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## COMPARISON OF RAPID TYPHIDOT TEST BASED ON SANITATION ENVIRONMENT IN HEALTHY INDIVIDUALS IN MEDAN

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### ABSTRACT

**Background:** Typhoid fever is a gastrointestinal tract infectious disease frequently found endemic in Indonesia, which can be transmitted by oro-fecal from food or water contaminated directly or vector carries these bacteria. This study aimed to compare the presence of antibodies from the Typhidot Test in people in poor and healthy sanitation conditions.

**Method:** This study is an observational study with a cross-sectional design. The population is all people around the Health center of Medan Petisah which subdistrict has fewer diarrhea cases and a high percentage of the health-house model, while the Health center of Medan Belawan has a high rate of diarrhea cases and a low percentage of healthy housing model. All data were calculated using SPSS and analyzed with Chi-Square and Regression Logistics tests.

**Results:** The sex and occupation did not show any significant difference in poor and healthy sanitation conditions, and no significant association between the presence of IgM and IgG from the results of the Typhidot test on both environmental sanitation ( $p>0.05$ ).

**Conclusion:** Sex and occupation did not show any significant difference in both conditions, and sanitation conditions did not correlate with the formation of either IgG or IgM among healthy people.

**Keyword:** Typhoid Fever, Typhidot, Rapid test, Sanitation

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### ABSTRAK

**Latar belakang.** Demam tifoid adalah penyakit menular saluran pencernaan yang sering ditemukan endemik di Indonesia, yang dapat ditularkan melalui tinja dari makanan atau air yang terkontaminasi secara langsung atau vektor membawa bakteri tersebut. Penelitian ini bertujuan untuk membandingkan keberadaan antibodi dari Tes Typhidot pada orang dalam kondisi sanitasi yang buruk dan sehat.

**Metode.** Penelitian ini merupakan penelitian observasional dengan desain cross-sectional. Penduduknya adalah seluruh masyarakat di sekitar Puskesmas Medan Petisah yang kecamatan memiliki kasus diare yang lebih sedikit dan persentase model rumah kesehatan yang tinggi, sedangkan Puskesmas Medan Belawan memiliki tingkat kasus diare yang tinggi dan persentase model perumahan sehat yang rendah. Semua data dihitung menggunakan SPSS dan dianalisis dengan uji Chi-Square dan Regresi Logistik.

**Hasil.** Jenis kelamin dan pekerjaan tidak menunjukkan perbedaan yang signifikan dalam kondisi sanitasi yang buruk dan sehat, dan



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tidak ada hubungan yang signifikan antara IgM dan IgG dari hasil uji Typhidot pada kedua sanitasi lingkungan ( $p > 0.05$ ).

**Kesimpulan.** Jenis kelamin dan pekerjaan tidak menunjukkan perbedaan yang signifikan dalam kedua kondisi tersebut, dan kondisi sanitasi tidak berkorelasi dengan pembentukan IgG atau IgM di antara orang sehat.

**Keyword:** Demam Tifoid, Typhidot, Tes cepat, sanitasi

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## 1. Introduction

Typhoid fever is an acute gastrointestinal tract infection caused by *Salmonella Typhi* [1]. This infectious disease has become a public health burden, accounting for 22 million cases yearly. The severe complication affects around 10% of patients, especially those without adequate therapy for over two weeks [2].

The high incidence and prevalence of typhoid fever affect the resistance of agents against the antibiotic. The study reported that the incidence of confirmed typhoid fever in 5 different hospitals in India was 44%, and the other 35% was *S. Paratyphoid*. Interestingly, around 82%, 9%, and 1% of these cases showed quinolone, cephalosporin, and macrolide resistance strain bacteria, respectively. Due to this reason, it becomes essential to use antibiotics for typhoid infections judiciously. Thus, it can reduce the resistance rate; on the other, it is also required to diagnose typhoid fever properly. There have been various diagnosis modalities to investigate typhoid infection, including the widal test and rapid test for typhoid (Typhidot) [3].

The Widal test and other serological tests are still widely used in developing countries which have proven to be quite sensitive, specific, and practical in endemic areas of this disease. Recent advances in molecular immunology study have led to the identification of potentially more sensitive and specific markers in the blood and urine of patients with typhoid fever and have made it possible to develop practical and inexpensive tools for typhoid infection diagnosis. Olsen et al. (2004) reported that the sensitivity and specificity values of several typhoid serodiagnostic tests were as follows: 89% and 53% for Multi-Test Dip-S-Ticks, 79% and 89% for Typhidot, 78% and 89% for TUBEX, and 64% and 76 % for the Widal test at the hospital and 61% and 100% for the Widal test at the Pasteur Institute.

