COMPARISON OF THE DIAGNOSTIC VALUE OF LOCAL WIDAL SLIDE TEST WITH IMPORTED WIDAL SLIDE TEST

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Abstract. A laboratory study comparing the Widal slide agglutination test using local antigens produced by Mekar Jaya Diagnostica (SAT-MJD) with imported antigens (Murex, Abbott) was carried out on 55 sera of typhoid fever patients with positive blood culture and 56 sera of non-typhoid febrile patients. The SAT-MJD antigens consisted of a mixture of 5 different phage-types of *S. typhi* dominantly found in Indonesia. This study revealed the following results: the diagnostic sensitivity of local and imported antigens was 83.93%, the diagnostic specificity of local antigens was higher than the imported antigens *ie* 82.14% compared with 64.28%, the diagnostic efficiency of local antigens was 82.88% compared with 73.87% of the imported antigens. The diagnostic positive and negative predictive values of the local antigens were 80.70% and 83.63%, respectively. The imported antigen revealed diagnostic positive and negative predictive values of 69.69% and 80%, respectively. The diagnostic specificity and efficiency of local antigens were significantly different ($p < 0.02$ and $p < 0.05$) from the imported antigens. The local antigens have some advantages. There was no variation in within-run and between-day test, compared with a 6.6% variation shown by the imported antigen. The test results obtained 5 minutes after mixing the serum with antigens reduced the possibility of false-positive and false-negative results. The cost of local antigens is lower than the imported antigens. Based on these data, the Widal SAT-MJD has a reliable diagnostic value and can be used in small laboratories, such as primary health centers (Puskesmas).

INTRODUCTION

Typhoid fever is an endemic infectious disease, which continues to be a serious public health problem in Indonesia. Based on the data released by the Ministry of Health, the number of typhoid fever cases in 1990 was 9.2 per 10,000, escalating to 17.4 per 10,000 in 1994 (Muliawan and Surjawidjaja, 1999).

Immuno-vaccination, case finding, and contact tracing, followed by adequate anti-microbial treatment, appear to be the mainstay in the fight against the disease. For case finding, a reliable, practicable and low cost diagnostic tool is very important (Handojo, 1996). To date, the conventional Widal test is widely used as an approach to diagnose typhoid fever, although its sensitivity and specificity are still doubtful, especially for endemic areas like Indonesia. The advantages of the Widal slide test are the high degree of practicability and the low cost of the test (Hoffman *et al*, 1986). The aim of this study is to compare the diagnostic value of the Widal slide agglutination test produced by Mekar Jaya Diagnostika (SAT-MJD) with the imported Widal slide agglutination test (Murex, 1998).

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MATERIALS AND METHODS

This laboratory study was performed on sera obtained from 121 adult patients, consisting of 55 patients with typhoid fever (positive blood culture for *S. typhi*), 56 non-typhoid febrile patients (negative blood, urine and stool cultures for *S. typhi*), and 10 paratyphoid A fever patients (positive blood culture for *S. paratyphi* A) attending the Out-patient Department of Gotong Royong and Waluyo Jati health centers or who were hospitalized in the Tropical Disease Ward of the Dr Soetomo Hospital; all residing in Surabaya.

In the list of non-typhoid diseases with fe-

ver, 19 patients were entered with dengue fever (primary and secondary), 23 patients with malaria (positive blood smear), and 14 patients with urinary tract infection. The population under study consisted of male and female adults, older than 15 years, suffering from fever for more than 7 days, who had signed informed consent.

None of the patients were under treatment with corticosteroids or with other immunosuppressive drugs during the previous month, or suffered from diseases that could interfere with the generation of a humoral immune response. Furthermore, none suffered from malnutrition.

The diagnostic criteria of the local and imported Widal slide agglutination test for typhoid fever used in this study were as follows (Setyawati, 1996):

1. If the titer of O agglutinin or O and H agglutinin was/were equal or higher than 2 times their cut-off value (≥1 : 160).

2. If, within an interval of 5-7 days, there was a 4-fold increment of the agglutinin titer.

Widal SAT-MJD test (1999) procedure

The Widal SAT-MJD antigen solution should be kept at room temperature for 20-30 minutes following refrigeration before performing the test. The test was performed on an object glass concave at the center. For optimal serum dilution in obtaining the antibody titer in the test sera, a ratio of serum and phosphate buffer saline solution (PBS) was made available by the manufacturer of the test kit, as can be seen in Table 1.

Table 1
Ratio of serum and phosphate buffer saline (PBS) for optimal serum dilution to obtain the antibody titer for the Widal SAT-MJD.

Titer	Ratio of		
	Serum (μl)	Phosphate buffer saline (μl)	Antigen (μl)
1:20	10	30	40
1:40	7	34	40
1:80	5	35	40
1:160	4	36	40
1:320	3	38	40

Serum dilution for O and H agglutinins began with a titer of 1 : 40, for PB agglutinin 1 : 80, and PA agglutinin 1 : 20.

