DOT-EIA TYPHOID TEST USING OMP *Salmonella typhi* LOCAL PHAGE TYPE ANTIGEN TO SUPPORT THE DIAGNOSIS OF TYPHOID FEVER

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ABSTRACT

A laboratory study was carried out on 44 sera of typhoid patients (age: 15-45 years, 24 females and 20 males) with positive blood culture for S. typhi, 41 sera non typhoid febrile patients (age: 16-51 years, 17 females & 24 males) with negative blood culture and negative Widal test, 136 sera of healthy persons as normal controls (age: 20-49 years, 95 females & 41 males). The basic principle of the Dot-EIA typhoid test was an indirect Elisa method using nitrocellulose as a solid phase and OMP S. typhi from five locally prevalent phage types as the antigens. Goat anti-human IgM alkaline phosphatase for the Dot-EIA typhoid IgM test and goat anti-human IgG alkaline phosphatase for the Dot-EIA typhoid IgG test were used as conjugates. BCIP/NBT was used as a chromogenic substrate and the results were visible as gray-blue spots on the surface of the nitrocellulose. The Dot-EIA typhoid test could produce results within 3 hours. The result of the Dot-EIA typhoid test showed a good diagnostic value for typhoid fever, a diagnostic sensitivity of 66 % for IgM and 86.4% for IgG and when both were determined (IgM and IgG) the diagnostic sensitivity was 97.7%. The diagnostic specificity was 100% for IgM and 90.24% for IgG and if both were determined (IgM and IgG) the diagnostic specificity was 90.24%. The diagnostic efficiency was 82.35% for IgM and 88.24% for IgG and when both were determined (IgM and IgG) the diagnostic efficiency was 94.12%. The diagnostic positive predictive value was 100% for IgM and 90.48% for IgG and when both were determined (IgM and IgG) the diagnostic positive predictive value was 97.7%. The diagnostic efficiency was 82.35% for IgM and 90.24% for IgG and when both were determined (IgM and IgG) the diagnostic efficiency was 90.24%. The diagnostic positive predictive value was 100% for IgM and 90.48% for IgG and when both were determined (IgM and IgG) the diagnostic positive predictive value was 97.7%. From a practical point of view under field conditions, the performance of the Dot-EIA typhoid IgM and IgG test could be considered as moderately practicable and not very expensive (Rp 5.025,- per test). Thus this test could be performed in laboratories up to small hospitals (C type hospitals). Based on these results, it can be concluded that the Dot-EIA typhoid test has a good clinical validity, a moderate practicability and is not very expensive.

***Keywords**: Dot-EIA typhoid test, OMP S. typhi antigen, typhoid fever*

INTRODUCTION

Typhoid fever remains a health problem in developing countries. It is estimated that 7 million cases are found annually in East and South Asia, where the incidence is approximately 540 per 100,000 populations (Chaicumpa, 1988). Salmonella infection disperses worldwide, from cities to villages, particularly in countries where the people live with a low level of hygiene and less accessibility of clean water supply. The infection may also be transmitted by tourists visiting those countries (Losonsky, 1997; Sippel, 1987).

Salmonella infection may occur in all ages, with the highest incidence found particularly in children. Symptoms that may be observed in this age group are commonly varied, and are is different from those found among adults. Among these patients, the symptoms are similar to those of gastroenteritis, malaria, lobular pneumonia, or acute meningitis (Sippel, 1987). *S. typhi* and *S. paratyphi* may cause intestinal infection. Both penetrate epithelial cells of intestinal mucosa, entering the lamina propria and eventually develop in Peyer's patch. Through lymph nodes and thoracic ducts they enter the circulation, leading to the occurrence of bacteremia and multiply systemically. Systemic infection commences by insertion of Salmonella, which is subsequently being captured by gut-associated lymphoid tissues. Here, Salmonella resides and grows in the macrophage and eventually disperses to the whole body through lymph nodes and the circulation, and finally grow in reticuloendothelial system (RES) (Youmans, 1986).