Despite this, the patients that were enrolled differed little. At the Hospital for Tropical Diseases, patients have seen a median of two days later in their illness, were slightly older, and were more likely to be female. Starting in the first week of the sickness, the TyphiDot's sensitivity was high. We did not observe this effect with the TUBEX, which also detects IgM antibodies. This is likely due to the TyphiDot's greater reliance on IgM results, which rise later in the illness, while IgG results rise earlier. In the Widal test, the O and H agglutinins usually appear on days 8 and 10–12, respectively. Presumably, because the Multi-Test Dip-S-Ticks tests antibodies to five distinct infections, it was the most expensive assay. Despite the relative ease of use of all three assays, the TUBEX was the most straightforward. The possibility of trouble understanding the results of hemolyzed samples is a drawback of the TUBEX test, which employs a colorimetric response. The Widal test should preferably be run on sera from both the acute and convalescent stages to detect a rise in the agglutination titer. However, a single acute-phase serum sample is often performed to guide treatment decisions before the collection of convalescent samples. High background rates of circulating antibodies to serotype Typhi or other *Salmonella* serotypes can result in a false-positive result, making it challenging to evaluate data from a single sample [4].

All these tests show the highest sensitivity values in the second week of the disease course. The Widal test is not sensitive and shows inter-operator variability. Two rapid kits, Typhidot and TUBEX, are showing potential accuracy [5]. Various studies have been performed to estimate the risk factors of typhoid fever, including personal hygiene and sanitation. Nuruzzaman and Syahrul (2016) reported that the habit of washing hands and the condition of their fingernails also affected the incidence of typhoid fever at Dr. Abdoer Rahem Situbondo Regional Hospital. Meanwhile, the study also reported that respondents who did not use good processed water had a higher risk of being infected with typhoid fever. Hence, the study aims to compare the rapid Typhidot test based on the sanitation environment in healthy individuals in Medan.

## **2. Methods**

This study was an observational study with a cross-sectional design. Before the study, the Health Research Ethics Committee of Universitas Sumatera Utara approved all study procedures. This study used a vein blood sample from the sample, that was analyzed in the Laboratorium of Universitas Sumatera Utara from 23 July 2022-23 August 2022.

The population of this study was all people in both poor and healthy sanitation conditions in Medan. This study selected some people, who were nominated as the community members of the selected health center's working area from this population as the sample by Judgmental Sampling and Consecutive Sampling Methods. At the initial sample selection, representation of poor and well sanitation conditions was selected by judgmental sampling methods from some public health centers in Medan. These public health centers were the Public Health Center of Medan Petisah and Medan Belawan to represent the well and poor sanitation conditions, respectively. These public health centers were selected based on data from local public health departments as secondary data. After that, the consecutive sampling method was used to select some people from each working area of public health centers, that fulfilled the inclusion and exclusion criteria from each work area. The number of samples was estimated at 48 people who fulfilled the inclusion criteria: persons aged more than 17 years old and did not suffer from a fever that has a temperature lower than 37.5°C and exclusion criteria: the person has received the Typhoid vaccine, history of typhoid fever within the last two weeks (based on laboratory investigation)

This study evaluated the respondent's characteristics, sanitation conditions, and Typhidot test results. These characteristics included age, sex, and occupation. Environmental sanitation was determined by secondary data from the local public health department and it was categorized as well or poor sanitation. Finally, the typhoid test included the IgG and IgM detection, categorized as positive and negative.

Typhidot test was performed to investigate the presence of Immunoglobulin G (IgG) and Immunoglobulin M (IgM). This test required the blood serum from the respondent. The blood serum was obtained from the vein blood that had been withdrawn from the antecubital vein, and then the blood was centrifuged. The serum was obtained from the upper layer of centrifuged blood. The amount of 45µL serum was pipet and mixed with a drop of buffer solution from the Typhidot test kit. After that, it was placed into the sample well. Last, the Typhidot test revealed the test result as a qualitative result. The presence of test and control lines in IgM and IgG Typhidot test cassettes indicate a positive and negative result when the cassette only revealed the control line without the test line.

