm$  19 years. Of the total 60 patients examined with SD Bioline, 5 (8.3%) were positive and 55 (91.7%) were negative. Meanwhile, examination with TUBEX TF found 3 (5%) positive people and 57 (95%) negative people. And there was a significant comparison between the results of the SD Bioline and Tubex TF examination with a value ( $p = 0.000$ ) and a low level of conformity was obtained with a Kappa value of 0.000. A comparison of the results of the IgM anti-*Salmonella typhi* examination was obtained using the SD Bioline and Tubex TF. methods with a very low degree of conformity.

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### BACKGROUND

Typhoid fever is an acute infectious disease caused by infection with the bacterium *Salmonella enterica* serovar typhi (*S. typhi*), which is a Gram-negative bacterium that is an intracellular facultative pathogen (Kaur et al., 2012).

Typhoid fever is still a world health problem that is still widely found in several developing countries, especially in tropical and subtropical areas such as India, Nepal, Pakistan, Papua New Guinea, Indonesia and a number of areas in Sub-Saharan Africa that have limited access to clean water facilities and lack of sanitation (Siba et al., 2012). Based on data from the WHO website in 2021, there are 11 to 21 million cases of typhoid fever occurring worldwide annually. The mortality rate due to typhoid fever is estimated at

128,000 – 161,000 deaths per year, worldwide (WHO, 2021). In Elisabeth (2016) In 2008, the typhoid morbidity rate in Indonesia was 81.7 per 100,000 population, with a distribution by age group of 0.0/100,000 population (0–1 year), 148.7/100,000 population (2–10). 4 years), 180.3/100,000 (5–15 years), and 51.2/100,000 ( $\geq$ 16 years) (Elisabeth et al., 2016).

Typhoid fever begins with the ingestion of *S. typhi* into the human body, which then colonizes the small intestine and eventually spreads to the bloodstream and settles in various organs of the reticulo endothelial system (RES) such as the liver, spleen and bone marrow. This process usually takes 1 or 2 weeks. In the early stages, *S. typhi* is easily eradicated from the circulation, then IgM antibodies are formed. As the disease process progresses to an advanced

stage and eventually heals, usually 3-4 weeks, *S. typhi* rarely circulates in the circulation, while antibodies are abundant. When the disease ends, *S. typhi* is completely lost from the host body, the level of IgM antibodies specific to *S. typhi* continues to decline, but IgG levels can remain high for several months to come (Yan et al., 2011).

Early detection of anti-*S. typhi* antibodies is still not optimal, the cause is low levels of antibodies when laboratory tests are carried out, which will have an impact on misdiagnosis and disease management. Various diagnostic methods of typhoid fever based on antibody detection as a substitute for Widal and blood culture are still being developed and have been widely marketed in various countries, including Indonesia, their use has begun to be developed, including SD Bioline and Tubex TF.

SD Bioline *Salmonella typhi* IgG/IgM using the immunochromatographic method, detects IgM against *Salmonella typhi* LPS antigen present in the patient's serum/plasma/whole blood. The interpretation of the examination results is qualitative, namely by looking at the presence or absence of a purple line on the test strip (Standard Diagnostic, Inc., 2016).

Tubex TF is a rapid test with the Inhibition Magnetic Binding Immunoassay (IMBI) method that can detect IgM specific to the O9 *S. typhi* antigen present in the patient's serum. The interpretation of the results of this examination is semi-quantitative, namely by comparing the color that appears in the examination reaction results with the standard color that has a score found on the Tubex TF kit (IDL Biotech, 2021).

The interpretation of the results of the SD Bioline examination is qualitative, while the interpretation of the results of the Tubex TF examination is semi-quantitative which is read visually. so it is necessary to conduct a study to compare the IgM anti-*Salmonella typhi* examination with the SD Bioline and Tubex TF methods.

## SUBJECT AND METHOD

### Study Design

Cross-sectional study. This research was conducted in RSUD DR. Soedjono Selong, East Lombok Regency. This research was conducted in January-April 2022.

### Population and Sample

The target population is all patients who have been examined for typhoid fever in RSUD DR. Soedjono selong. The source population was patients who underwent typhoid fever examination who were hospitalized in January-April 2022. The sampling technique was consecutive sampling. Sample size based on Kappa Cohen test formula ie 60 samples.

### Research variable

The dependent variable in this study is IgM, while the independent variable is SD Bioline and Tubex TF. Immunoglobulin M (IgM) is a protein with a high molecular weight (macroglobulin) in a secreted form consisting of 5 to 6 subunits.

SD BIOLINE *Salmonella typhi* IgG/IgM is a test kit using the basic principles of immunochromatography. TUBEX TF is

a serological test that detects immunoglobulin M against the specific O9 antigen of *Salmonella typhi*.

### Study Instrument

The SD Bioline kit uses the immunochromatographic method to detect IgM against the LPS antigen of *Salmonella typhi* and the Tubex TF kit uses the Inhibition Magnetic Binding Immunoassay (IMBI) method to detect IgM against the O9 antigen of *Salmonella typhi*.