After an incubation period of 8 - 14 days, discomfort may occur in the abdomen, fever and headache. The secondary bacteriemia will occur after the symptoms last for several days, which are aggravated by fever and anorexia. These symptoms indicate acute typhoid fever, in which the specific antibody IgM will be induced and last for several weeks, after which it is replaced by IgG. During this period, sepsis and septic shock may be fatal if left untreated (found in roughly 15%) and in 10% recurrence may appear if accurate treatment is not delivered, while 1 - 4% of the patients may become carriers (depending on age and sex) (Losonsky, 1997).

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Up to date, the diagnostic tools to detect *S. typhi* effectively (sensitive and specific) as well as efficiently (practical and cost-saving) are still unavailable. Gall test is highly specific, but is less sensitive as the results of examination are mostly false negative. Additionally, it takes one week to obtain the results, while the patients should be promptly treated with specific antibiotics. Widal test is also less sensitive and specific, as it may provide false positive results in non typhoid patients infected with other Gram negative rods, and it may also be affected by the patients' immunological condition, so that even an insignificant development of antibody may already produce false negative results (Baron,1990; Prihatini,1982).

The objective of this study was to determine the diagnostic value, practicability, and costs needed for Dot-EIA typhoid *S. typhi* antigen test as a diagnostic tool for typhoid fever.

MATERIALS AND METHODS

Procedure of Dot-EIA Typhoid IgM
(Handojo, 1996; Purwaningsih, 1997)

1. 2 ml OMP *S. typhi* antigen, diluted with PBS with pH of 7.4 (the amount of dilution was obtained from evaluating chessboard titration results), was dropped onto nitrocellulose paper 7 x 10 mm and were incubated overnight at 4^{o}C.
2. Nitrocellulose paper was blocked using blocking solution for 1 hour in room temperature on the shaker.
3. Nitrocellulose paper was washed for 3 x 5 minutes with PBST.
4. The sera from patients with typhoid fever, non-typhoid fever, and normal controls were diluted with sample diluent (1 : 20) and incubated for 30 minutes, and re-diluted with blocking solution until reaching twice the upper threshold of the normal titer. 300 ml-diluted sera was dropped onto nitrocellulose paper, and incubated for 1 hour at room temperature on the shaker.
5. The paper was washed again 3 x 5 minutes with PBST.
6. 300 ml conjugate (alkaline phosphatase-labeled goat anti-human IgM) was added. The conjugate had been diluted with blocking solution (obtained from evaluating chessboard titration results), and incubated for 1 hour at room temperature on the shaker.
7. Nitrocellulose paper was washed 3 x 5 minutes with PBS pH of 7.4.
8. 300 ml substrate solution of BCIP/NBT was added, and incubated for 5 minutes in a dark place.
9. 300 ml-distilled water was added to stop the reaction, and the color was read using naked eye.
10. Results were regarded as positive when the formed color intensity was bluish violet ($\geq$ 2X cut off value), and was regarded as negative if no color was formed (as can be seen in Figure 1).

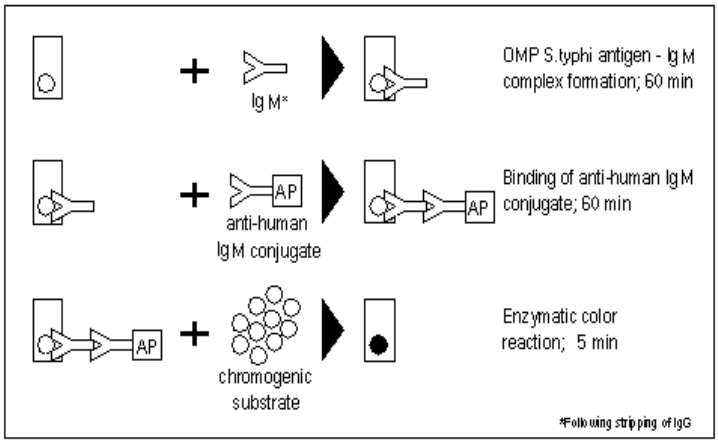


Figure 1. Reaction principles of Dot-EIA Typhoid IgM Test

Procedure of Dot-EIA Typhoid IgG
(Handojo, 1996; Purwaningsih, 1997)

