

# DEVELOPMENT OF THE RAPID AND SIMPLIFIED ELISA (WHOLE BLOOD-ELISA) USING SAMPLES OF *SCHISTOSOMA JAPONICUM*-INFECTED HUMAN WHOLE BLOOD

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**Abstract.** An ELISA technique was developed using samples of *Schistosoma japonicum*-infected human whole blood based on the conventional ELISA. In this study, the following were demonstrated. 1) Whole blood samples could be used. 2) The volume of whole blood and conjugate could be reduced to 0.05 ml. 3) The incubation time was shortened to 5 minutes. 4) The optical density could be measured at 10 minutes after transferring the substrate and the volume was reduced to 0.1 ml. 5) It did not require a fixed temperature setting. 6) The operation time was as short as 20 to 30 minutes. 7) The optical density values were almost the same as the conventional ELISA and were not influenced by other common intestinal helminthic infections. 8) The observed variations from day to day including effects of sampling in stool examination were negated by the results of this ELISA technique. 9) Based on correlation with stool examination results, criteria can be formulated in which optical density values of 0.3 and above as positive, 0.1 to less than 0.3 as doubtful, and less than 0.1 as negative. Whenever an immunological field survey is necessary, before and after a selective or a mass treatment control program, this WHOLE BLOOD-ELISA, which was shown to be rapid and simple, is recommended.

## INTRODUCTION

As one of various diagnostic methods of schistosomiasis, a parasitic helminthic infection, enzyme-linked immunosorbent assay (ELISA) has been the most widely used *in vitro* (Engvall and Perlmann, 1971). Thereafter, the microplate-based ELISA (Voller *et al*, 1974) and k-ELISA, a kinetic-dependent modification (Tsang *et al*, 1980, 1982) were developed. The Falcon assay screening test system using microsomal adult worm antigen, FAST-ELISA, was introduced by Hancock and Tsang (1986). The dot-ELISA blotted onto nitrocellulose paper was subsequently developed (Boctor *et al*, 1987), followed by the RAST-ELISA (Weiss *et al*, 1978; Ismail *et al*, 1989); transferable solid phase (TSP)-ELISA based on FAST-ELISA (Moser *et al*, 1990), and blood spotted on filter paper, DIG-ELISA, (Kamal *et al*, 1994) were recently introduced.

However, there have been few reports of a rapid, simple, and cost-effective ELISA method for a satisfactory screening test in a mass treatment of schistosomiasis (Hamilton *et al*, 1998). Here, we report on ELISA method using whole blood samples from *Schistosoma japonicum*-infected humans, and compared this to the conventional ELISA method using ABTS as a substrate for horseradish peroxidase (HPR) conjugate as described by Matsuda *et al* (1984).

## MATERIALS AND METHODS

### Parasite antigen

*S. japonicum* (Yamanashi strain) was maintained in *Oncomelania nosophora* snails and mice (ICR strain). *S. japonicum* eggs were isolated from the intestines of infected mice by a digestion technique with pronase and collagenase (Matsuda *et al*, 1984), lyophilized and kept at -80°C until use.

### ELISA procedure

**Conventional ELISA procedure.** Conventional ELISA was conducted according to Matsuda

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*et al* (1984). The optimal condition for ELISA was determined by checker-board titration. The wells of micro-titer-plates were treated (sensitized) with 0.1 ml of crude egg antigen at a concentration of 10 µg protein/ml of carbonate buffer (0.05 M, pH 9.6) in a moist box at 37°C for 2 hours. The plates were washed with PBS-Tween (0.15 M phosphate buffered-saline containing 0.05% Tween 20), air-dried, wrapped with a plastic bag and kept at -80°C.