### **Data Analysis**

Initially, all data were analyzed by descriptive statistics, shown as frequency and percentage. After that, the analysis continued to inferential statistics that were chi-square. If these data did not fulfill the assumption of Chi-Square, the data were analyzed by Fisher exact. One of these assumptions was expected value of the cells should be five or greater in at least 80% of cells.

## **3. Results**

This study was performed in two different sanitation conditions, which were poor and well sanitation. Based on the sanitation questionnaire was aimed to sample in either Medan Belawan or Medan Petisah among 24 respondents in each environment. Medan Belawan was defined as a poor sanitation environment, and Medan Petisah was defined as healthy sanitation conditions. Medan Belawan is the one sub-district from 21 sub-districts in Medan that was selected to represent the poor sanitation condition. Meanwhile, good sanitation was represented by Medan Petisah.

All respondents from Medan Petisah and Medan Belawan were evaluated for characteristics including age, sex, and occupation. Furthermore, this study evaluated not only the characteristics of respondents but also the presence of IgM and IgG from the Typhidot test.

Based on Table 1, only age was a significant difference in poor and healthy sanitation conditions ( $p < 0.05$ ). Other characteristics, which were sex and occupation, did not show any significant difference in poor and healthy sanitation conditions ( $p > 0.05$ ).

Table 1. Characteristics of All Respondents

Characteristics	Sanitation, n (%)		Total	P-Value
	Poor	Well		
Age (years old)				
Late Adolescent (17-25)	3 (6.3)	2 (4.2)	5 (10.4)	0.048
Early Adulthood (26-35)	9 (18.8)	5 (10.4)	14 (29.2)	
Late Adulthood (36-45)	9 (18.8)	5 (10.4)	14 (29.2)	
Early elderhood (46-55)	3 (6.3)	12 (25.0)	15 (31.3)	
Sex				
Male	2 (4.2)	3 (6.3)	5 (10.4)	1.000
Female	22 (45.8)	21 (43.8)	43 (89.6)	
Occupation				
Housewife	18 (37.5)	15 (31.3)	33 (68.8)	0.304
Employee	2 (4.2)	3 (6.3)	5 (10.4)	
Student	1 (2.1)	3 (6.3)	4 (8.3)	
Entrepreneur	1 (2.1)	2 (4.2)	3 (6.3)	
Fisherman	2 (4.2)	0 (0)	2 (4.2)	
Construction worker	0 (0)	1 (2.1)	1 (2.1)	

Based on Table 2, IgM was found in a respondent (2.4%) for both sanitation conditions. The Fisher's exact test showed that the P-value was 0.755 and indicated no significant relationship between the Typhidot Rapid Test IgM results and environmental sanitation

Table 2. Comparison of Typhidot Rapid Test IgM Results for Healthy Individuals in Both Sanitation Conditions in Medan.

Sanitation	Typhidot (IgM), n (%)		Total	P-Value	PR (95% CI)
	Positive	Negative			
Well	1 (2.4)	23 (47.9)	24 (50.0)	0.755	1.00 (0.066-15.081)
Poor	1 (2.4)	23 (47.9)	24 (50.0)		
Total	2 (4.8)	46 (95.8)	48 (100.0)		

Based on Table 3, this study also compared the presence of other types of Immunoglobulins, in health respondents from both sanitation environments IgG was found in two respondents (4.2%) for both sanitation conditions. The Fisher exact test showed that the P-value was 0.755, indicating no significant relationship between the Typhidot Rapid Test IgG Results and environmental sanitation. Meanwhile, the Prevalence Ratio (PR) and 95% CI values support the results of the Fisher's exact test analysis, where the 95% CI value shows an inconsistent value where the lower limit value of PR  $< 1$  (0.153) and the upper limit value  $> 1$  (6.531).

Based on Table 3, the PR value also shows no relationship between the presence of IgG through the Typhidot test and sanitation conditions.