1. 2 ml OMP *S. typhi* antigen, diluted with PBS with pH of 7.4 (the amount of dilution was obtained from evaluating chessboard titration results), was dropped onto nitrocellulose paper 7 x 10 mm and incubated overnight at 4°C.
2. Nitrocellulose paper was blocked using blocking solution for 1 hour in room temperature on the shaker.
3. Nitrocellulose paper was washed for 3 x 5 minutes with PBST.
4. The sera from patients with typhoid fever, non-typhoid fever, and normal controls were diluted with sample diluent (1 : 20) and incubated for 30 minutes, and re-diluted with blocking solution until reaching twice the upper threshold of the normal titer. 300 ml diluted serum was dropped onto nitrocellulose paper, and incubated for 1 hour at room temperature on the shaker.
5. The paper was washed again 3 x 5 minutes with PBST.
6. 300 ml conjugate (alkaline phosphatase-labeled goat anti-human IgM) was added. The conjugate had been diluted with blocking solution (obtained by evaluating chessboard titration results), and incubated for 1 hour at room temperature on the shaker.
7. Nitrocellulose paper was washed 3 x 5 minutes PBS pH of 7.4.
8. 300 ml substrate solution of BCIP/NBT was added, and incubated for 5 minutes in a dark place.
9. 300 ml distilled water was added to stop the reaction, and the read using naked eye.
10. Results were regarded as positive when the formed color intensity was bluish violet ($\geq$ 2X cut off value), and was regarded as negative if no color was formed (as can be seen in Figure 2).

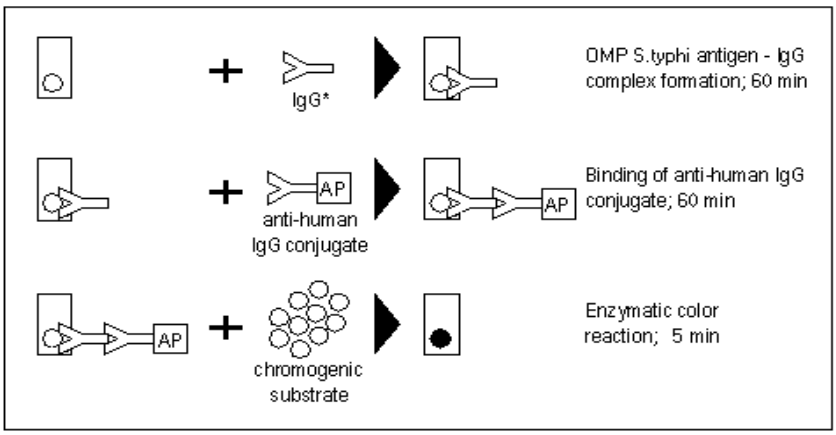


Figure 2. Reaction principles of Dot-EIA Typhoid IgG Test

Determining The Degree of Reliability
(Sensitivity and Specificity)

Using the criteria of Handojo I (1988) as follows:
Very high : if the value of a laboratory test characteristic was ≥ 95%
High : if the value of a laboratory test characteristic ranging between 80 - 94.9%
Moderate : if the value of a laboratory test characteristic ranging between 65 - 79%
Low : if the value of a laboratory test characteristic ranging between 50 - 64%
Very low : if the value of a laboratory test characteristic was < 50%

RESULTS

Table 1 Chessboard Titration in Dot-EIA Typhoid IgM Tests (Sera dilution 1 : 1,200)