The wells were washed three times for 5 minutes each with PBS-Tween after each addition with antigen, antibody, and conjugate. The optimal dilutions for sera and conjugates were determined by a checker-board titration. A test sample (serum) and a conjugate were diluted with BSA-Tween, containing 1% bovine serum albumin (Fraction V, Sigma Chemical, USA). Test samples diluted to the optimal concentration were placed in sensitized wells and incubated at 37°C for 45 minutes and washed. Then, 0.1 ml aliquot of anti-human IgG conjugated with horseradish peroxidase (ICN Pharmaceuticals, USA) diluted to the optimal concentration, was added and the plate incubated at 37°C for 1 hour. After washing, 0.2 ml aliquot of ABTS [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) di-ammonium salt; Sigma Chemical, USA] used as a substrate was added to each well and the plate was kept at room temperature for 10 to 60 minutes to allow enzyme reaction to occur. ABTS stock solution was prepared as follows: 30 mg of ABTS was dissolved in a mixture of 50 ml each of 0.1 M citric acid and 0.1 M sodium dihydrogen phosphate, with 0.01 ml of 30% H<sub>2</sub>O<sub>2</sub>. The optical density of the product was measured by a micro-plate-reader (Corona, Japan) at a wavelength of 415 nm.

**Simplified ELISA procedure.** A 0.05 ml aliquot of whole blood or serum was used. The volume of conjugate was 0.05 ml, and that of the substrate was 0.1 ml. Incubation time of blood sample and conjugate was 5 minutes, and time of the enzyme reaction was 10 minutes. After each addition of antibody and conjugate, wells were successively washed for three to five times with PBS-Tween. This rapid and simplified ELISA procedure was carried out at room temperature.

### Optimizing the simplified ELISA procedure

In the simplified ELISA method, human whole blood was diluted at 1:5, 1:10, 1:20, 1:40, 1:80, and 1:160, and 0.05 ml of each dilution was used as blood samples. The optimal concentration of anti-human IgG conjugated with a horseradish peroxidase (ICN Pharmaceuticals, USA) was determined by a checker-board titration in the dilutions of 1:1000, 1:3000, 1:5000, 1:10000 and 1:20000. Then 0.05 ml of the optimal conjugate dilution (1:1000) was used as described above.

*S. japonicum*-infected human sera from residents of endemic areas of the Philippines were mixed with an equal volume of normal human whole blood, and were used as the infected whole blood samples. Negative control human sera were collected from students of Dokkyo University School of Medicine who have no history of exposure to schistosomiasis. These were also mixed with equal volume of normal human whole blood and were used as normal whole blood reference.

### Collection of blood and stool samples

Human whole blood samples were collected from residents of Leyte, the Philippines, treated with heparin and used immediately or were stored in a refrigerator after adding 5% sodium azide. The human serum samples were used after being centrifuged at 1,710g for 5 minutes at room temperature and the remaining were kept at -80°C. Human sera used as positive and negative references were also stored at -80°C. Simultaneous with the blood collection, stool examinations were performed by Kato-Katz thick smear technique (Katz *et al*, 1972). After one month, feces were again collected from the residents who were stool negative for *S. japonicum* ova successively for 5 days and examined by both Kato-Katz thick smear and formalin-detergent techniques (Moody *et al*, 1986). The cumulative positivity for the whole duration of stool examinations was then graphed.

### Statistical method

For statistical study, analysis of variance was determined using Student's *t*-test. A maximum 2-tailed probability value of 5% ( $p < 0.05$ ) was considered statistically significant.

Table 1

Field screening test of optical density values<sup>a</sup> against *S. japonicum* given by human blood samples of the residents in Leyte, the Philippines in the rapid and simplified ELISA and the conventional ELISA.

