Bulletin of the World Health Organization, 65 (2): 233-244 (1987)

Collaborative study on antigens for immunodiagnosis of *Schistosoma japonicum* infection*

K. E. MOTT,[1] H. DIXON,[2] C. E. CARTER,[3] E. GARCIA,[4] A. ISHII,[5] H. MATSUDA,[6] G. MITCHELL,[7] M. OWHASHI,[8] H. TANAKA,[6] & V. C. TSANG[9]

Six research laboratories in Australia, Japan, the Philippines and the USA participated in a collaborative evaluation of immunodiagnostic tests for Schistosoma japonicum *infections. The serum bank consisted of 385 well-documented sera from Brazil, Kenya, Philippines, Republic of Korea and Europe. Twelve* S. japonicum *antigen/test system combinations were evaluated.*

Crude S. japonicum *egg antigens showed the highest sensitivity and specificity. The defined or characterized antigens showed no advantage over the crude antigens. Quantitative seroreactivity of all* S. japonicum *antigens showed a positive correlation with faecal egg counts (log x + 1) in all age groups. The performance of the circumoval precipitin test was satisfactory within the same laboratory but with differences in the results between laboratories. A monoclonal antibody used in a competitive radioimmunoassay test system performed as well as the crude egg antigens.*

The high sensitivity of crude S. japonicum *antigens now permits further evaluation for wide-scale use in public health laboratories of endemic areas to support control efforts.*

The control of schistosomiasis due to *Schistosoma japonicum* is progressing rapidly in China (*1*) and the Philippines (*2*), while transmission has now ceased in Japan (*3*). Since the availability of praziquantel, large-scale chemotherapy is now recognized to be a cost-effective approach for the control of this disease through the general health care system. However, because of the reduced prevalence and intensity of this infection, there is now need for a simple, reliable and reproducible diagnostic technique. Current methods for stool examination such as the modified Kato cellophane faecal thick-smear technique or the merthiolate-iodine-formaldehyde concentration (MIFC) technique are insensitive for low egg counts, while the miracidial hatching technique, although sensitive and cheap, has not been carefully standardized (*4*). These methods are generally laborious and time-consuming, especially when the case detection rate is low owing to high rates of negative stool examinations.

In a previous collaborative study on antigens for immunodiagnosis of schistosomiasis, the three *S. japonicum* egg antigens evaluated showed high sensitivity and satisfactory specificity (*5*). Since that study, many of its findings have been confirmed; for example, crude antigens may perform as well as or better than "purified" antigens (*6*), and cross-reactivity occurs between the *S. mansoni* egg antigens and *S. japonicum* infected sera (*7*, *8*). These results indicated that an immunodiagnostic technique may be a useful diagnostic tool in the maintenance phase of control of schistosomiasis. It was thus decided that a further evaluation of the available *S. japonicum* antigens would help determine whether a large-scale evaluation of a limited number of antigens was warranted.

* Sponsored by the Scientific Working Group on Schistosomiasis of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

[1] Schistosomiasis and other Trematode Infections, World Health Organization, 1211 Geneva 27, Switzerland. Requests for reprints should be sent to this address.

[2] Health Statistics and Trend Assessment, World Health Organization, Geneva, Switzerland.

[3] Department of Biology, Vanderbilt University, Nashville, TN, USA.

[4] Department of Parasitology, Institute of Public Health, University of the Philippines, Manila, Philippines.

[5] Department of Parasitology, Okayama University Medical School, Okayama, Japan.

[6] Department of Parasitology, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

[7] Immunoparasitology Unit, Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia.

[8] Department of Parasitology, Miyazaki Medical College, Miyazaki, Japan.

[9] Helminthic Diseases Branch, Centers for Disease Control, Atlanta, GA, USA.

The objectives of the present collaborative study were to evaluate the sensitivity and specificity of the available *S. japonicum* antigens for immunodiagnosis as related to:

— intensity of infection;
— treatment status;
— cross-reactivity with sera from persons infected with *S. mansoni*;
— cross-reactivity with sera from persons infected with other trematodes or helminths present in *S. japonicum* endemic areas.

