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DIAGNOSTIC VALUE OF WIDAL SLIDE ASSAY USING ONE PHAGE TYPE LOCAL ANTIGEN COMPARED WITH FOUR PHAGE TYPES LOCAL ANTIGEN IN TYPHOID FEVER PATIENTS IN SURABAYA

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ABSTRACT

Widal slide test is one of the serologic tests which are practical, inexpensive and not time consuming. The Widal slide test can be performed in small laboratories, but the sensitivity and specificity depend on the antigen. Salmonella typhi in Indonesia consists of five phage types (D2, A, B1, D6 and E1), however, in Indonesia, it is difficult to differentiate between these phage types. Our objectives is to compare the diagnostic value of Widal slide test with one phage type and four phage types local antigen in typhoid fever patients. The method in this research is an observational, cross sectional study. Carried out on 78 patients consisting of 39 patients with positive gall culture and 39 non typhoid fever patients with fever and negative gall culture diagnosed as dengue hemorrhagic fever, malaria, B Hepatitis and Salmonella paratyphi A fever. The results come out that cut off value of Widal slide determined by O or H titer agglutinin was 1/80. The result of Widal slide was considered as positive if O agglutinin titer was more than the cut off value (≥1/160). The sensitivity of Widal slide one phage type was 30.76 % (very low) and the specificity was 94.87 % (high). Positive Predictive Value was 85.71 % (high) and Negative Predictive Value was 57.81 % (low), diagnostic efficiency was 62.82% (low). The sensitivity of Widal slide four phage types was 82.05 % (high) and the specificity was 82.05 % (high). PPV was 82.05 % (high) and NPV was 82.05 % (high), diagnostic efficiency was 82.05 % (high). Mc. Nemar statistics showed a significant difference in results between Widal slide one phage type and four phage types. So, we conclude that Widal slide four phage types showed a higher sensitivity and specificity than one phage type. For the future, we suggest Widal slide test to use four or five phage type's local antigen.

Keywords: *Widal slide local, phage type, Salmonella typhi*.

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INTRODUCTION

Laboratory role in establishing the diagnosis of typhoid fever is very important. Laboratory facilities to help make the diagnosis of typhoid fever were classified into three groups, namely: isolation of germs that cause clinical specimens like blood, bone marrow, urine, feces and duodenal fluid; immunoassay track the increase in antibody levels against specific antigens of *Salmonella typhi* and *Salmonella typhi* determine specific antigen; *Salmonella typhi* DNA tracking of patient specimens, done in two ways, namely by tracking DNA hybridization (DNA probes) and doubling strands of nucleic acid from *Salmonella typhi* by polymerase chain reaction technique (PCR).

Typhoid fever serology tests that are part of the examination immunoassay intended to track the increasing level of antibodies against *Salmonella typhi* antigen or to determine the specific antigens of *Salmonella typhi*. Widal test is a serological test which is still widely used in Indonesia. Widal test plate using antigens from local bacterial strains (endemic areas) gave sensitivity and specificity higher than those originating from outside the antigen (antigen imports).

In Indonesia there are five types of *Salmonella typhi* phage (D2, A, B1, D6, and E1). It is known from joint research conducted by the University of Malaya, Clinical Pathology Faculty of Medicine Airlangga and Microbiology Faculty of Medicine UI. Making the five phage antigen levels in Indonesia are expected to provide high sensitivity because it has a broad spectrum, but the difficulty to make this antigen was determine phage must be performed beforehand. Determination of phage has yet to be done in Indonesia.

Making antigens derived from the isolation of patients with typhoid fever germ can not represent all the phage (phage 5) that exist in Indonesia that provides low sensitivity because the spectrum is not broad. Clinicians often complain about the low sensitivity of Widal examinations by local antigen has been done. This low sensitivity may be due to local manufacture of antigen by bacteria isolated from patients does not represent the 5th phage found in Indonesia. Storage also affects the stability of stock bacteria *Salmonella typhi*. *Salmonella*

typhi is better stored at -80 ^{o}C in order to avoid mutations (WHO 2003). This study aimed to compare the local single four phage widal test plate.