Ag	++	+	-	PBS	++	+	-	PBS	++	+	-	PBS
10 µg	++	++	+	-	++	++	+	-	++	++	+	-
	++	++	+	-	++	++	+	-	++	++	+	-
7.5 µg	++	++	+	-	++	+	+	-	++	+	+	-
	++	++	+	-	++	+	+	-	++	+	+	-
5.0 µg	++	++	+	-	++	++	+	-	+	+	-	-
	++	++	+	-	++	++	+	-	+	+	-	-
2.5 µg	++	++	+	-	++	+	-	-	+	+	-	-
	++	++	+	-	++	+	-	-	+	+	-	-
1.0 µg	++	+	+	-	+	-	-	-	+	-	-	-
	++	+	+	-	+	-	-	-	+	-	-	-
Conjugate		1 : 15,000				1 : 20,000				1 : 25,000		

Results of optimization, as seen in Table 1, the optimal dilution of local phage type OMP *S. typhi* antigen was 2.5 µg and the optimal dilution of conjugate goat anti-human IgM alkaline phosphatase for Dot-EIA typhoid IgM test was 1 : 20,000.

Table 2 Chessboard Titration in Dot-EIA Typhoid IgG Tests (Sera dilution 1 : 1,500)

Ag	++	+	-	PBS	++	+	-	PBS	++	+	-	PBS
10 µg	++	++	+	-	++	++	+	-	++	++	+	-
	++	++	+	-	++	++	+	-	++	++	+	-
7.5 µg	++	++	+	-	++	++	+	-	++	++	+	-
	++	++	+	-	++	++	+	-	++	++	+	-
5.0 µg	++	+	+	-	++	+	+	-	++	+	+	-
	++	+	+	-	++	+	+	-	++	+	+	-
2.5 µg	++	+	+	-	++	+	+	-	++	+	+	-
	++	+	+	-	++	+	+	-	++	+	+	-
1.0 µg	++	+	+	-	++	+	-	-	+	+	-	-
	++	+	+	-	++	+	-	-	+	+	-	-
Conjugate		1 : 15,000				1 : 20,000				1 : 25,000		

From the results of optimization as seen in Table 2, the optimal dilution of local phage OMP *S. typhi* antigen was 1 µg and the optimal dilution of conjugate goat anti-human IgG alkaline phosphatase for Dot-EIA typhoid IgG test was 1 : 20,000.

Table 3 Determination Cut off Value of the Titer in Dot-EIA Typhoid IgM test

Dot-EIA Typhoid IgM Test				
Serum Titer	1 : 600	1 : 1,200	1 : 1,800	1 : 2,400
Σ individuals	85	46	15	2
%	62.50	33.82	11.03	1.47

Based on the percentile estimation as suggested by de Savigny (1980) to a number of 136 normal control sera, it could be concluded that the upper cut off value of the titer for IgM was 1: 1,800, which was the highest IgM titer (highest sera dilution) in 98.53% of the sera from healthy individuals. Based on these facts, would use sera dilution twice than the upper cut off value of the titer, i.e., 1: 3,600. Therefore, the titer of 1: 3,600 indicated a pathologic increase of specific IgM in a local phage type of OMP *S. typhi* antigen.

Table 4 Determination Cut Off Value of the Titer in Dot-EIA Typhoid IgG Test

Dot-EIA Typhoid IgG Test				
Serum Titer	1 : 750	1 : 1,500	1 : 2,250	1 : 3,000
Σ individuals	88	57	35	2
%	64.7	41.9	25.7	1.47

Based on the percentile estimation as suggested by de Savigny (1980) to a number of 136 normal control sera, it could be concluded that the upper cut off value of the titer for IgG was 1 : 1,2250, which was the highest IgG titer (highest sera dilution) from specific IgG found in healthy individuals. Based on these facts, would use serum dilution twice than the upper cut off value of the titer, i.e., 1 : 4,500. Therefore, the titer of 1 : 4,500 indicated a pathologic increase of specific IgG value towards a local phage type of OMP *S. typhi* antigen.