In addition, the reproducibility of the test system on the same sera was to be assessed.

MATERIALS AND METHODS

Study design

This collaborative study was designed like the one previously reported (*5*). Material to the serum bank was contributed by five laboratories in Brazil, Kenya, Philippines, Republic of Korea and Switzerland. These laboratories were selected on the basis of having participated in the previous collaborative study and/or having published reports of *S. japonicum* antigens that had potential application for the immunodiagnosis of *S. japonicum* infection.

The sera were stored by and distributed through WHO headquarters, Geneva, to each participating laboratory in October 1983. Each laboratory forwarded the results of the serological tests on the data entry forms used in the previous collaborative study to WHO headquarters where data processing and analysis were carried out. The analysed results from each laboratory were returned within 4 months. Staff in Geneva prepared a comparative analysis of the data which forms the basis of this report.

Serum bank

The serum bank was designed so that (1) the serological results could be compared with corresponding parasitological data, (2) sera from both untreated and previously treated persons with or without *S. mansoni* or *S. japonicum* eggs in the stool could be assessed, (3) sera from persons with parasitologically confirmed *Clonorchis sinensis*, *Paragonimus* and *Ascaris* infections could be evaluated, and (4) the results of duplicate and triplicate samples of sera could be compared.

Among the 385 sera in the serum bank, the control sera included were:

— 24 sera from persons never exposed to schistosomiasis or who had not resided in an endemic area;
— 11 sera from persons residing in urban areas of endemic countries and never exposed to schistosomiasis;
— 59 sera from persons never exposed to schistosomiasis or who had not resided in an endemic area but had parasitologically confirmed *Clonorchis sinensis*, *Paragonimus* or *Ascaris* infection.

There were 59 sera from persons with *S. japonicum* eggs in the stool, 45 of which had other intestinal parasites, and 44 serum specimens from persons without *S. japonicum* eggs in the stool (20 of which had other intestinal parasites), but who had either been previously treated or who had resided in an endemic area. Of those with *S. japonicum* eggs in the stool, 45 sera had associated faecal egg counts by the Kato

Table 1. Combinations of *Schistosoma japonicum* (*S.j.*) antigens and test systems evaluated in the collaborative study

Antigen	Antigen code	Test[a]
Whole *S. japonicum* eggs	33 (*20*)[b]	COPT (*20, 26*)[b]
Whole *S.j.* eggs	30 (*19*)	COPT (*19*)
Crude *S.j.* eggs	28 (*14*)	ELISA (*14*)
Crude *S.j.* eggs	31 (*23*)	ELISA (*14*)
Crude fraction *S.j.* eggs	20 (*20*)	ELISA (*5*)
Heat-treated *S.j.* eggs	29 (*22*)	ELISA (*14*)
Allergenic fraction *S.j.* eggs	32 (*13*)	ELISA (*14*)
Purified fraction *S.j.* eggs	21 (*21*)	ELISA (*5*)
Partially purified *S.j.* urea eggs	25 (*24*)	K-ELISA (*25*)
Crude cytosolic *S.j.* adult worm	22 (*24*)	K-ELISA (*25*)
Purified microsomal *S.j.* adult worm	23 (*24*)	K-ELISA (*25*)
S.j. monoclonal antibody I.134	27 (*18*)	RIA[c] (*18, 27*)

[a] COPT (circumoval precipitin test); ELISA (enzyme-linked immunosorbent assay); K-ELISA (kinetic-dependent ELISA); RIA (radioimmunoassay).

[b] Figures in parentheses are references describing the antigen preparation or the test.