Table 3. Comparison of Typhidot Rapid Test IgG Results for Healthy Individuals in Both Sanitation Conditions in Medan City

Sanitation	Typhidot (IgG), n (%)		Total	P-Value	PR (95% CI)
	Positive	Negative			
Well	2 (4.2)	22 (45.8)	24 (50.0)	0.755	1.00 (0.153-6.531)
Poor	2 (4.2)	22 (45.8)	24 (50.0)		
Total	4 (8.3)	44 (91.7)	48 (100.0)		

Based on Figure 1, the distribution of IgG is bigger than IgM in both sanitation area

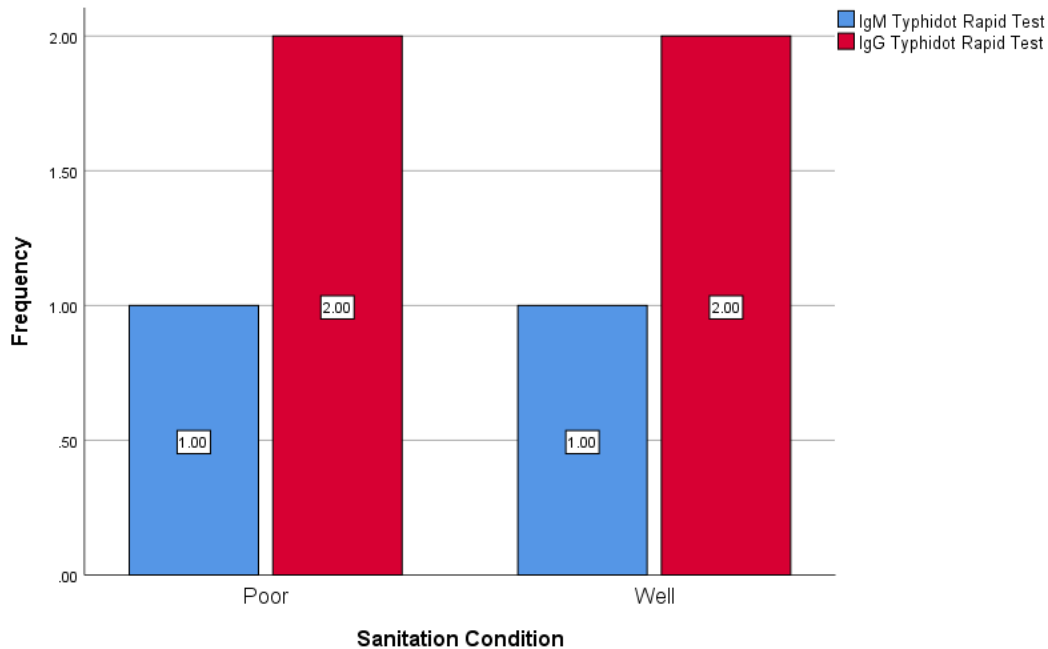


Figure 1. Distribution of IgM and IgG presence in Healthy Individuals in Both Sanitation Conditions in Medan City

#### 4. Discussions

Based on the study result, it is seen that there was no relationship between the presence of Immunoglobulin G or Immunoglobulin M against the sanitation condition among all respondents in the working area of Medan public health centers. However, the number of respondents tended to form IgG than IgM. It can be seen from the result of the study that the number of respondents who formed IgG was a respondent for both sanitation conditions, and two respondents for both sanitation conditions formed IgM. Several modalities of rapid tests for typhoid infection include Tubex TF and Typhidot. The sensitivity and specificity of Tubex TF were 78% and 87%, respectively. Meanwhile, the sensitivity and specificity of Typhidot were 78% and 77%, respectively. These rapid tests were based on the presence of immunoglobulin formed as an acquired immune response against the bacteria [6]–[8].

This study demonstrated that a respondent with IgG formation is more common than an IgM. It indicated that *S. Typhi* suffers from typhoid carriers. On the other hand, it also affects the half-life of either IgG or IgM. The half-life of IgG is longer, up to 12 months, compared to IgM, which is only around four months. It leads to a higher number of healthy patients with IgG than IgM.

IgM and IgG are components of the acquired immune system secreted by B cells as the process of humoral immunity. These B cells differentiate into plasma cells that actively produce IgM and IgG according to cytokines and inflammatory mediators that stimulate B cells' development. IgG has a shorter half-life longer in the blood serum than IgM or other Immunoglobulins. Hence IgG indicates primary and secondary immune responses to an antigen. IgM is similar to IgG, that was the process of humoral immunity. Furthermore, IgM can transform into another type of Immunoglobulin with a similar structure, Immunoglobulin A (IgA), found in body fluids such as saliva, intestinal fluid, and blood serum [9], [10].