Table 5 Sensitivity of Dot-EIA Typhoid IgM and IgG Tests

Patients Group	Dot-EIA Typhoid IgM Test					Dot-EIA Typhoid IgG Test				
	Positive		Negative		Σ	Positive		Negative		Σ
	N	%	N	%	N	N	%	N	%	N
Typhoid Fever	29	66	15	34	44	38	86.4	6	13.6	44

As seen in Table 5, the diagnostic sensitivity of Dot-EIA typhoid IgM test was 29/44 X 100% = 66%, and for IgG was 38/44 x 100% = 86.4%. According to the criteria of Handojo was moderate, and that of IgG was high. The difference was statistically (McNemar) not significant ($p > 0.05$).

Table 6 Sensitivity of Combined Dot-EIA Typhoid IgM and IgG Tests

Group of patients	Interpretation of Combined Results of Dot-EIA Typhoid IgM and IgG Tests									
	True Positive		True Negative		Σ	False Positive		False Negative		Σ
	N	%	N	%	N	N	%	N	%	N
Typhoid Fever	43	97.7	0	0	43	0	0	1	2.3	1

As seen in Table 6, if the results of Dot-EIA typhoid IgM and IgG tests were combined, the diagnostic sensitivity was as much as 97.7%. was categorized as high.

Table 7 Specificity of Dot-EIA Typhoid IgM and IgG Tests

Group of Patients	Diagnosis	Dot-EIA Typhoid IgM Test					Dot-EIA Typhoid IgG Test				
		Positive		Negative		Σ	Positive		Negative		Σ
		N	%	N	%		N	%	N	%	
Nontyphoid fever	*ParatyphiA*	0	0	4	100	4	0	0	4	100	4
	UTI	0	0	7	100	7	4	57.1	3	42.9	7
	DHF	0	0	8	100	8	0	0	8	100	8
	Malaria	0	0	22	100	22	0	0	22	100	22
Total				41	100	41	4	9.8	37	90.2	41

As seen in Table 7, the diagnostic specificity of Dot-EIA typhoid IgM test was 41/41X 100% = 100%. The diagnostic specificity of Dot-EIA typhoid IgG test was 37/41 X 100% = 90.24%, which was high. False positive results were found in 4 patients with urinary tract infection. The difference of specificity between both tests was statistically (McNemar) not significant ($p > 0.05$).

Table 8 Specificity of the Combined Results of Dot-EIA IgM and IgG Tests

Group of Patients	Interpretation of the Combined Results of Dot-EIA IgM and IgG Tests					
	True Negative		Σ	False Positive		Σ
Nontyphoid fever	N	%		N	%	
	37	90.24	41	4	9.76	41

From 41 patients with non-typhoid fever, a number of 37 patients showed negative combined results of Dot-EIA typhoid IgM and IgG tests. Therefore, the diagnostic specificity of combined Dot-EIA typhoid IgM and IgG tests was 90.24%, which was high.

Table 9. Efficiency of Dot-EIA Typhoid IgM and IgG Tests

Group of patients	Dot-EIA Typhoid IgM Test					Dot-EIA Typhoid IgG Test				
	Positive		Negative		Σ	Positive		Negative		Σ
	N	%	N	%		N	%	N	%	
Typhoid fever	29	66	15	34	44	38	86.4	6	13.6	44
Nontyphoid fever	0	0	41	100	41	4	9.8	37	90.2	41
Total	29	34.1	56	65.9	85	42	49.4	43	50.6	85

Table 9, shows that the diagnostic efficiency of IgM was 29+41/ 85 X 100% = 82.35%, which was high, while the diagnostic efficiency of IgG was 38+37/85 X 100% = 88.24%, as high. The difference of diagnostic efficiency of both test was statistically (McNemar) significantly different ($p < 0.05$). As seen in Table 9, the diagnostic positive predictive value of Dot-EIA typhoid IgM test was 29/29 X 100% = 100%, which was very high, while the diagnostic positive predictive value of Dot-EIA typhoid IgG test was 38/42 X 100% = 90.48%, as high.