[c] *RIA with antigen 27.* The protocol for this standard type of competitive radioimmunoassay using a labelled monoclonal antibody was described by Mitchell et al. (*18*) and Cruise et al. (*27*): 50 µl of crude *S. japonicum* adult worm antigen, at a predetermined desired dilution in borate buffer pH 9.5 was incubated on poly (vinyl chloride) plates overnight at room temperature. Post-coating involved a 1–2h incubation with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline pH 7.3 (PBS). After washing 3 times in 0.05% Tween 20 in PBS, 25 µl serum dilutions of 1:10, 1:100 and 1:1000 in Tween 20 : PBS : BSA were added per well followed by 25 µl ^{125}I-labelled monoclonal antibody, I.134, corresponding to approximately 20 000 cpm. After overnight incubation at room temperature, the plates were washed and cut and radioactivity in the wells determined in an autogamma counter. Data were expressed as percentage inhibition of maximum binding of radioactivity. In this assay, approximately 0.2 µg/ml unlabelled I.134 results in 50% inhibition of binding of ^{125}I-I.134, with maximum binding being approximately 3000 cpm and background about 50 cpm. It has been determined previously that this assay is only suitable for schistosomiasis japonica.

cellophane faecal thick-smear technique and 14 had confirmed qualitative parasitological diagnosis by the MIFC technique.

There were 168 sera from persons with *S. mansoni* eggs in the stool (64 of which had other intestinal parasites) and 20 sera from persons without *S. mansoni* eggs in the stool who had previously been treated or had resided in an endemic area.

The methodology for collection of the sera and storage has been described previously (*5*). The serum bank contained 37 duplicate specimens and 3 triplicate specimens. The maximum number of serological results for any single antigen/test combination among the participating laboratories was 364.

The combinations of antigens and test systems evaluated in the collaborative study are shown in Table 1.

Statistical analysis

The methodology of the statistical analysis has been described in the report of the previous collaborative study (*5*). Multiple regression analysis of the data was carried out to determine the contribution of egg count, degree of organomegaly, effect of treatment, age and sex to the quantitative serological results. The equation fitted to the data was:

$$y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4 + b_5 x_5$$

where y is the quantitative laboratory result expressed as either the optical density, or units, or the $\log_{10}$ (titre) or the $\log_2$ (titre);
x_1 is $\log_{10}$ (egg count + 1);
x_2 is 1 for no organomegaly, 2 for hepatomegaly, 3 for splenomegaly and 4 for hepatosplenomegaly;
x_3 is 1 for no treatment for schistosomiasis and 2 for subject treated for schistosomiasis;
x_4 is age in years;
x_5 is 1 for a male subject and 2 for a female subject;
b_1, b_2, b_3, b_4, and b_5 are the partial regression coefficients.

The multiple regression coefficient, "multiple R", was also computed and all coefficients were tested in order to determine if they were significantly different from zero. As in the previous study, it was not possible to compare directly the coefficients of different antigen/test combinations, but it was possible to compare the significant findings.

Table 2. Percentage specificity of *S. japonicum* antigens in sera from persons from non-endemic areas of schistosomiasis and according to three other types of parasite infections

Antigen	Antigen code	Test	Percentage specificity				
					Type of parasite		
			Group No. 1[a] (23-24)[b]	Group No. 2[a] (58-59)[b]	*Ascaris* (14-15)	*Paragonimus* (22)	*Clonorchis* (23)
Whole *S. japonicum* eggs	33	COPT	100.0	100.0	100.0	100.0	100.0
S. japonicum monoclonal antibody I.134	27	RIA	100.0	98.3[c]	100.0	95.5[c]	100.0
Whole *S. japonicum* eggs	30	COPT	100.0	98.3	93.3	100.0	100.0
Crude *S. japonicum* eggs	28	ELISA	100.0	94.9[c]	93.3[c]	100.0	90.9[c]
Heat-treated *S. japonicum* eggs	29	ELISA	100.0	94.9[c]	93.3[c]	100.0	90.9[c]
Crude *S. japonicum* eggs	31	ELISA	100.0	94.9	93.0	100.0	90.9
Purified fraction *S. japonicum* eggs	21	ELISA	95.8	93.2	86.7	100.0	90.9
Allergenic fraction(s) *S. japonicum* eggs	32	ELISA	100.0	93.2	86.7	100.0	90.9
Crude cytosolic *S. japonicum* adult worm	22	K-ELISA	100.0	83.1	93.3	68.2	90.9
Purified microsomal *S. japonicum* adult worm	23	K-ELISA	100.0	71.2	80.0	54.5	81.8
Partially purified *S. japonicum* urea eggs	25	K-ELISA	100.0	61.0	80.0	31.8	77.3
Crude fraction *S. japonicum* eggs	20	ELISA	100.0	57.6	73.3	27.3	77.3