### Research Flow Summary

The sample in this study were patients who were hospitalized and were willing to sign the consent form to become respondents. Blood and serum for examination were obtained from patients who underwent typhoid fever examination who were hospitalized and brought to the Clinical Pathology Laboratory of RSUD DR. Soedjono Selong. Serum was obtained from venous puncture, centrifuged at 5000 rpm for 10 minutes, at room temperature. The supernatant was taken carefully, and if resuspension occurred, it was centrifuged again. The supernatant taken can be used immediately or stored at -20 0C.

The SD Bioline kit used in this study was for the examination of anti-*Salmonella typhi* IgM/IgG. The method for examination is as follows: put 4 drops (90-120 l) of the diluent reagent into an empty test tube, then add 1 L of blood into the same test tube. The strip is inserted into the diluent and sample reagent mixture, and the results can be read after 15-30 minutes. Results are read and adjusted according to the guidelines on the reagent box.

The Tubex TF kit used in this study was for the examination of IgM anti *S. typhi*. The method for examination of IgM anti-*S. typhi* is 45 l of brown reagent (detector) put into a Tubex TF reaction well strip, then 45 l of patient sample is added, after which it is stirred 10 times by pipetting. Then the mixture was incubated on a rack for 2 minutes. Then 90 l of blue reagent (indicator) was added, then the Tubex TF reaction well strip was closed using Tubex TF adhesive tape, then the Tubex TF reaction well strip was tilted and shaken for 2 minutes. Then the Tubex TF reaction well strip was placed on the Tubex TF color scale. Allow separation to occur for 5 minutes, Then the results can be read by comparing the color produced at the end of the reaction with the Tubex TF color scale. The Tubex TF color scale ranges from 0 (test negative, color clear pink) to 10 (test positive, color dark blue) (IDL Biotech, 2021).

### Data analysis

All data analysis was performed using SPSS® version 25 (IBM Corp., Chicago). The data obtained on each variable are presented descriptively in tabular form. Data were analyzed using Chi-Square test with a significance level of  $p < 0.05$ . The Kappa suitability test was conducted to determine the level of conformity of the results between the two tests.

### Research Ethics

The protocol of the study was approved by the medical and health research ethic committee Faculty of Medicine, Al-Azhar Islamic university with certificate No. 12/EC-03/FK-06/UNIZAR/II/2022, on 9th of February 2022.

## RESULTS

### Sample Characteristics

Table 1. Sample characteristics

Characteristic	Category	Frequency	Percentage
Gender	Man	26	43.3%
	Woman	34	56.7%

Table 2. Baseline Characteristics

Variable	mean	SD	Min.	Max.
1. Age (Years)	25,425	19.3985	2	64

The sample in this study were patients who were hospitalized at RSUD DR. Soedjono Selong numbered 60 people. There were 26 (43.3%) males and 34 (56.7%) females. The youngest is 2 years old and the oldest is 64 years old with an average age of 25 years  $\pm$  19 years. Of the total 60 patients examined with SD Bioline, 5 (8.3%) were positive and 55 (91.7%) were negative. Meanwhile, examination with Tubex TF found 3 (5%) positive people and 57 (95%) negative people.

### Bivariate Analysis

Bivariate analysis in this study was to compare the results of the SD Bioline examination with Tubex TF, it was found that there was a comparison of the results of the

examination between SD Bioline and Tubex TF on the examination of IgM anti-Salmonella typhi ( $p < 0.000$ ). While the Kappa test obtained a very low level of conformity, namely 0.000

Table 3. Cross tabulation of positive and negative results of anti-S. typhi IgM examination using SD Bioline and Tubex TF methods

Results	Bioline Elementary	Tubex TF
Positive	5 (8.3%)	3 (5%)
Negative	55 (91.7%)	57 (95%)
Total	60 (100%)	60 (100%)

Table 4. The relationship between positive and negative results of anti-Salmonella typhi IgM examination with SD Bioline and Tubex TF (Chi-Square) methods

		TUBEX TF		Total	$p$	Kappa
		Positive	Negative			
Bioline Elementary	Positive	3	2	5	0.000	0.000
	Negative	0	55	55		
Total		3	57	60		

## DISCUSSION

This research was conducted from January to April 2022 at RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province. In the first stage of the study, 60 samples were collected, consisting of men and women with an age range of 2-64 years. The next stage is the first examination, an IgM/IgG anti-Salmonella typhi examination is carried out using the immunochromatographic method using the SD Bioline kit from SD (Standard Diagnostics, Inc). Then a second examination was carried out, namely the examination of IgM anti-Salmonella typhi with the inhibition magnetic binding immunoassay (IMBI) method using the Tubex TF kit from IDL Biotech.

Rapid detection of the presence of anti-Salmonella typhi IgM is still a challenge in establishing a laboratory diagnosis of typhoid fever. One of the causes of the obstacles in detecting these antibodies is the low level of IgM antibodies when laboratory tests are carried out.