Some previous studies have evaluated the presence of various Immunoglobulins that specifically recognize various antigens present. The rate of IgG and IgM elimination was different. IgG can persistently last up to 12 months after infection, while IgM and IgA have a faster rate of decline. The concentration of IgM and IgA decreases within 3-4 months after infection [10], [11].

Serologic tests are widely used to investigate the presence of specific antibodies to identify foreign antigens, especially among typhoid carrier cases. However, this test has low accuracy in identifying the antibodies. It is associated with a high rate of false positives due to cross-reactivity to other infections. Therefore, the detection of antigens, not antibodies, appears to be a suitable approach for developing a future Rapid Diagnostic Test for typhoid fever.

The serologic test detects the presence of a specific antibody against an antigen, and it still has many disadvantages, especially in the case of typhoid carriers. Because of cross-reaction against some other infection, thus it increases the false positive rate. This study reported no significant difference in the formation of IgG and IgM between poor and well sanitation. It may be due to many factors, one of which is the reagent used for this study. This study used commercial reagents commonly used in the market to identify the presence of Immunoglobins G and M, leading to inaccurate IgG and IgM identification. Therefore, further study is required for molecular identification of IgM and IgG molecules such as Two-Dimensional Gel Electrophoresis, Affinity Gel, and SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis). Other factors that may also affect the result of this study were the sampling methods. This study used non-representative sampling methods, that decreased the representative of the sample. Nur Nasri also reported that the host, agent, and environment also affect the nature of a disease. A recent study showed that the environment did not affect the nature of *S. Typhii* infection when the other factors from the host and agent did not fully control in this study and it may skew the recent result study. Some host factors like household condition, annual earnings, comorbid, and personal hygiene were not fully controlled as research variables. Moreover, agent factors like resistance and biochemical profile of *S. Typhii* also did not control as a research variable [12], [13].

Sanitation is the control of the physical, biological, social, and economic environment that affects human health. Furthermore, the World Health Organization (WHO) defines sanitation as an effort to surveillance several physical environmental factors that affect humans, especially physical development, health, and survival. The environment is helpful for physical and spiritual health and good social conditions for family and individual health. Indonesia is one of the countries that still has poor sanitation conditions. It can be proven by some slum places to live, sewage disposal, and a lack of clean water supply. This study selected poor and healthy sanitation from the Health Center of Medan Belawan and Medan Petisah, respectively [14]–[16].

This study showed no relationship between sanitation conditions and the presence of IgM or IgG in healthy people. None of the previous studies detected the presence of IgM or IgG among healthy people in several sanitation conditions. Several previous studies only analyzed serological tests with a history of typhoid fever or patients with symptoms of typhoid fever. House et al. (2001) and Arora et al. (2019) reported that various serological tests could be useful for diagnosing patients with typhoid fever who are clinically symptomatic with negative or unconfirmed culture results. Furthermore, this serological test also needs to be performed in combination with other serological tests to increase the accuracy of the diagnostic test. Furthermore, recent studies have shown that serological tests that detect the presence of specific antigens on *S. Typhii* show better results than serological tests for anti-*S. Typhii* antibodies [17], [18].

Some risk factors have been reported for *S. Typhii* infection, one of them is sanitation conditions. Siddiqui et al. (2018) also reported a similar result on whether hand-washing habits significantly reduce the incidence of typhoid fever, especially in developing countries. Some previous studies have investigated the relationship between *S. Typhii* infection against hand wash habits and sanitation conditions. However, none of these studies was performed to investigate the relationship between serologic results against hand washing habits and sanitation conditions. Another study also showed. Thus, the recent study demonstrated that there was no relationship between serologic results (IgM and IgG) and sanitation conditions (P-Value > 0.05) [19], [20]. The strength of this test in preliminary data has shown sensitivity and specificity of 95% and 86% of Typhidot tests. The limitation of this test still has many disadvantages, especially in the case of typhoid carriers. Because of cross-reaction against some other infection, thus it increases the false positive rate. This study reported no significant difference in the formation of IgG and IgM between poor and well sanitation. It may be due to many factors, one of which is the reagent used for this study.

## 5. Conclusions

In this study, we found that sanitation conditions did not correlate with the formation of either Immunoglobulin G or Immunoglobulin M among healthy people. Sex and occupation did not show any significant difference in both conditions.

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