MATERIALS AND METHODS

This research is observational research, as there is no specific treatment of the sample and was performed by cross sectional study. This research was conducted on patients with typhoid fever and non typhoid fever in adult who outpatient at 'Gotong Royong' clinic in Manyar Kartika IV/80-10 Surabaya, outpatient and inpatient care at Dr. Soewandhie Hospital Surabaya and inpatient unit of internist at Dr. Soetomo Hospital Surabaya that meet the inclusion and exclusion criteria. Calculation of the minimum representative sample size in this study are based on the prevalence of typhoid fever in internal medicine sections in Dr. Soetomo period 1996-2000, i.e. 2.5%.

For the calculation of minimum sample size, use the following formula (Basuki 1999): n=number of minimum sample, p=probability of an event (percentage of the estimated percentage of items to be checked is 2.5%), q=100-p, For accuracy of 95%, the minimum sample size is: n = (4xpxq)/25 ; n=(4 x 2,5 x (100 - 2,5))/25 ; n=39.

Samples were taken from the serum of typhoid patients and non typhoid that meet the criteria for inclusion and exclusion samples. Inclusion criteria of typhoid patients are males and females aged 12-60 years, have fever more than 7 days, accompanied by one or more symptoms of typhoid fever, in blood cultures found *S. typhi*, during the last two years had never received vaccinations anti typhoid, willing to participate in this study.

Inclusion criteria for non typhoid fever sufferers are males and females aged 12-60 years, have fever for more than seven days with a diagnosis of another disease as the cause of their fever, In blood culture *S. typhi* was not found, during the two years do not ever get a vaccination anti typhoid and never experienced the heat of more than seven days during last one year, willing to participate in this research. Exclusion criteria are patients with typhoid fever or fever non typhoid who got treatment with corticosteroids or other immunosuppressive drugs, patients with typhoid fever or fever non typhoid with severe malnutrition, patients with typhoid fever or fever accompanied by another disease non typhoid disturbing immunologist humoral system (selected by clinical data).

Variable measured in this study is the local plate Widal test and four one phage. The dependent variable is typhoid fever. Research conducted in the infectious disease unit and the research and development (R & D) Clinical Pathology in Dr. Soetomo Hospital. The study began in August 2007 until January 2007.

Diagnostic sensitivity of Widal test in adult patients with typhoid fever is determined by the formula: (∑typhoid patients with a positive Widal test/∑all typhoid patients in the study)x100%. Diagnostic spesitivity of Widal test in adult patients with typhoid fever is determined by the formula: (∑non typhoid patients with a positive Widal test/∑ all non typhoid patients in the study)x100%.

Widal test positive predictive value is determined by the formula: (∑ typhoid patients with a positive Widal test/□ all patients with positive Widal test)x100%. Widal test negative predictive value is determined by the formula: (∑ non typhoid fever patients with a negative Widal test/∑ all patients with a negative Widal test)x100%. Efficiency of diagnostic Widal test in typhoid fever is determined to find out the percentage of patients correctly classified as sick or not sick. Widal test plate efficiency is determined by the formula: ((∑typhoid patients with a positive Widal test + ∑non typhoid fever patients with a negative Widal test)/ ∑all patients in this study)x100%.

Diagnostic value of widal test plate 4 phage with a phage plate widal test in terms of sensitivity, specificity, and diagnostic efficiency by using non-parametric statistical tests Mc Nemar. To assess the degree of diagnostic sensitivity, diagnostic specificity, positive predictive value of diagnostic, negative predictive value of diagnostic criteria used from Handojo (1988) as follows: very high if the value of a laboratory test characteristics equal or greater than 95%, high if the value of a laboratory test characteristics between 80-94%, medium if the value of a laboratory test characteristics between 65-79%, low if the value of a laboratory test characteristics between 50-64%, very low if the value of a laboratory test characteristics of less than 50%.

PROCEDURE

The Blood Samples

Every patient non typhoid fever and typhoid were 10 ml of blood taken at the time of admission in Dr. Soewandhie Hospital or outpatient at 'Gotong Royong' Foundation clinics and the inpatient at internal medicine Dr. Soetomo Hospital, 5 ml of blood for seed, 5 ml syringe which was then left in serum taken. Serum is

stored in several small aliquot (200 µl per tube) at a temperature of -20 ^{o}C until all samples are collected and treated simultaneously.