[a] Group No. 1 were Europeans and group No. 2 were Koreans.
[b] Figures in parentheses give the number of sera tested in each group.
[c] Remaining percentages were uncertain results.

Table 3. Sensitivity and specificity of *S. japonicum* antigens in sera from persons from *S. japonicum*-endemic areas

Antigen[a]	Test	Sensitivity (%)[b]	Antigen[a]	Test	Specificity (%)[c]
22	K-ELISA	100.0	33	COPT	78.0
25	K-ELISA	100.0	27	RIA	70.7
31	ELISA	100.0	28	ELISA	68.6
32	ELISA	100.0	29	ELISA	68.6
21	ELISA	98.3	30	COPT	68.6
23	K-ELISA	98.3	31	ELISA	60.9
30	COPT	98.2	32	ELISA	60.9
28	ELISA	96.4	21	ELISA	50.0
27	RIA	87.9	23	K-ELISA	45.5
20	ELISA	87.4	20	ELISA	31.6
29	ELISA	85.5	25	K-ELISA	15.9
33	COPT	77.2	22	K-ELISA	0.0

[a] Antigen code as given in Table 1.
[b] Tested on 48–59 sera from persons with *S. japonicum* eggs in their stool.
[c] Tested on 23–90 sera from persons with negative stool examination.

Table 4. Percentage sensitivity of *S. japonicum* antigens according to the number of eggs in the stools, in sera from persons infected by *S. japonicum* (*S.j.*) and *S. mansoni* (*S.m.*) parasites

	Percentage sensitivity									
	1–100 eggs[a]		101–200 eggs[a]		201–400 eggs[a]		401–800 eggs[a]		>800 eggs[a]	
Antigen (code)/test system	*S.j.* [23–27][b]	*S.m.* [63–70]	*S.j.* [2]	*S.m.* [28–29]	*S.j.* [9]	*S.m.* [27–30]	*S.j.* [2–3]	*S.m.* [14–16]	*S.j.* [2–4]	*S.m.* [17–21]
Whole egg (30)/COPT	100.0	3.0	100.0	6.9	100.0	14.3	100.0	0.0	100.0	11.1
Whole egg (33)/COPT	65.4	7.6	100.0	0.0	100.0	0.0	100.0	0.0	100.0	21.1
Crude egg (31)/ELISA	100.0	34.9	100.0	31.0	100.0	40.7	100.0	7.1	100.0	64.7
Crude fraction egg (20)/ELISA	100.0	90.0	100.0	75.9	100.0	86.7	100.0	93.8	100.0	90.5
Crude egg (28)/ELISA	96.3	1.5	100.0	3.4	100.0	3.6	100.0	0.0	100.0	5.6
Monoclonal antibody I.134 (27)/RIA	85.2	18.6	100.0	6.9	100.0	10.7	100.0	18.8	100.0	26.3
Allergenic fraction egg (32)/ELISA	100.0	25.4	100.0	20.7	100.0	33.3	100.0	7.1	100.0	41.2
Partially purified urea egg (25)/K-ELISA	100.0	98.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Crude cytosolic adult worm (22)/K-ELISA	100.0	95.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Purified microsomal *S. japonicum* adult worm (23)/K-ELISA	100.0	98.6	100.0	93.1	100.0	96.6	100.0	93.8	100.0	95.0
Purified fraction egg (21)/ELISA	96.3	61.4	100.0	62.1	100.0	76.7	100.0	56.3	100.0	81.0
Heat-treated egg (29)/ELISA	81.5	7.6	100.0	6.9	88.9	7.1	100.0	0.0	100.0	22.2

[a] Egg counts are per gram of faeces.
[b] Figures in square brackets give the number of sera tested in each group.