Table 10. Predictive value of Dot-EIA Typhoid IgM and IgG Tests

Group of patients	Interpretation of Dot-EIA Typhoid IgM and IgG Tests											
	True Pos		Σ	True Neg		Σ	False Neg		Σ	False Pos		Σ
Typhoid fever	N	%		N	%		N	%		N	%	
	43	97.7	44	37	90.2	41	1	2.3	44	4	9.8	41

As seen in Table 10, combined diagnostic positive predictive value of Dot-EIA typhoid IgM and IgG Tests were 43 /47 X 100% = 91.49 %, which was high. As seen in Table 10, the diagnostic efficiency of combined Dot-EIA Typhoid IgM and IgG test was 43+37/85 X 100% = 94.12%, as high. As seen in Table 9, the diagnostic negative predictive value of Dot -EIA typhoid IgM test was 41 /56 X 100% = 73.21%, as moderate, while the negative predictive value of IgG was 37/43 X 100% = 86.05%. Table 10, shows that the diagnostic negative predictive value of combined Dot-EIA Typhoid IgM and IgG Test was 37/38 X 100% = 97.37%, high according to Handojo.

Table 11. Reproducibility in One Series of Dot-EIA Typhoid IgM and IgG Tests

Examination series	Pooled sera IgM						Pooled sera IgG					
	A	B	C	D	E	F	A	B	C	D	E	F
1	++	++	+	+	0	0	++	++	+	+	0	0
2	++	++	+	+	0	0	++	++	+	+	0	0
3	++	++	+	+	0	0	++	++	+	+	0	0
4	++	++	+	+	0	0	++	++	+	+	0	0
5	++	++	+	+	0	0	++	++	+	+	0	0

The above table shows that the results of reproducibility test in one series of examination (within run) in Dot-EIA Typhoid IgM and IgG tests were 100%. This indicated that the test that still produced positive results although each test was repeated 5 times in one series of examination. Similar outcome was also found in tests with less positive and negative results. Thus, results of reproducibility in one series of examination were 100%, or coefficient variation of 0%.

Table 12. Reproducibility Between Days of Dot-EIA Typhoid IgM and IgG Tests.

Examination day	Pooled sera IgM						Pooled sera IgG					
	A	B	C	D	E	F	A	B	C	D	E	F
1	++	++	+	+	0	0	++	++	+	+	0	0
2	++	++	+	+	0	0	++	++	+	+	0	0
3	++	++	+	+	0	0	++	++	+	+	0	+
4	++	++	+	+	0	0	++	++	+	+	0	0
5	++	++	+	+	0	0	++	++	+	+	0	0

Table 12, demonstrates that results of reproducibility between days of examination of Dot-EIA Typhoid IgM and IgG tests reveal remarkable results, IgM was 100% (coefficient 0%) and IgG was 97% (coefficient variation 3%).

DISCUSSION

The Dot-EIA typhoid tests, both IgM and IgG, had been subjected to optimization by means of chessboard titration in order to obtain an optimal test condition. Antigen concentration, optimal conjugate and serum dilution had been undertaken earlier to provide a clear and distinct difference between the sera of typhoid fever patients with strong positive Widal test (titre 1/320 or 1/640) and those of healthy individuals (negative Widal test) and blank (PBS). After chessboard titration had been undertaken, one more standardization procedure was needed, i.e., the determination of cut off value in minimally 120 healthy individuals (de Savigny, 1980).

In this study, cut off value was determined in 136 healthy individuals with negative Widal test for Dot-EIA typhoid IgM and 136 other healthy individuals for Dot-EIA typhoid IgG test. Since patients involved in this study were from a typhoid fever endemic area, they often suffered from sub clinical infection with *S. typhi.* To prevent competition with anti-typhoid IgG, sera used in Dot-EIA typhoid IgM test was treated with a sample diluent of Zeus Scientific product that contained anti-human IgG (chain specific) which bound to IgG and formed a precipitate. This procedure was undertaken to prevent false low or false negative results of Dot-EIA typhoid IgM test. To ascertain the accuracy of the results, quality control in each tests always involved positive and negative controls in each examination series. If there was any deviation found in the results of control tests, the examination series must be repeated.