No.	Sex	Age	Rapid and simplified ELISA		Conventional ELISA	Stool examination <sup>e</sup>	Treatment
			Whole blood <sup>b</sup>	Serum <sup>c</sup>	Serum <sup>d</sup>		
1	Male	14	0.931	0.931	1.001	S(2-2), A, T	2001
2	Female	18	0.825	0.875	1.006	A	None
3	Female	17	0.531	0.625	0.738	T, H	None
4	Female	32	0.232	0.331	0.421	S(1-1), A	Nov 7, 2000
5	Female	31	0.540	0.605	0.597	A, T	None
6	Female	35	0.732	0.833	1.015	S(0-1), A, T	Nov 7, 2000
7	Male	14	1.028	1.073	1.081	S(1-0), A, T	Nov 7, 2000
8	Male	10	0.926	ND <sup>f</sup>	ND	S(1-0), T	Nov 7, 2000
9	Male	44	0.224	0.326	0.380	Negative	Nov 7, 2000
10	Female	44	0.158	0.296	0.153	Negative	Nov 7, 2000
11	Female	49	0.805	0.974	0.907	S(0-1), H	Nov 7, 2000
12	Male	43	0.543	0.661	0.788	S(3-0), A, T	Nov 7, 2000
13	Female	47	0.387	0.639	0.681	A, H	Nov 7, 2000
14	Male	40	0.127	0.211	0.152	H	1997
15	Female	18	0.647	0.869	0.683	T	Nov 7, 2000
16	Female	39	0.237	0.301	0.402	A, T	Nov 7, 2000
17	Male	21	0.576	0.764	0.751	Negative	None
18	Male	5	NS <sup>g</sup>	0.748	0.797	S(2-0), A	None
19	Male	4	ND	0.938	0.942	S(2-0), A, T	None
20	Female	33	0.745	ND	ND	S(1-0), A, H	April, 2000
21	Male	36	0.355	0.511	0.713	A, H	April, 2000
22	Male	12	0.489	0.629	0.941	S(2-2)	April, 2000
23	Female	17	0.504	0.706	0.790	S(1-1), A	April, 2000
24	Female	11	0.305	0.412	0.568	S(1-0)	None
25	Female	13	0.469	0.742	0.859	S(1-0), T	Nov 7, 2000
26	Female	13	0.779	ND	ND	S(0-1), A, T	Nov 7, 2000
27	Female	14	0.455	0.535	0.673	S(2-1), A	Nov 7, 2000
28	Male	40	0.170	0.267	0.320	H	None
29	Male	11	0.420	0.534	0.939	S(0-1), A, T	March, 2000
30	Male	18	0.302	0.421	0.829	S(3-15), T, H	Nov 7, 2000
31	Female	39	0.132	0.179	0.401	T, H	Nov 7, 2000
32	Female	7	ND <sup>f</sup>	ND	ND	S(2-4), A	None
33	Male	10	0.375	ND	ND	S(1-3), T	Nov 7, 2000
34	Female	9	0.652	0.587	1.042	S(26-15), T	Nov 7, 2000
35	Female	7	0.788	0.881	1.180	S(1-0), A, T	None
36	Female	5	0.681	NS <sup>g</sup>	NS	S(19-22), A, T	None
37	Male	9	1.248	0.888	1.256	S(2-5), A, T	None
38	Male	34	1.301	0.855	1.190	S(1-0), A,	Nov 7, 2000
39	Female	34	0.610	0.665	0.888	A, T	None
40	Female	62	0.189	NS	NS	Negative	Nov 7, 2000
41	Male	67	0.384	0.393	0.610	Negative	Nov 7, 2000
42	Female	50	0.214	0.166	0.231	Negative	Nov 7, 2000
43	Male	11	1.013	0.855	1.114	H	Nov 7, 2000
44	Male	17	0.768	0.792	0.977	S(1-0)	2001
45	Male	9	0.139	0.158	0.253	Negative	Nov 7, 2000

Table 1 (Continued).

Field screening test of optical density values<sup>a</sup> against *S. japonicum* given by human blood samples of the residents in Leyte, the Philippines in the rapid and simplified ELISA and the conventional ELISA.