RESULTS

Evaluation of control sera

The serum bank contained 24 sera from Europeans who had never resided in an endemic area. The specificity of all antigen/test combinations in these sera was 100% except one highly purified *S. japonicum* egg antigen (Table 2).

Cross-reactivity with other trematodes/helminths

Cross-reactivity was observed mostly in sera from persons infected with *Ascaris*. The least cross-reactivity was observed in sera from persons infected with *Paragonimus*. The least cross-reactivity was observed with the circumoval precipitin test (COPT) and in the radioimmunoassay (RIA) using an antigen isolated by a monoclonal-antibody-based technique (Table 2). No difference in the quantitative ELISA or RIA readings between the sera from persons infected with *Ascaris*, *Clonorchis sinensis* and *Paragonimus* was observed ($P<0.0001$).

Evaluation of antigens

S. japonicum egg and adult worm antigens were evaluated in a total of 12 antigen/test combinations. Sensitivity was evaluated by examination of all sera from untreated persons who had *S. japonicum* eggs in the stool, as well as those who were not cured after specific treatment. Specificity was evaluated in all sera from untreated persons from endemic areas with consistently negative stool examinations and sera from previously treated persons with negative stool examinations (Table 3).

In two groups of antigen/test systems from the same laboratory (antigen numbers 31, 32 and 28, 29, 30) the crude egg antigens, either as crude soluble egg antigen (SEA) or whole eggs, were as sensitive and specific as the purified egg antigens. In another series (antigen 20, 21) the purified egg antigen was more sensitive, but the specificity of both was low.

Seroreactivity and faecal egg count

Although the maximum number of sera tested (18) was small the sensitivity of each antigen/test system

Table 5. Sensitivity of *S. japonicum* antigen/test systems in sera from persons with *S. japonicum* and *S. mansoni* infection

		Percentage sensitivity			
		S. japonicum infection		*S. mansoni* infection	
Antigen (code)	Test	Treated group [6–7][a]	Untreated group [5–7]	Treated group [34–37]	Untreated group [62–66]
Partially purified urea egg (25)	K-ELISA	100.0	100.0	100.0	98.5
Purified microsomal adult worm (23)	K-ELISA	85.7	100.0	97.3	93.9
Crude cytosolic adult worm (22)	K-ELISA	100.0	100.0	94.6	98.5
Crude fraction egg (20)	ELISA	100.0	100.0	86.5	89.4
Purified fraction egg (21)	ELISA	100.0	100.0	75.7	60.6
Crude egg (31)	ELISA	100.0	100.0	31.11	35.2
Allergenic fraction egg (32)	ELISA	100.0	100.0	23.6	25.8
Monoclonal antibody I.134 (27)	RIA	85.7	71.4	16.2[b]	16.9[c]
Heat-treated egg (29)	ELISA	85.7	100.0	8.9[d]	7.7[e]
Whole eggs (30)	COPT	85.7	100.0	8.8	4.6
Whole eggs (33)	COPT	85.7	71.4	8.8	3.1
Crude egg (28)	ELISA	85.7[f]	85.7[f]	5.9[g]	0.0[h]

[a] Figures in square brackets give the number of sera tested in each group.

[b] 2.7%, [c] 3.1%, [d] 17.6%, [e] 16.9%, [f] 14.3%, [g] 23.5% and [h] 26.2% were unsure results.