The diluted serum and antigen suspension were mixed using an applicator and the object glass was afterwards rotated gently for 5 minutes at room temperature. The result of the test (agglutination) was read using the naked eye above a 10 Watt neon light or with the aid of sunrays near a window. Together with examination of patient sera, control sera were also diluted two-fold the cut-off value of the test. Control sera for Widal SAT-MJD were enclosed with the kit.

As stated in the leaflet, the cut-off values for adults were determined as follows: for O and H agglutinins corresponding with a titer of 1 : 80, for PA agglutinin a titer of 1 : 40, for PB agglutinin, a titer of 1 : 160.

If the Widal SAT-MJD showed a negative result, the test had to be terminated, and the result of the test was reported as negative.

Widal Murex test (1998) procedure

The Widal Murex antigen solution should be kept at room temperature after refrigeration. The test was performed on a white tile. Serum quantities of 80 μl, 40 μl, 20 μl, 10 μl and 5 μl were dropped onto the tile using a micro-pipette. Then, 1 drop (40 μl) of O, H, PA or PB antigen solution was added, respectively, using a pipette from the kit. The mixture was homogenized with an applicator and the tile was rotated manually. The results were read in one minute under sunlight with the naked eye. For all agglutinins, the cut-off value was 1 : 80. Control sera, positive and negative, must be run for each examination. For the Widal Murex, control sera were not included in the kit.

For examination of the Widal SAT-MJD and Murex, 3 laboratory technicians were assigned to read each slide. The reported end-results were those approved by at least 2 of the 3 readers. The examination of positive control sera should give a positive result while negative control sera should give a negative result. If there is a discrepancy in the result of control sera, these series of examination should be repeated.

The diagnostic values of Widal SAT-MJD and Murex were assessed based on the determi-

Table 2
Results of Widal Murex and Widal SAT-MJD in 55 patients with typhoid fever and 56 patients with non-typhoid fever.

Type of disease	NA	Widal Murex				Widal SAT-MJD			
		Positive		Negative		Positive		Negative	
		NB	%	NC	%	NY	%	NZ	%
Typhoid fever	55	46	83.63	9	16.37	46	83.63	9	16.37
Non-typhoid fever	56	20	35.72	36	64.28	10	17.86	46	82.14

NA : number of patients; NB : number of patients positive by Widal Murex; NC : number of patients negative by Widal Murex; NY : number of patients positive by Widal SAT-MJD; NZ : number of patients negative by Widal SAT-MJD.

nation of the diagnostic sensitivity, diagnostic specificity, diagnostic efficiency, diagnostic positive predictive value and negative predictive value. If there were differences in the diagnostic values of sensitivity, specificity, and efficiency, between the Widal SAT-MJD and the Murex, McNemar's test was used to recognize the significance ($p < 0.05$) (Siegel, 1988).

RESULTS

The results of the study are summarized in Table 2. Of 55 patients with typhoid fever admitted to this study, 46 showed a positive result by Widal SAT-MJD, and 46 were positive by Widal Murex. Based on that result, the diagnostic sensitivity of both Widal tests was 83.63%.

Forty-four samples were positive by both Widal SAT-MJD and Murex, 2 samples were positive by Widal SAT-MJD but negative by Widal Murex, and 2 other samples were positive by Widal Murex but negative by Widal SAT-MJD.

Nine samples of typhoid fever patients (16.37%) showed a negative result by Widal SAT-MJD and Murex. From these 9 samples, 4 samples did not show any agglutination to O and H antigen of *S. typhi*, and the remaining 5 samples showed positive agglutination only to H antigen of *S. typhi*.

Examination of 56 samples of non-typhoid patients showed that 46 gave a negative result by Widal SAT-MJD, and 36 samples were negative by Widal Murex. Based on these results, the diagnostic specificity of Widal SAT-MJD was 82.14%, which was higher ($p<0.02$) than the diagnostic specificity of Widal Murex, at 64.28%.

Table 3
Results of Widal SAT-MJD and Murex in 55 samples of typhoid fever and 56 samples of non-typhoid fever.

	SAT-MJD		Murex	
	NPA (+)	NPB (+)	NPA (+)	NPB (+)
Typhoid fever	17	0	19	16
Non-typhoid fever	3	0	8	16

NPA = Number of patients positive for paratyphoid A antigen.
NPB = Number of patients positive for paratyphoid B antigen.

Sixteen samples of typhoid fever and non-typhoid fever, respectively, showed false-positive results to PB in the Widal Murex. Moreover, 9 samples of non-typhoid patient showed false-positive results to 2-4 agglutinins (positive agglutination to O and H antigen of *S. typhi* and also positive to PA and/or PB antigen).

The Widal Murex also showed higher false-positive results to PA, as shown in Table 3. The diagnostic efficiency for Widal SAT-MJD found in this study was 82.88% compared with 73.87% for Widal Murex.

The diagnostic positive and negative predictive values of Widal SAT-MJD were 80.70% and 83.63%, respectively, while the Widal Murex val-

ues were lower, at 69.69 and 80%, respectively.

