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Comparison of Widal Test Using Serum and Plasma Samples

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ABSTRACT

The widal test is a test for diagnostic of thyfoid fever serology. Widal test aims to detect the presence of antibodies against Salmonella antigen, by measuring the degree of agglutination of antibody titer to antigen O and H in sample. In this research Widal test was performed using serumand plasma samples. This study aims to determine whether there is a difference in the value of titer on the Widal test between serum samples and plasma EDTA samples. This type of research is an experimental study. Samples were taken from patient suspected of thyfoid fever of 10 sample in the Laboratory of SM Raja Amplas in 2018. The result of comparative titer Widal test using serum and plasma samples obtained results 30% of the 10 samples examined there are differences in the value of Widal titer but does not affect the final diagnosis of typhoid fever. The value of the Widal test titer using plasma is lower than the value of Widal Titer with the serum samples. The use of plasma samples to determine the Widal titer is particularity susceptible to mingling with the erythrocytes due to the presence of anticoagulant particles but does not affect the result of the Widal test. EDTA anticoagulants do not damage the antibodies contained in the serum, this can be seen in the results of the study, there were no differences in each sample examined, so the difference in results obtained due to other factors, namely the error of researchers in the Widal test. The Widal test using serum samples can gives results according to the actual circumstances because serum is not contaminated by EDTA anticoagulants.

1. Introduction

Salmonella is a major cause of food-borne diseases (foodborn deseases). In general, Salmonella serotypes cause diseases of the digestive organs. The disease caused by Salmonella is called salmonellosis. The characteristics of a person who has salmonellosis are diarrhea, stomach cramps, and fever within 8-72 hours after eating food contaminated with Salmonella.[1]

The three main serotypes of Salmonella species are Salmonella typhi, Salmonella typhimurium, and Salmonella enteritidis. Salmonella typhi causes typhoid fever (typhoid fever), due to the invasion of bacteria into the blood vessels. Salmonella typhi is unique only to humans, and no other host. Salmonella typhi infection can be fatal to infants, toddlers, pregnant women and their wombs and the elderly. This is due to their decreased immunity.[1]

Laboratory examinations are currently very important because of the shift in the function of laboratory examination results from diagnostic support to diagnostic enforcer. In order for the examination results to be accurate, quality and accountable, in stages laboratory examinations which include pre-analysis, analytic and post-analytic must be carried out according to the procedure.[2]

The Widal test is a test used in the serological diagnosis of typhoid fever. The principle of the Widal

test is that patients with typhoid fever or enteric fever will have antibodies in their serum that can react and agglutinate multiple dilutions. The Widal test aims to detect the presence of antibodies (immunity) against Salmonella by measuring the agglutination levels of antibody titers against O and H antigens in the body's blood sample. Our bodies will form antibodies if we are exposed to Salmonella typhi germs, both germs that enter naturally and cause illness or germs that enter but do not show symptoms (careers) or through vaccination.[3]

The Widal test is still widely used today, especially in developing countries including Indonesia. Interpretation of the Widal test must pay attention to several factors, including sensitivity, specificity, disease stage, patient factors such as immunity status and nutritional status that can affect the formation of antibodies, immunological features of local community (endemic or non-endemic areas), antigen factors, techniques and reagents used.[4]

Widal test interpretation must pay attention to several factors, namely sensitivity, stage of the disease; Patient factors such as immunity status and nutritional status that can affect the formation of antibodies, immunological features of the local community (endemic or non-endemic areas), antigen factors, techniques and reagents used. The Widal test has the advantage that the costs required for Widal's examination are relatively cheap. The drawbacks of the Widal test are that the results are inaccurate, a positive result does not mean that it is always sick, and likewise a negative result does not mean that it is not sick. A positive result of a Widal test with a titer of more than 1/320 and supporting clinical symptoms is considered sufficient to establish a diagnosis of fever. Typhoid. What is still often wrong is the timing of this Widal examination. The Widal test should be done after the 5th day of fever. The specimens that can be used in the Widal test are blood serum, blood plasma and blood. The difference between serum and plasma is that serum does not contain anti-coagulants while plasma contains anti-coagulants, one of which is Ethylene Diamin Tetra Acetate (EDTA).[5]

Plasma and serum are the yellowish parts of the blood which will be clearly visible when the blood cells settle to the bottom of the tube. Some laboratories rarely use EDTA plasma samples, but that does not mean they never use them at all. Plasma is only used as an alternative to serum if very little serum is obtained during an emergency. In addition, the use of plasma that is susceptible to being mixed with erythrocytes due to the presence of anticoagulant particles of EDTA will affect the results of the examination. EDTA is a complex acid, a polymino carboxylic acid which is commonly used as a chelating agent for several metal ions / elements, especially Fe3 + and Ca2 +. These particles in EDTA are likely to affect the results on Widal's examination.[6]

Based on data and surveys obtained from the field, checking the Widal titer level using serum often gets into trouble because of insufficient blood volume or lysis of the serum due to inaccurate serum intake. Poor sample conditions will certainly affect the results of Widal's titer examination, if that happens, Widal's examination can use an EDTA plasma sample. The use of EDTA plasma samples is preferred because of time efficiency considerations, plasma samples are obtained more quickly than serum samples. This study aims to determine the differences in titer levels in Widal's examination using serum and plasma samples.