Blood Seed

5 ml of blood were aseptically inserted into the media bile broth, then stir average, incubated at 37 ^{o}C and every morning for 14 days the media whipped the bottle. Sub cultured on agar media Salmonella-Shigella (SS) on 3 days to 5. The growth of microorganism colonies in the form of fine, transparent, which is suspected as Salmonella sp, followed by Gram staining, biochemical tests and agglutinin test with specific anti sera. Kuamn growth morphology that is incompatible with *S. typhi* bacteria on agar medium of the SS, then the respective liquid media Oxgall discarded. Planting on Salmonella-Shigella agar media was repeated on days 5.7 and 14, if no germ growth. After day 14 remained negative, the liquid media and otherwise disposed Oxgall no growth or negative (Baron et al. 1994)

Biochemical Test

Biochemical tests are carried out by planting a colony of bacteria on solid media Indole Lysine Iron (LIM). Simmons Citrate (SC) and Triple Sugar Iron (TSI) and incubated at 37 ^{o}C for 18-24 hours. Features of germ *S. typhi* on the following biochemical tests (Baron et al. 1994) are explain in the following: *S. typhi* is the bacteria that ferment glucose, so the media is acidic (yellow). Aerobic atmosphere on the surface of medium TSI (slant) would alter the acidic media became alkaline (red). On the inside of an anaerobic atmosphere Butt media so that media remains acidic and colored yellow. *S. typhi* does not produce CO_2, it produce H_2S which marked the formation of black color on TSI medium after binding to the sodium trisulfat H_2S. At Simmons Citrate media only source of carbon is citrate, so that no color changes in the media.

S. typhi has decarboxylase enzymes that will break down the amino acid lysine. Acid lysine results of solving a fixed LIM medium purple. *S. typhi* can not break down the amino acid tryptophans, which are not formed yield components of the solution containing tryptophan called indole nitrogen. On the addition of Kovac reagent is not formed on the surface of the red color LIM media. Motility *S. typhi* looks like a brush (brush-like) at about the line of inoculation in the media LIM.

Table 1. Reaction of *Salmonella typhi*, Salmonella paratyphi A, Salmonella paratyphi B, and E. coli on medium TSI, LIM and SC after 24 hours incubation at 37 ^{o}C

Germs Type	TSI				LIM			SC
	Slant	*Butt*	Gas	H_2S	Lisin	Indol	Motility	Citrate
S. typhi	K	A	-	+	+	-	+	-
S. paratyphi A	K	A	+	-	_	-	+	-
S. paratyphi B	K	A	+	+	+	-	+	-
E.coli	A	A	+	-	+	+	+	-

Note: K= alkali, A = *Acid*, + = positif, (-) = negative, LIM = *Lysine Indole Motility*, TSI = *Triple Sugar Iron*, SC = *Simmons Citrate*

Antisera Test

Antisera tests performed on suspected colonies of bacteria which is *S. typhi* bacteria on Salmonella-Shigella medium and biochemical tests gave results consistent with *S. typhi*. This test is done to identify the antigen of *S. typhi* bacteria colonies, namely the O antigen, H or both O and H created in the glass objects by mixing the two suspensions antigen little colony of germs with physiological solution.

The first suspension drops with anti sera against the O antigen, whereas the second suspension drops with anti sera against antigens of H. This mixture was homogenized by rotating glass objects by hand, and then viewed under a microscope. Otherwise positive test when there is absence of agglutination and agglutination otherwise negative. Anti sera which are used are made by Biofarma Bandung.

Widal Test Plate Inspection Procedures

Widal reagent plate removed from the refrigerator and left at room temperature until the temperature is equal to room temperature (30 minutes). Patient serum was diluted in serum diluents solution in the glass objects as listed in the table (using micro pipette).

For aglutinin O, H titer dilutions starting from 1/40, followed by micro pipette 40 ul drop of antigen suspension and stirred until flat, then the object glass shook shaken for five minutes, after which the results read. The result of the positive test readings followed by dilution and if the test result is negative, no need to

proceed with the dilution and immediately reported as the results of widal test negative plate.

Reagents should be stored in a refrigerator at 4°C. The reading test is performed with the naked eye using light of the sun, near a window or at a distance of 20 cm above the 10 Watt fluorescent lamps. See is there any agglutination. The upper limit/threshold above (the cut-off) of normal titer plates widal test in adults is as follows (Handojo 2004) agglutinin O and H according to the titer 1/80. Positive to say when the titer ≥2 times the upper limit of normal titer for agglutinin O

Table 2. Comparison of serum and diluents volume of serum for antibody titer in widal test plate made of Clinical Pathology laboratory of Dr. Soetomo Hospital.