No.	Sex	Age	Rapid and simplified ELISA		Conventional ELISA	Stool examination <sup>e</sup>	Treatment
			Whole blood <sup>b</sup>	Serum <sup>c</sup>	Serum <sup>d</sup>		
46	Male	36	0.601	0.471	0.631	Negative	Nov 7, 2000
47	Female	60	0.401	0.393	0.546	Negative	1958
48	Male	19	0.944	0.962	1.043	S(2-2), H	Nov 7, 2000
49	Female	10	1.035	0.592	1.136	S(0-3), A	Nov 7, 2000
50	Female	39	0.125	0.132	0.216	Negative	March, 2000
51	Female	8	0.699	0.597	0.924	A	Nov 7, 2000
52	Male	28	0.877	0.666	1.068	H	Nov 7, 2000
53	Male	21	1.029	0.586	1.142	T	March, 2000
54	Male	38	0.585	0.337	0.848	T	March, 2000
55	Male	69	0.413	0.325	0.642	T	March, 2000
56	Female	15	0.280	0.262	0.587	T	March, 2000
57	Female	15	0.255	0.127	0.506	Negative	1998
58	Male	16	0.629	0.407	0.928	A, T	2000

<sup>a</sup>Values in duplicate were averaged and the mean values were converted in case OD value of positive control was 1.000.

<sup>b</sup>OD values of negative control were 0.033 or 0.034; <sup>c</sup>OD values of negative control were 0.017 or 0.024.

<sup>d</sup>OD values of negative control was -0.039 or 0.001.

<sup>e</sup>S; *S. japonicum*, A; *Ascaris lumbricoides*, H; hookworm, T; *Trichuris trichiura*. The examination was done by Kato-Katz method using 10 mg aliquots.

<sup>f</sup>ND; Not done; <sup>g</sup>NS; No sample

## RESULTS

### Optimal concentration of samples in the rapid and simplified ELISA procedure

Optimal concentration of samples of *S. japonicum*-infected human whole blood was examined by the rapid and simplified ELISA at dilutions of 1:1, 1:5, 1:10, 1:20, 1:40, 1:80, and 1:160, and the optical density values decreased gradually from undiluted blood to the dilution of 1:160 (Fig 1). The values for normal human whole blood also decreased, but became zero at dilution of 1:10 and remained zero up to the dilution of 1:160 (Fig 1). The optical density values of *S. japonicum*-infected human whole blood were highly significantly different from the values of normal human whole blood ( $p < 0.001$ ). Therefore, the 1:10 dilution of *S. japonicum*-infected human whole blood was the most suitable concentration to be used in the rapid and simplified ELISA, WHOLE BLOOD-ELISA.

### Rapid and simplified ELISA used in mass screening

In the mass screening, the rapid and sim-

plified ELISA was carried out using blood samples (whole blood and serum) from 58 residents in Leyte, the Philippines. In comparison of the rapid and simplified ELISA with the conventional ELISA, the optical density values in the rapid and simplified ELISA using whole blood (WHOLE BLOOD-ELISA) were nearly the same as those in the rapid and simplified ELISA using serum and were slightly less than the ones of the conventional ELISA (Table 1). It was observed that there were not any extremely big differences in optical density values between WHOLE BLOOD-ELISA and the conventional ELISA. It was further observed that *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* infection did not influence the optical density values in WHOLE BLOOD-ELISA.

From the results of the stool examination, the positive rate of schistosomiasis was 48.3% (28/58). After one month, repeat stool examinations performed for 5 days showed that 18 of the 24 people (75%) previously negative turned out to be positive (Table 2). When the results of the first and repeat stool examina-

Table 2  
Comparison of detection rate against *S. japonicum* between WHOLE BLOOD-ELISA and stool examinations in human samples from Leyte, the Philippines.