2. Method

Research design

The type of research used is experimental, by comparing the results of Widal's examination on serum and plasma samples. The place of research and sampling was carried out at the SM Primary Clinical Laboratory. Raja Amplas Medan. The research was conducted in May-June 2018. The population in this study were patients suspected of having typhoid fever who came to the SM Primary Clinical Laboratory. Raja Amplas Medan during June 2018. The samples used were 10 samples from suspected typhoid fever patients who came to the SM Primary Clinical Laboratory. Raja Amplas Medan.

Vein Blood Collection

Blood is the main ingredient in Widal's examination. The blood used was the Mediana Cubiti vein. 7 Torniquete was placed on the inner side of the elbow crease, which is 3 cm above the median cubital vein. The patient is asked to make a fist. The part of the mediana cubital vein that will be taken for blood is felt, then disinfected with 70% alcohol by pressing the vein with a 70% alcohol cotton, then cleaning in one direction. With the needle hole facing up, the vein is slowly poked until the blood comes out at the end of the vein. The suction syringe is slowly withdrawn and stopped until the amount of blood is 3 ml. Then remove the torniquete and ask the patient to open his fist. An alcohol swab is placed on the needle and then the syringe is pulled out slowly. Blood from the syringe is inserted into the frozen blood tube (red tube) and EDTA tube (purple tube) by opening the nald from the syringe, then slowly inserting the blood through the tube wall into the red tube and the purple tube (EDTA blood) slowly. land through the walls of the tubes 1.5 ml each. The blood that has been drawn is allowed to stand for 30 minutes. Then centrifug the blood at 3000 rpm for 15 minutes, the clear yellow layer at the top of the red tube is serum and in the purple tube is plasma. Serum and plasma are taken using a micro pipette and put into a new, clean and sterile tube for examination.

Widal Test Examination

The Widal test is performed to see if there are antibody titres in serum and plasma in suspected typhoid fever sufferers. Agglutination reactions will occur when the Salmonella typhi antigen suspension is mixed with serum suspected of having typhoid fever containing antibodies.

In addition to preparing the patient's serum or plasma samples, positive and negative controls were also made as a reference. Each drop one drop of reagent onto the ring slide. A total of 50 µl of patient serum was added to each side of the reagent in single ring 1 and 50 µl of plasma in each reagent in single ring 2, then homogenized with a stirring rod. Rotator for 1 minute, and check for agglutination. If agglutination is formed then Widal is positive. If one of the ring slides is formed agglutination, serum and plasma are diluted to determine the patient's titer.

If there is agglutination (positive) on one of the ring slides / plates with serum and plasma samples, dilute 80 µl of serum, serum and plasma pipette then drop 20 µl of reagent then rotator for 1 minute. If coarse agglutination is still formed, continue with serum and plasma depletion of 40 µl then drop 20 µl of reagent, then rotator for 1 minute. If agglutination is still formed, continue to thin out the serum and plasma by 20 µl, then drop 20 µl of reagent, then rotator for 1 minute. If agglutination is still formed, continue to thin out the serum and plasma as much as 10 µl, then drop 20 µl of reagent, then rotator for 1 minute. If agglutination is still formed, continue to thin out the serum and plasma by 5 µl, then drop 20 µl of reagent, then rotator for 1 minute. If agglutination is formed, serum and plasma depletion is stopped at 5 µl depletion and the patient is tested positive with an antibody titer of 1/320. Dilution is carried out until no more agglutination occurs, the last agglutination is used as the titer.