Titer	Comparison		
	Serum (µl)	Diluent (µl)	Antigen Serum (µl)
1/40	7	34	40
1/80	5	35	40
1/160	4	36	40
1/320	3	38	40
1/640	2	40	40

Reproducibility Widal Test plate

Reproducibility is determined by using two pools of sera in the three groups. Group 1 is the sera which gave a positive widal test result with a high titer of 1/320. Group 2 are sera that gave positive results with the widal test titer 1/160. Group 3 are sera which gave negative widal test results. The assessment was conducted as following reproducibility within run, sera examined five times in a one-time inspection. And between day reproducibility, sera examined one times a day for five consecutive days.

RESULTS

In this research, Widal test plate of 39 samples of typhoid patients and 39 patients with fever non typhoid samples, which were included in the sample inclusion and exclusion criteria. The sample consisted of 14 of typhoid fever (35.90%) adult men and 25 (64.10%) adult women, whereas fever non typhoid sample consisted of 18 (46.15%) adult men and 21 (53.85%) adult female . Patient with non typhoid fever consisted of three malaria patients (7.69%) samples, patients with urinary tract infection (UTI) caused by E. coli as many as three (7.69%) samples, patients with dengue hemorrhagic fever (DHF), 30 (76.92%) samples, one chronic hepatitis patients (2.56%) samples, patients with paratyphoid fever 2 (5.13%) samples (determined based on clinical and laboratory criteria).

Table 3. Tabulation of local plate widal test results one phage in 39 patients with typhoid fever and 39 patients with fever non typhoid.

	Typhoid	Non typhoid	Total
Positive	12	2	14
Negative	27	37	64
Total	39	39	78

The results of diagnostic widal test local plate one phage for diagnostic sensitivity: 12/39 x 100% = 30.76%, diagnostic specificity: 37/39 x 100% = 94.9%, diagnostic positive predictive value: 12/14 x 100% = 85.7%, negative predictive value of diagnostic: 37/64 x 100% = 57.8%, diagnostic efficiency: 49/78 x 100% = 62.82%

Table 4. Tabulation of local plate widal test results of four phage at 39 and 39 patients with typhoid fever sufferers non typhoid.

	Typhoid	Non typhoid	Total
Positive	32	7	39
Negative	7	32	39
Total	39	39	78

The results of diagnostic widal test local plate four phage for diagnostic sensitivity: 32/39 x 100% = 82.05%, diagnostic specificity: 32/39 x 100% = 82.05%, diagnostic positive predictive value: 32/39 x 100% = 82.05%, negative predictive value of diagnostic: 32/39 x 100% = 82.05%, diagnostic efficiency: 64/78 x 100% = 82.05%.

Mc Nemar statistical test showed significant differences for one phage widal local plate with four phage ($p<0.05$). Reproducible results of widal test local plate on one phage between the within run and day in accordance with the expected titer.

Reproducible results of widal test local plate run within a single phage in accordance with the expected titer while on the run between examination showed there were two (6.6%) sera from pool III and IV give results higher than the expected titer.

Table 5. The diagnostic value of widal test local plate single phage and four phage with reliability

Diagnostic value	Widal plate single phage		Widal plate four phage	
	Percentage	Reliability	Percentage	Reliability
Diagnostic sensitivity	30.76 %	Very low	82.05 %	High
Diagnostic specificity	94.87 %	High	82.05 %	High
Positive predictive value	85.71 %	High	82.05 %	High
Negative predictive value	57.81 %	Low	82.05 %	High
Diagnostic efficiency	62.82 %	Low	82.05 %	High

Table 6.Comparison of the results of a single phage widal test local plate with four phage

One phage / Four phage	Widal Positive	Widal Negative	Total
Widal Positive	15	22	37
Widal Negative	0	41	41
Total	15	63	78

Table 7. Results of local plate widal test reproducibility of phage

Pool	*Within run*			*Between day*		
Sera	Negative	1/160	1/320	Negative	1/160	1/320
I	5	0	0	5	0	0
II	5	0	0	5	0	0
III	0	5	0	0	5	0
IV	0	5	0	0	5	0
V	0	0	5	0	0	5
VI	0	0	5	0	0	5

Table 8.Results of widal test reproducibility local plate four phage

Pool	*Within run*			*Between day*		
Sera	Negative	1/160	1/320	Negative	1/160	1/320
I	5	0	0	5	0	0
II	5	0	0	5	0	0
III	0	5	0	0	1	4
IV	0	5	0	0	1	4
V	0	0	5	0	0	5
VI	0	0	5	0	0	5