No.	Whole blood-ELISA OD	Stool examinations <sup>a</sup>						Others	Treatment
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>		
53	1.029	○	○▲					H, T	Mar 2000
43	1.013	○	○▲					H, T	Nov 2000
52	0.877	○	○▲					H	Nov 2000
2	0.825	○	○△	○▲				A, T	None
51	0.699	○	○△	●					Nov 2000
39	0.610	○	●					A, T	None
46	0.601	○	●					H	Nov 2000
5	0.540	○	●					A, T	None
3	0.531	○	○△	○△	●			H, T	None
55	0.413	○	●					T	Mar 2000
47	0.401	○	○△	○△	○△	○△	○△	H, T	1958
13	0.387	○	○▲					H	Nov 2000
41	0.384	○	○△	○△	○△	○▲		A, H, T	Nov 2000
21	0.355	○	●					H, T	Apr 2000
56	0.280	○	○▲					H	Mar. 2000
57	0.255	○	○△	○△	○△				1998
16	0.237	○	○△	○△				A, T	Nov 2000
9	0.224	○	○△	○▲					Nov 2000
42	0.214	○	○△	○▲				H, T	Nov 2000
40	0.189	○	○△	○△	○△	○△	○△	A, H, T	Nov 2000
10	0.158	○	○△	○▲				H	Nov 2000
45	0.139	○	○△	○△	○△	○△	○△	T	Nov 2000
31	0.132	○	○△	○△	○△	○△	○△	H, T	Nov 2000
50	0.125	○	●						Mar 2000

OD of positive control : 1.000, OD of negative control : 0.033, 0.034

○●=Kato-Katz thick smear technique, ▲△=Formalin-detergent technique.

●▲=*S. japonicum* eggs were detected. ○△=*S. japonicum* eggs were not detected.

A=*Ascaris lumbricoides*, H=hookworm, T=*Trichuris trichiura*

<sup>a</sup>The first and the 2<sup>nd</sup>-6<sup>th</sup> stool examinations were done in August and September, 2002, respectively.

tions among the negatives were taken into account, the total positive rate of schistosomiasis went up to a high value of 79.3 % (Fig 2).

When the optical density values of WHOLE BLOOD-ELISA were 0.3 and above, schistosome eggs were found in almost all stool samples of people examined (the OD value of positive control was 1.000 and that of negative control was 0.033/0.034). When the optical density value was comparatively high (0.415) and the result of stool examination was negative, this indicated that the person might have had schistosome infection in the past. When

the optical density values of WHOLE BLOOD-ELISA were over 0.1 but less than 0.3, schistosome eggs were detected only in the stools of some people (Fig 3). When the optical density values were less than 0.1, no schistosome eggs could be found in the stool (Fig 3).

It would be worth noting that the optical density values in WHOLE BLOOD-ELISA were nearly the same as the ones in the rapid and simplified ELISA using serum, and there were no fluctuations in case detection rate by the number of samplings and the duration of examinations as seen in the stool examinations.

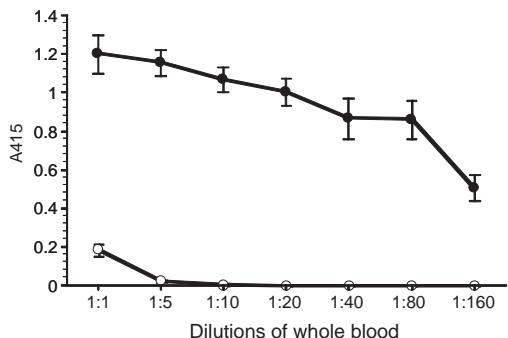


Fig 1—Mean optical density values of *S. japonicum*-infected and normal human whole blood as examined by the rapid and simplified ELISA. (●) *S. japonicum*-infected human whole blood, (○) normal human whole blood. Bar = standard deviation, n = 5.