Table 1. Interpretation of Widal Test Results[8]

Serum volume (µl)	Dilution
80	1/20
40	1/40
20	1/80
10	1/160
5	1/320

3. Result and Discussion

Widal's examination using serum samples and plasma samples showed that out of 10 samples found 100% contained antibodies to Salmonella antigen (positive for typhoid fever and paratyphoid fever) either using serum samples or using plasma samples. Serum samples and plasma samples can be used in Widal's examination because they both contain the antibodies needed in Widal's examination where the antibodies in the serum sample and plasma sample agglutinate with the antigens in the O and H reagents.[9]

In this study, in patient sample number 3 (S3) there is a difference in the titer value of the Salmonella paratyphi B antigen H suspension where the titer value in the serum sample is 1/80 and the titer value in the plasma sample is 1/40, in the patient sample number 4 (S4) there is a difference in the titer value of Salmonella typhi O antigen where the serum sample titer value is 1/160 and the titer value in the plasma sample is 1/80, and in patient sample number 8 (S8) there is also a difference in titers in Salmonella typhi O antigen where the The serum sample titer was 1/160 and the plasma sample titer was 1/80 (Table 2 and Table 3).

Table 2. Typhoid fever examination results with Serum samples

No	Sample Code	Age (Year)	O	AO	BO	CO	H	AH	BH	CH	Information
1	S1	20	320	80	160	160	320	160	160	80	+ Typhoid
2	S2	21	320	160	320	320	320	80	160	80	+ Typhoid
3	S3	10	80	40	40	160	160	80	**80**	40	+ Paratyphoid
4	S4	17	**160**	80	80	160	160	160	160	80	+ Paratyphoid
5	S5	20	160	40	80	160	80	320	160	160	+ Paratyphoid
6	S6	14	320	40	80	320	320	80	80	320	+ Typhoid
7	S7	1	80	40	160	320	160	80	40	160	+ Paratyphoid
8	S8	8	**160**	80	80	160	160	80	80	160	+ Paratyphoid
9	S9	11	320	40	80	40	80	80	40	160	+ Typhoid
10	S10	16	320	80	320	80	80	80	160	80	+ Typhoid

Information: ■ *= different Widal titer values*

Table 3. Widal examination results with Plasma samples

No	Sample Code	Age (Year)	O	AO	BO	CO	H	AH	BH	CH	Information
1	S1	20	320	80	160	160	320	160	160	80	+ Typhoid
2	S2	21	320	160	320	320	320	80	160	80	+ Typhoid
3	S3	10	80	40	40	160	160	80	**40**	40	+ Paratyphoid
4	S4	17	**80**	80	80	160	160	160	160	80	+ Paratyphoid
5	S5	20	160	40	80	160	80	320	160	160	+ Paratyphoid
6	S6	14	320	40	80	320	320	80	80	320	+ Typhoid
7	S7	1	80	40	160	320	160	80	40	160	+ Paratyphoid
8	S8	8	**80**	80	80	160	160	80	80	160	+ Paratyphoid
9	S9	11	320	40	80	40	80	80	40	160	+ Typhoid
10	S10	16	320	80	320	80	80	80	160	80	+ Typhoid

Information: ■ *= different Widal titer values*

Plasma samples gave lower titer results than the use of serum samples, this was due to the researchers' error in the processing technique in the Widal examination process, so that the results of Widal's examination using

the same plasma sample were lower than the serum samples. Ethylene Diamin Tetra Acetat anticoagulant content (EDTA) contained in the plasma tube does not affect the stability of antibody antigen bonds because the function of EDTA anticoagulants is to inhibit the action of activators on blood clotting and get rid of calcium ions so that the blood remains liquid. EDTA anticoagulant does not damage the antibodies contained in the serum, this can be seen in the results obtained that there is no difference in each sample examined, so that the difference in the results obtained is caused by other factors, namely the error of the researcher in carrying out the Widal test.[10-12]

From the results of the comparative study of Widal's examination using serum and plasma samples, it was found that of the 10 samples studied

there was a 30% difference in the results of the titer obtained, where the titer results in the plasma sample were lower than the titer results in the serum sample. The difference in serum sample titer and plasma sample on Widal's examination was not so significant because it did not affect the final result reading for the diagnosis of typhoid fever.

Widal's examination should use a serum sample to get a more accurate result, because the use of a plasma sample in this examination gives a lower titer than using a serum sample. So for a good Widal examination accuracy, the sample used should be a serum sample. This is because Widal's examination is very sensitive to the condition of the specimen. Use of plasma samples is recommended at certain times when serum samples are not available.

4. Conclusion

From the results of the comparison examination of Widal's test in 10 patients with suspected typhoid fever using serum samples and plasma samples, the same results were obtained where 10 patients were positive for antibodies to Salmonella antigen (positive for typhoid fever and paratyphoid fever). The use of serum samples and plasma samples in Widal's examination did not have a significant difference, although there were differences in titers, they did not affect the reading of the final diagnosis. However, it is better if Widal's examination is to use a serum sample and a plasma sample is used only at certain times if a serum sample is not available.

5. Thank You Note

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