Table 6. Specificity of *S. japonicum* antigen/test systems in sera of persons from *S. japonicum* and *S. mansoni*-endemic areas without eggs in the stool, according to treatment

		Percentage specificity			
		S. japonicum infection		*S. mansoni* infection	
Antigen (code)	Test	Treated group [4–6][a]	Untreated group [13–18]	Treated group [7–12]	Untreated group [8]
Crude egg (28)	ELISA	0.0	92.3	100.0	100.0
Whole eggs (30)	COPT	0.0	92.3	100.0	100.0
Whole eggs (33)	COPT	33.3	93.3	100.0	100.0
Heat treated egg (29)	ELISA	0.0	92.3	87.5[b]	100.0
Monoclonal antibody I.134 (27)	RIA	16.7	93.3	81.8	100.0
Allergenic fraction egg (32)	ELISA	0.0	87.5	71.4	100.0
Crude egg (31)	ELISA	0.0	87.5	57.1	100.0
Purified fraction egg (21)	ELISA	0.0	68.7	45.5	100.0
Crude fraction egg (20)	ELISA	0.0	62.5	18.2	50.0
Crude cytosolic adult worms (22)	K-ELISA	0.0	0.0	0.0	0.0
Purified microsomal adult worm (23)	K-ELISA	0.0	67.7	0.0	87.5
Partially purified urea egg (25)	K-ELISA	0.0	27.8	0.0	12.3

[a] Figures in square brackets give the number of sera tested in each group.
[b] 12.5% were unsure results.

Table 7. Cross-reactivity between *S. japonicum* antigens and sera from *S. mansoni*-endemic areas

S. mansoni egg-positive[a]			*S. mansoni* egg-negative[b]		
Antigen[c]	Test	Seroreactivity (%)	Antigen[c]	Test	Seroreactivity (%)
20	ELISA	100.0	22	K-ELISA	100.0
25	K-ELISA	99.4	25	K-ELISA	95.0
22	K-ELISA	97.6	20	ELISA	69.0
23	K-ELISA	96.4	23	K-ELISA	65.0
21	ELISA	65.9	21	ELISA	31.6
31	ELISA	35.8	31	ELISA	20.0
32	ELISA	25.8	32	ELISA	13.3
27	RIA	16.0	27	RIA	10.5
29	ELISA	8.3	29	ELISA	6.2
30	COPT	6.4	30	COPT	0.0
33	COPT	5.7	28	ELISA	0.0
28	ELISA	2.6	33	COPT	0.0

[a] Tested on 151–167 sera from persons with *S. mansoni* eggs in their stool.
[b] Tested on 15–20 sera from persons from the endemic area with negative stool examinations.
[c] Antigen code given in Table 1.

was 100% (except antigen 29) when the faecal egg count was higher than 100 eggs per gram of faeces. Below 101 eggs per gram of faeces, the sensitivity was 100% except with three purified *S. japonicum* egg antigens (21, 27, 29) and two crude antigens (28 and 33) (Table 4).

Effect of treatment on seroreactivity

In individuals excreting *S. japonicum* eggs prior to or after treatment, the sensitivity of most test systems was high (Table 5). The heat-treated *S. japonicum* egg antigen, the labelled monoclonal antibody to *S. japonicum* and the COPT did not react to two sera (the same sera in each case) from persons who had been treated with praziquantel but whose stool was still positive.

The specificity of all the antigen test systems was low in the small group (7–9 sera) of six-month post-treatment sera from persons whose stool examinations were negative (Table 6). The mean ELISA and RIA readings in sera from persons who were egg-negative after treatment were consistently significantly higher than in sera from persons who were egg positive after treatment ($P<0.0001$).

Cross-reactivity between S. japonicum *antigens and* S. mansoni *sera*

Qualitative results: some crude egg antigens showed the lowest rate of seroreactivity in sera from persons from *S. mansoni* endemic areas, with or without eggs in the stool (Table 7). Cross-reactivity rates did not increase according to increasing *S. mansoni* faecal egg counts (Table 4).

The rates of seroreactivity of *S. japonicum* antigens was similar in sera from treated and untreated persons who excreted *S. mansoni* eggs (Table 5). The mean ELISA and RIA readings in these groups of sera were not significantly different (P always >0.07). However, post-treatment sera of those who were egg-negative were seroreactive in several antigen/test systems (Table 6).