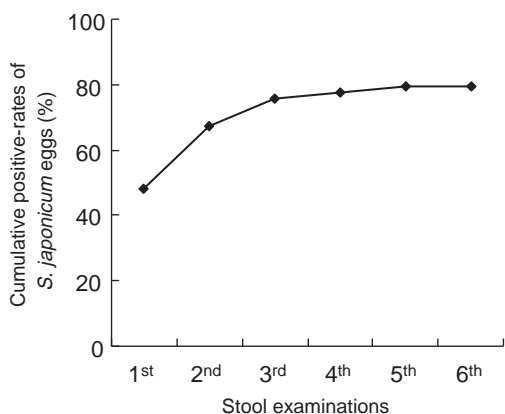


Fig 2—Cumulative positive rates of *S. japonicum* eggs of individuals after successive stool examinations.

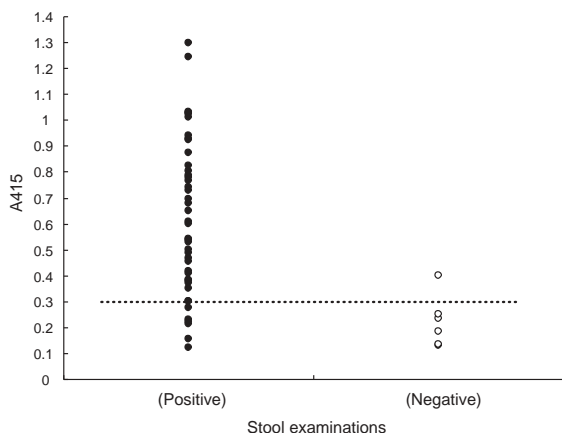


Fig 3—Relationship between optical density values against *S. japonicum* given by the rapid and simplified ELISA using human whole blood (WHOLE BLOOD-ELISA) and the detection of *S. japonicum* eggs in stool examinations. Dashed line, indicates OD values of over and under 0.3.

## DISCUSSION

Enzyme-linked immunosorbent assay (ELISA) has been widely used as a screening test for selective or mass treatment of schistosomiasis. Various methods of ELISA have been introduced. The k-ELISA and RAST-ELISA have to be conducted in a special facility for radio-immunoassay. The k-ELISA, furthermore, requires a temperature of 25°C. The FAST-ELISA has almost the same characteristics as the WHOLE BLOOD-ELISA as far as incubation and operation times are concerned. However, there is a difference in the type of samples examined, that is, serum samples in the FAST-ELISA and whole blood samples in the WHOLE BLOOD-ELISA. The latter is, therefore, simpler as centrifugation is not necessary and less volume has to be used.

We have, therefore, introduced an ELISA method that is simple and more rapid than the other forms including the conventional ELISA. This rapid and simplified ELISA, WHOLE BLOOD-ELISA, has the following characteristics. 1) It is possible to use whole blood instead of sera, and since the dilution of the whole blood has a comparatively high concentration, the use of a dilution of 1:10 is very convenient. 2) The volume of whole blood and conjugate is 0.05 ml compared to 0.1 ml of serum and conjugate used in the conventional ELISA. 3) The incubation time of whole blood and conjugate is as short as 5 minutes compared to 45 minutes necessary for the incubation time of serum and 60 minutes for conjugate in the conventional ELISA. 4) The optical density can be measured at 10 minutes after transferring the substrate (0.1 ml) compared to 30 minutes after transferring the substrate (0.2 ml) in the conventional ELISA. 5) The WHOLE BLOOD-ELISA does not require constant temperature setting whereas a temperature of 37°C is necessary in the conventional ELISA. 6) The operation time of WHOLE BLOOD-ELISA is 20-30 minutes compared to over 3 hours in the conventional ELISA.

The observed variations from day-to-day and of the samplings in the stool examinations (Utzing *et al*, 2001) were revised by the results of the WHOLE BLOOD-ELISA. Furthermore, in comparison with the conventional ELISA, the optical density values of WHOLE BLOOD-ELISA

were almost the same and were not influenced by other common intestinal helminthic infections.