Procedure for producing and purifying OMP antigen used for Dot-EIA typhoid test in this study had been adapted to the method employed in the Institute of Postgraduate Studies and Research, University of Malaya, although not totally similar.

Internal Validity of Dot-EIA Typhoid IgM and IgG Tests

A. Detectability of Dot-EIA typhoid test is satisfactory. Dot-EIA typhoid IgM test is able to detect the presence of IgM anti-OMP *S. typhi* up to 1,600 x serum dilution, while Dot-EIA typhoid IgG test is able to detect the presence of IgG anti-OMP *S. typhi* up to 2,250 x serum dilution in healthy individuals.
B. Precision in within run of examination, either for Dot-EIA typhoid IgM or IgG tests, was highly reliable (100%), and so was the reproducibility between days of examination (100%). However, for IgM there was one among 30 test (3.3%) which produced a deviated result. Thus, the reproducibility between days of examination for Dot-EIA IgM test was 96.7% (coefficient variance 3.3%), which was highly reliable.
C. The accuracy of Dot-EIA typhoid tests, both IgM and IgG, as other semi quantitative serological tests, was assured by the presence of either positive or negative controls in each series of examination. If there was a deviation in positive and negative control, the series of examination should be repeated.

External (Clinical) Validity/Reliability/Test Diagnostic Value

A. Dot-EIA Typhoid IgM Test

As revealed in the result of this study, the diagnostic sensitivity of the test was only 66%, approaching the lower limit of diagnostic sensitivity moderate, although it has a very high diagnostic specificity (100%). This was because the patients involved in this study were those who lived in an endemic area and in the first or second week of the course of disease. Therefore, the IgM antibody remained less abundant, so that a proportion of 34% of the patients showed false negative results of Dot-EIA typhoid IgM test.

Since the test specificity was 100%, even though the sensitivity was only 66%, all sera that produced positive results were obtained from patients suspected as having typhoid fever, so that the diagnostic positive predictive value was very high (100%) and the diagnostic efficiency value was also high (82.35%), while the diagnostic negative predictive value was moderate.

The above data lead to the conclusion that the results of positive Dot-EIA typhoid IgM test was reliable in establishing the diagnosis of typhoid fever in group of patients suspected with typhoid fever as an indicator of typhoid fever.

B. Dot-EIA Typhoid IgG Test

As seen in the results of this study, the diagnostic sensitivity of Dot-EIA typhoid IgG test was 86.4%, which was regarded as high. Compared to the diagnostic sensitivity of Dot-EIA typhoid IgM test (66%), that of Dot-EIA typhoid IgG test was higher, although statistically not significant ($p > 0.05$).

The difference resulted from the fact that patients involved in this study lived in endemic area, so that most of them had likely ever experienced sub clinical infection with *S. typhi*, so they already had memory cells. At the time they suffered from typhoid fever, induced immune response was secondary one, so that the increase of IgG production (presence of memory

cells) became accelerated in a higher level compared to that of IgM. Consequently, IgG level increased beyond the upper threshold of referred titer in a relatively shorter time than that of IgM, with the result that more typhoid fever patients showed positive results of Dot-EIA typhoid IgG test, thereby the diagnostic sensitivity became higher as well.

The diagnostic specificity of Dot-EIA typhoid IgG test, as found in this study, was 90.24%, was high. This was because samples of non-typhoid patients met the inclusion and exclusion criteria (negative Gall and Widal tests). If the Widal test was not performed, out of 51 non-typhoid patients, 13 patients showed false positive results, so that the diagnostic specificity was only 74.5%.