Morbidity

Neither the sensitivity nor the specificity of the antigens was related to morbidity. On the other hand, a significant correlation between the mean ELISA optical density (OD) or RIA titres and morbidity was observed. The highest OD readings were noted in sera from persons with hepatosplenomegaly and the lowest readings were observed in those without organomegaly. These findings paralleled increasing faecal *S. japonicum* egg counts; as expected, the lowest egg counts were observed in persons without organomegaly. These findings are summarized in Table 8. Intermediate readings and egg counts were usually observed among persons with either hepatic or splenic enlargement alone.

For *S. japonicum* egg-positive persons the mean

Table 8. Comparison of quantitative readings, age and *S. japonicum* egg counts in sera from persons without organomegaly and with hepatosplenomegaly[a]

Antigen[b]	Test	Reading[c]			Age (years)			Egg count[d]		
		Without organomegaly	Hepatosplenomegaly	Significance (P)	Without organomegaly	Hepatosplenomegaly	Significance (P)	Without organomegaly	Hepatosplenomegaly	Significance (P)
20	ELISA	0.42	0.82	0.001	31.02	23.13	0.081	0.57	1.43	0.003
21	ELISA	0.48	0.88	0.001	31.02	23.13	0.081	0.57	1.43	0.003
22	K-ELISA	2.85	7.01	0.001	31.31	24.35	0.115	0.53	1.51	0.001
23	K-ELISA	5.10	14.37	0.001	31.31	24.35	0.115	0.55	1.51	0.001
25	K-ELISA	6.26	11.54	0.001	31.31	24.35	0.115	0.55	1.512	0.001
28	ELISA	5.21	12.06	0.001	30.65	25.93	0.285	0.66	1.53	0.005
29	ELISA	4.70	9.79	0.001	30.65	25.93	0.285	0.66	1.53	0.005
31	ELISA	2.57	4.53	0.004	33.60	26.85	0.172	0.80	1.38	0.107
32	ELISA	2.91	5.23	0.004	33.60	26.85	0.172	0.80	1.38	0.107
27	RIA	1.35	3.56	0.001	30.51	24.94	0.215	0.58	1.44	0.003

[a] Tested on 30–50 sera from persons without organomegaly and 13–17 sera from persons with hepatosplenomegaly.

[b] Antigen code given in Table 1.

[c] Optical density for antigens 20, 21, 31 and 32, units for antigens 22, 23 and 25, $\log_2$ titre for antigens 28 and 29, and $\log_{10}$ titre for antigen 27.

[d] $\log_{10}$ (egg count + 1).

Table 9. Correlation coefficients between quantitative readings and $\log_{10}$ (egg count + 1) according to type of infection

Antigen[a]	Test	Correlation coefficients: *S. mansoni* infection (151-167)[b]	Correlation coefficients: *S. japonicum* infection (48-59)[b]
20	ELISA	0.20[c]	0.62[d]
21	ELISA	0.21[c]	0.64[d]
22	K-ELISA	0.30[d]	0.59[d]
23	K-ELISA	0.36[d]	0.53[d]
25	K-ELISA	0.24[d]	0.64[d]
28	ELISA	0.14	0.70[d]
29	ELISA	0.13	0.66[d]
31	ELISA	0.18	0.58[d]
32	ELISA	0.18	0.58[d]
27	RIA	0.05	0.56[d]

[a] Antigen code given in Table 1.
[b] Figures in parentheses are the number of sera tested in each group.
[c] Significantly different from 0; $P<0.01$.
[d] Significantly different from 0; $P<0.001$.

quantitative readings with antigens 27 (RIA) and 28 (ELISA) were significantly higher in the group with hepatosplenomegaly than in the group without organomegaly ($P<0.01$ and <0.001, respectively); on the other hand, the mean age and mean egg counts of these two groups were not significantly different (age, $P=0.12$ and $P=0.16$; egg count, $P=0.07$ and $P=0.10$, respectively).

Correlation coefficient analysis

All of the antigen/test systems providing quantitative data showed a significant correlation with faecal egg count (log $x+1$), i.e., as the egg count increased, the quantitative seroreactivity increased (Table 9). Correlation coefficients of the crude antigens were similar to those of the purified antigens in the same test system.