Based from the mass screening of the residents in Leyte, the Philippines, the diagnosis of schistosomiasis was made possible using the criteria in which the optical density values of 0.3 and over as positive, from 0.1 to less than 0.3 as doubtful, and less than 0.1 as negative. Therefore, the WHOLE BLOOD-ELISA is a satisfactory screening method for determining schistosomiasis infection particularly in areas such as Leyte, the Philippines, where the prevalence may vary with the administration of praziquantel. Whenever an immunological field survey is necessary, particularly before and after a selective or a mass treatment control program, the WHOLE BLOOD-ELISA is recommended.

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#### REFERENCES

- Boctor FN, Stek MJ, Peter JB, Kamal R. Simplification and standardization of dot-ELISA for human schistosomiasis mansoni. *J Parasitol* 1987; 73: 589-92.
- Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry* 1971; 8: 871-4.
- Hamilton JV, Klinkert M, Doenhoff MJ. Diagnosis of schistosomiasis: antibody detection, with notes on parasitological and antigen detection methods. *Parasitology* 1998; 117 Suppl: S41-57. Review.
- Hancock K, Tsang VCW. Development and optimization of the FAST-ELISA for detecting antibodies to *Schistosoma mansoni*. *J Immunol Methods* 1986; 92: 167-76.
- Ismail MM, Bruce JI, Attia M, Tayel SE, Sabah AA, El-Ahmedawy BA. The detection of IgE by radioallergosorbent technique (RAST) and ELISA in Egyptian cases of schistosomiasis. *J Egypt Soc Parasitol* 1989; 19: 29-34.
- Kamal KA, Shaheen HI, El-Said AA. Applicability of ELISA on buffer-eluates of capillary blood spotted on filter papers for the diagnosis and clinical staging of human schistosomiasis. *Trop Geogr Med* 1994; 46: 138-41.
- Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Rev Inst Med Trop São Paulo* 1972; 14: 397-400.
- Matsuda H, Tanaka H, Blas BL, Noseñas JS, Tokawa T, Ohsawa S. Evaluation of ELISA with ABTS, 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid), as the substrate of peroxidase and its application to the diagnosis of schistosomiasis. *Jpn J Exp Med* 1984; 54: 131-8.
- Moody AH. An introduction to selected diagnostic methods for parasites available as kits for small health units. Laboratory in health care-developing country. Proceedings of the 17<sup>th</sup> Congress of the International Association of Medical Laboratory Technologists. Dordington: Tropical Health Technologic, 1986: 28-9.
- Moser D, Doumbo O, Klinkert M. The humoral response to heat shock protein 70 in human and murine *Schistosomiasis mansoni*. *Parasite Immunol* 1990; 12: 341-52.
- Tsang VCW, Wilson BC, Maddison SE. Kinetic studies of a quantitative single-tube enzyme-linked immunosorbent assay. *Clin Chem* 1980; 26: 1255-60.
- Tsang VCW, Tao Y, Qui L, Xue H. Fractionation and quantitation of egg antigens from *Schistosoma japonicum* by the single-tube kinetic-dependent enzyme-linked immunosorbent assay (k-ELISA): higher antigenic activity in urea-soluble than in aqueous-soluble fractions. *J Parasitol* 1982; 68: 1034-43.
- Utzinger J, Booth M, N'goran EK, Müller I, Tanner M, Lengeler C. Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. *Parasitology* 2001; 122: 537-44.
- Voller A, Bidwell D, Huldt G, Engvall E. A microplate method of enzyme-linked immunosorbent assay and its application to malaria. *Bull WHO* 1974; 51: 209-11.
- Weiss N, Stürchler D, Dietrich FM. Radioallergosorbent and indirect fluorescent antibody tests in immunodiagnosis of schistosomiasis. *Lancet* 1978; 2: 1231-3.