The proportion of false positive result was 25.5%, because local phage type OMP *S. typhi* antigen used in this study still contained a large number of bands. According to Ismail (1991), bands located at 92 kDa and 62 kDa could induce a cross-reaction with DHF virus, plasmodium malaria, and other Gram-negative bacteria (*E. coli, S. albus, P. aerogenes*, etc.), except 50 kDa band, which was specific only for OMP *S. typhi* antigen.

For Indonesians, the band should be determined first by means of Western blot test using sera of patients with typhoid fever in Indonesia. The result is likely not different from that in Malaysia. Thus, if the local phage type OMP *S. typhi* antigen used contain only protein with certain kDa that was in agreement with the results of Western blot test, for example, 50 kDa, false positive results obtained in non typhoid fever patients will be less than 25.5% and its specificity will be higher although Widal screening test is not performed as an inclusion criteria. Antigen purification in that kDa can be undertaken using electroelution.

Additionally, since 9 patients with non typhoid fever showed positive Widal test, it was possible that these patients had previous typhoid fever in less than 2 years or obtained previous anti-typhoid vaccination, with the result that IgG and *S. typhi* concentration remained above the threshold of referred titer. Due to its high sensitivity (86.4%) and specificity (90.2%), Dot-EIA typhoid IgG can be used as a reliable diagnostic tool for typhoid fever. The diagnostic efficiency rate of Dot-EIA typhoid IgM test was 82.35%, and that of Dot-EIA typhoid IgG test was 88.24% which was significantly different ($p < 0.05$).

The diagnostic positive predictive value of Dot-EIA typhoid IgG test was 90.48% and the diagnostic negative predictive value of Dot-EIA typhoid IgG test was 86.5%, both of which were categorized as high. From these data, it can be concluded that the result of Dot-EIA typhoid IgG test had a high reliability.

C. Combined Dot-EIA Typhoid IgM and IgG Tests

As has been revealed in this study, the diagnostic sensitivity of combined Dot-EIA IgM and IgG tests (one or both was positive) was 97.72. This indicated that combined Dot-EIA Typhoid IgM and IgG tests were a reliable typhoid fever screening test, although the cost of examination became more expensive.

The high sensitivity of the combination was likely due to local phage type of the antigens used in those tests, so that they were homologue to anti-typhoid antibody in the sera of the patients. Antibody binding to homologue antigen is far stronger than that to heterologue antigen, which is easily detached during the washing phase. The diagnostic specificity of combined Dot-EIA typhoid IgM and IgG test was 90.24%, so that if the result of combined Dot-EIA typhoid IgM and IgG test was negative for one or both, it is almost certain that the patients had no typhoid fever.

The combined diagnostic efficiency or truth of IgM and IgG in this study was 94.12%. The combined diagnostic positive predictive value of IgM and IgG was 91.49% while the combined diagnostic negative predictive value of IgM and IgG was 97.37%. According to Handojo's criteria (1988) those rates belonged to the category of high and very high, so that combined Dot-EIA typhoid IgM and IgG tests was a reliable diagnostic tool to detect the presence of typhoid fever.

CONCLUSION

From data analysis obtained in this study, it can be concluded that Dot-EIA typhoid IgM test is highly specific (100%) but less sensitive (66%), Dot-EIA typhoid IgG test was moderately sensitive (86.4%) and moderately specific (90.24%). The combined Dot-EIA typhoid IgM and IgG tests is a diagnostic tool for typhoid fever with moderate degree of practicability. The Dot-EIA typhoid test is a relatively inexpensive laboratory test.

RECOMMENDATION

To obtain a higher diagnostic specificity, in the antigen processing in Dot-EIA typhoid test, the following two steps should be taken: after SDS-PAGE has been carried out, Western blot test should be performed by using the sera of typhoid fever patients in Indonesia to find which

part or which kDa from the local phage type of OMP *S. typhi* is specific for those patients in Indonesia. Subsequently, antigen purification is done to obtain antigen with the expected molecular weight by electroelution. To shorten examination time, it is recommended to use immunochromatography.

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