In this study, a comparison of the interpretation of the results of the IgM/IgG anti Salmonella typhi examination was carried out using the SD Bioline kit (Standard Diagnostics, Inc., 2016) which detects IgM/IgG against Salmonella typhi

LPS antigens found in serum/plasma/whole blood of patients using the immunochromatographic method. The results of the examination are stated by seeing whether or not a purple line appears on the test strip. Meanwhile, using the Tubex TF kit (IDL Biotech, 2021) which detects IgM anti-O9 Salmonella typhi by inhibition magnetic binding immunoassay (IMBI). The results of the examination are stated based on the visible color scale score, which is said to be negative with a score of 2, weak positive 4-5 and strong positive if it has a score of 6.

From a total of 60 patients who were included in this study, it was found that 26 male patients (43.3%) and female patients were 34 (56.7%). The results obtained in this study were more women and in accordance with research conducted by Jayadi et al., (2015) obtained 17 male patients and 28 female patients. In this study, it was also found that the age range varied, namely the youngest was 2 years old and the oldest was 64 years old, with an average age of 25 years  $\pm$  19 years. Age is associated with factors for the occurrence of typhoid fever including lack of individual hygiene, very dense living environment, insufficient clean water supply, decreased patient's immune system,

From the examination that has been carried out using the SD Bioline kit on the IgM anti S. typhi examination, the

results showed positive results for 5 (8.3%) samples and negative results for 55 (91.7 %) samples. The positive percentage of typhoid fever based on the results of the SD Bioline examination was quite low, namely 5 (8.3%). This may be because the SD Bioline examination is very subjective where a positive result is indicated by seeing whether or not a purple line appears on the test line and the control strip line. While negative results are stated by looking at the appearance of the control line only on the strip. SD Bioline uses the immunochromatographic method to detect IgM/IgG specific serum antibodies against Salmonella typhi LPS antigens found in the patient's serum/plasma/whole blood.

From the results of the tests that have been carried out using the Tubex TF kit on the IgM anti-Salmonella typhi examination, the results showed positive results of 3 (5%) and negative results of 57 (95%). The positive percentage of typhoid fever based on the results of the Tubex TF examination is quite low, namely 3 (5%). This may be because Tubex TF has limited accuracy because the reading is subjective where the test results are based on the results seen by the examiner's eyes. While the negative results obtained can be caused by the samples being examined from patients who are recovering or suffering from chronic typhoid fever. This is because the immunoglobulin circulating in the blood is IgG, while the Tubex TF test can only detect IgM as a sign of acute infection and cannot detect IgG.

From the cross tabulation of the results of the SD Bioline examination with Tubex TF, 3 (5%) samples were positive in the SD Bioline and Tubex TF kits, 2 (3.3%) samples were positive in the SD Bioline kit but negative on the Tubex TF kit in detecting anti-Salmonella IgM typhi, 0 (0%) samples were negative on SD Bioline, but 55 (91.7%) samples were negative on the Tubex TF kit in detecting IgM anti Salmonella typhi. The results of Chi-Square analysis obtained p value = 0.000 <0.05 which indicates that there is a significant (significant) relationship between the results of the SD Bioline kit examination and the results of the Tubex TF examination on the IgM anti-Salmonella typhi examination. The Kappa suitability test was conducted to determine the level of conformity between the SD Bioline and Tubex TF test results. From the Kappa test, the Kappa value is 0.000. This value indicates that the results of the examination of the SD Bioline and Tubex TF kits have a very low level of conformity. According to Dahlan (2014) that the interpretation of Kappa values and the degree of conformity varies greatly from 0-0.2 (very low), <0.2-0.4 (low), >0.4-0.6 (moderate), > 0.6-0.8 (strong) and >0.8-1 (very strong). This study is not in accordance with the research conducted by Wahyuniati (2014) that there was no Kappa value between the SD Bioline and Tubex TF tests because none of the results of the SD Bioline examination were positive, so there was no agreement at all between these two types of tests. >0.4-0.6 (moderate), >0.6-0.8 (strong) and >0.8-1 (very strong). This study is not in accordance with the research conducted by Wahyuniati (2014) that there was no Kappa value between the SD Bioline and Tubex TF tests because none of the results of the SD Bioline examination were positive, so there was no agreement at all between these two types of tests. >0.4-0.6 (moderate), >0.6-0.8 (strong) and >0.8-1 (very strong). This study is not in accordance with the research conducted by Wahyuniati (2014) that there was no Kappa value between the SD Bioline and Tubex TF tests because none of the results of the SD Bioline examination were positive, so there was no agreement at all between these two types of tests.

## CONCLUSION

Based on the results of the study, it can be concluded that there is a comparison of the results of the IgM anti-Salmonella typhi examination with the SD Bioline and Tubex TF methods with a very low level of conformity between the two tests.

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## CONFLICT OF INTEREST

There was non conflict of interest in this study

## AUTHOR'S CONTRIBUTION

Herlinawati contributed in making proposals and data collection, Resna Hermawati contributed in data collection and I Gede Angga Adyana contributed in data processing.

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