DISCUSSION

The diagnostic sensitivity of Widal SAT-MJD and Murex, as revealed in this study, were equal, at 83.63%. This result is classified as high (80%-90%) according to the criteria of Handojo (1988). Of the 9 typhoid samples that showed false negative results to Widal SAT-MJD and Murex, 4 samples showed no agglutination to O and H antigen. It is assumed that these 4 samples were drawn in the first week of fever, so that the antibody was not yet formed, or these patients had already been given antibiotics prior to the Widal test (Tandra and Soewandojo, 1986). The remaining 5 samples showed positive results only to H antigen and therefore could not be used to diagnose typhoid fever.

Increment of H antigen, not accompanied by an increment in O agglutinin, can occur in several conditions (Handojo, 2001). Firstly, the patients have been exposed to a low dose ($<10^5$) of infection with *S. typhi* thereby resulting in the generation of memory cells to H antigen (T-cell dependent). If these patients fall ill due to *S. typhi*, the immune response is inherent to the pattern of a secondary immune response. In secondary infection with *S. typhi*, the production of H agglutinin and O agglutinin takes place at approximately the same, or even higher, rate due to the presence of memory cells to the H antigen in these patients. Secondly, the patients have just recovered (6-24 months) from typhoid fever and are now reinfected with *S. typhi*. The H agglutinin can be maintained for a longer period (2 years) compared with the O agglutinin, which is about 5 months. Thirdly, the patients have been vaccinated with anti-typhoid vaccine, but are now suffering from typhoid fever. For these 9 samples, it is suggested that the examination be repeated in one week. If there is an increment of O and H agglutinin 4-fold or more from the initial titer, the diagnosis of typhoid fever can be established.

Nineteen typhoid samples showed false-positive results to PA antigen with Widal Murex and 17 with Widal SAT-MJD. We compared these results with 10 samples proven to be paratyphoid A fever. Of these, 5 samples were false-positive for typhoid fever. We assumed that there was a cross reactivity among the *Enterobacteriaceae* examined in this Widal test (Rockhill *et al*, 1981; Pang and Puthucheary, 1989). From the clinical view point this raised no difficulties in treating the patient, as typhoid fever and paratyphoid fever can be treated with the same medicines.

The diagnostic specificity of Widal SAT-MJD revealed in this study was 82.14%, compared with 64.28% by Widal Murex. The difference was statistically significant ($p<0.02$). The low diagnostic specificity of Widal Murex in this study was caused by the high false-positive samples to PB antigen. There were 16 non-typhoid samples false-positive to Widal Murex, but none of these samples showed a false-positive result with Widal SAT-MJD.

The examination by Widal SAT-MJD in non-typhoid samples showed that 10 samples gave a false-positive result. False-positive results to typhoid fever occurred in one malaria patient, 3 DHF patients, and 3 UTI patients. Two patients with UTI were false-positive for paratyphoid A, and 1 patient with UTI was false-positive to both typhoid fever and paratyphoid A. Using Widal Murex, we found difficulties in classifying the non-typhoid patients with false-positive results because, out of 16 samples that were false-positive, 9 samples were false-positive to 2-4 agglutinins.

Several factors can increase the O and H agglutinin of *S. typhi* in non-typhoid patients. Firstly, as has already been mentioned above, the possibility of cross-reactivity among the *Enterobacteriaceae*. Secondly, activation of polyclonal B-cells in viral infections, such as DHF. Infection with a subclinical dose of *S. typhi* can stimulate B lymphocyte or plasma cells, activated by the dengue virus, to provide O agglutinin or O and H agglutinins above the cut-off value. Thirdly, anamnestic reaction stimulated by non-typhoid fever, resulting in an increment of O and H agglutinin of *S. typhi*, which occurs in patients already infected by other salmonellae or who have been injected by anti-typhoid vaccine (Pang and Puthucheary, 1989). The diagnostic efficiency of Widal SAT-MJD became higher than the diagnostic efficiency of Widal Murex because it was influenced by its diagnostic specificity. The dif-

ference in these two diagnostic efficiencies were proven to be statistically significant ($p<0.05$).

From the practical view point, the Widal SAT-MJD has some advantages. The control sera included in the Widal SAT-MJD kit are of great benefit, because when purchased separately, the costs become high. For examination of all agglutinins, 120 μl of serum are required. According to the leaflet, Widal SAT-MJD can also be performed using a Pasteur pipette. The ratio of serum and antigen is stated on the leaflet. For this purpose, a very simple pipette is enclosed, so it is easy to perform in public health centers. The incubation period for this test is very short, *ie* not longer than 5 minutes, showing no false-positive agglutination after 5 minutes. The low cost of the Widal SAT-MJD is an added advantage.

For comparison with the Widal SAT-MJD, the Widal Murex does not include the control sera; 620 ml of serum is required to examine all agglutinins, and should be drawn by micro-pipette. The results should be read in 1 minute and many false-positive agglutinations are revealed after 1 minute. The cost of Widal Murex is 2 to 3 times that of Widal SAT- MJD.

Analysis of data obtained in this study indicate that the Widal slide agglutination test produced by Mekar Jaya Diagnostika Research Laboratory is an eligible and low-cost screening test for typhoid fever, and can be performed at public health centers in rural areas.

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