DISCUSSION

In Indonesia there are five types of *Salmonella typhi* phage (D2, A, B1, D6, and E1). It is known from joint research conducted by the University of Malaya, Clinical Pathology Faculty of Medicine Airlangga and Microbiology Faculty of Medicine UI, but when the five types of phage was brought to Indonesia , one phage die in the way so that the antigens used in this study consisted of four types of phage. Assessment of

quality of a laboratory test contains several elements as follows: diagnostic sensitivity, diagnostic specificity, positive predictive value and negative predictive value, diagnostic efficiency, reproducibility and practicality.

LABORATORIES RELIABILITY TEST

Diagnostic Sensitivity

Widal test diagnostic sensitivity of local plate of 39 samples of a single phage typhoid patients 30.76%. According to the Handojo criteria (1988) the diagnostic sensitivity of this test is very low. Widal test diagnostic sensitivity of four local plate phage against 39 samples of typhoid patients 82.05%. This diagnostic test according to the criteria of Handojo (1988) has high sensitivity. McNemar statistical test results (ά=0.05) showed that differences in diagnostic sensitivity was significantly ($p<0.05$). Differences in the sensitivity analysis results for four phage local antigens has a broader spectrum that has the possibility to bind antibodies which are homologous with five phage types of *S. typhi* in Indonesia bigger.

Local antigen consisting of a phage-spectrum is not wide, so it was likely that the patients infected by *S. typhi* phage with different types of antibodies that are formed so that no homologue of the antigen in the reagent. Storage of antigen at room temperature can cause mutations in bacteria, so that was not homologous antigen with antibody in patients. Germs *S. typhi* stable stored at - 80^{o}C (WHO 2003).

Diagnostic Specificity

The study of 39 samples of non-typhoid with local plates widal test result of one phage gave a diagnostic specificity of 94.87% which is high, while the local plate widal test four phage 82.05% which is also high. Widal test local plate one phage gave a positive result on a quasi-one patients with UTI and DHF patients. Widal local plate test four phage gave false positive results on two patients with UTI and four DHF patients and one patient S.paratyphi A. This false positive results can occur because of widal test is a serologic test using polyclonal rather than monoclonal antigen so that the possibility of cross reaction can occur with other antigens including those from other bacteria. Differences in diagnostic specificity of the widal test plate with four phage and single phage tested statistically with Mc.Nemar, test showed no significant difference with $p<0.05$

Diagnostic Positive Predictive Value and Negative Predictive Value of Diagnostic

Positive predictive value and negative predictive value of the local plate single widal test phage in this study is 85.71% and 57.81%, while the local plate widal test four phage positive predictive value and negative predictive value equal to 82.05%. widal test result indicates a local four-phage plates are superior in diagnosing typhoid fever in the group of typhoid fever and typhoid fever are not in the group of non typhoid patients.

Diagnostic Efficiency

Widal test diagnostic efficiency of the local plate one phage 62.82% (low), while for the four phage for 82.05% (high). Widal test the ability of local plate one phage to diagnose typhoid fever low with the possible errors of diagnosis 37.18% while for the four phage the ability to diagnose typhoid fever high with the possibility of diagnostic error 17.95%.

Reproducibility Widal test

Widal test is reproducible in a single phage good local plate. The results of repeatedly examination of 30 pools of sera as expected. Widal test is reproducible in local plate four phage repeated examination of 30 pools of sera obtained two checks on the titer of 1/160 and of higher level 1/320. This may be influenced by subjectivity reading factors even though so this result does not cause the fault diagnosis.

PRACTICALITY OF WIDAL TEST PLATE

Widal test reagents local plates are easy to make and relatively good stability. This test is also easy to implement and requires no special power. Widal test local plate also does not require a long time, the results can be read after 5 minutes a mixture of antigen, serum and diluents. Time required for this test is 10-15 minutes.

CONCLUSION

Widal test local plate four phage provide diagnostic value (sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency) higher than the local plate widal test one phage, which concluded that: local plate widal test is better than four phage test widal local plate one phage. We suggest that widal test plates should be put on local antigen-containing phage 4-5, for it is necessary for the determination of phage in the making of local antigens. *S. typhi* germs used to manufacture the antigen should be stored at - 80 ^{o}C to prevent mutations that would affect the sensitivity and specificity of widal test.

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