Multiple regression analysis

The positive correlation between quantitative ELISA OD readings and RIA titres and faecal egg counts (log $x+1$) was highly significant. A lower, but significant, positive correlation with hepatosplenomegaly was also observed. Most antigens also showed a significant correlation after treatment, i.e., higher readings after treatment. There was no significant correlation between the readings and age or sex. The data are summarized in Table 10.

Reproducibility of results

The analysis of the results of duplicate and triplicate serum samples was provided to each partici-

Table 10. Coefficients obtained by multiple regression analysis between quantitative readings and $\log_{10}$ (egg count + 1), organomegaly, and treatment in *S. japonicum* infection

Antigen code	Test	Egg count	Degree of organomegaly	Treatment	Coefficient
20	ELISA[a]	0.126[b]	0.077[c]	0.200[b]	0.655[b]
21	ELISA[a]	0.129[b]	0.079[b]	0.200[b]	0.683[b]
22	K-ELISA[e]	0.850[b]	0.917[b]	1.206[d]	0.645[b]
23	K-ELISA[e]	1.672[b]	2.370[b]	1.825	0.683[b]
25	K-ELISA[e]	1.895[b]	0.954[c]	2.718[b]	0.666[b]
28	ELISA[f]	2.178[b]	1.335[b]	3.804[b]	0.749[b]
29	ELISA[f]	1.970[b]	0.906[c]	2.656[b]	0.689[b]
31	ELISA[a]	0.854[b]	0.410[d]	1.176[c]	0.671[b]
32	ELISA[a]	1.016[b]	0.470[d]	1.227[c]	0.657[b]
27	RIA[g]	0.584[b]	0.555[b]	0.821[c]	0.687[b]

[a] Result expressed as OD reading.
[b] Significantly different from 0; $P<0.001$.
[c] Significantly different from 0; $P<0.01$.
[d] Significantly different from 0; $P<0.05$.
[e] Result expressed as units.
[f] Result expressed as $\log_2$ (titre).
[g] Result expressed as $\log_{10}$ (titre).

pating laboratory. The data were analysed according to the qualitative results and the quantitative readings. The data from all laboratories were internally consistent, showing good reproducibility of both quantitative and qualitative results.

DISCUSSION

The major conclusion of this collaborative study is that the crude *S. japonicum* egg antigens have high sensitivity and specificity. The defined or characterized antigens of this study showed no advantage over the crude antigens. This conclusion is supported by independent studies (*6*). In two series of egg antigens in this study the purified antigens showed lower sensitivity than the crude antigens although specificity remained unchanged. In another pair of egg antigens, although the sensitivity of the purified antigen was higher, the specificity of both antigens was low.

A direct positive correlation between seroreactivity of *S. japonicum* antigens and faecal egg count (log $x+1$) was confirmed in the quantitative test systems. The positive correlation was not as strong as that observed with purified *S. mansoni* antigens as shown in a previous collaborative study (*5*). The small number of sera in this study did not permit a valid statistical evaluation of the quantitative readings in different egg count groups. However, all antigen/test systems detected all those infected with more than 100 *S. japonicum* eggs per gram of faeces. An HLA association with low or high IgG antibody response to *S. japonicum* has been demonstrated (*9*). The relationship between HLA and faecal egg counts has not yet been assessed. It is of interest that in individuals with low egg counts some antigen/test systems detected high levels of antibody to the *S. japonicum* antigen. This aspect may be further investigated in large cohorts, family studies and in relation to specific treatment.

The usefulness of immunodiagnosis to establish post-treatment cure of *S. japonicum* infection was not shown in this study. The six-month post-treatment sera from egg-negative persons showed higher quantitative readings than sera from egg-positive persons before treatment. Comparative pre- and post-treatment studies indicate that after treatment the changes in the general immune response (IgG, IgM, lymphocyte transformation) are related to the duration of the infection prior to treatment (*10*). No assessment of the role of the intensity of infection on the post-treatment immune response or seroreactivity has yet been reported.

The interpretation of the data on specificity was discussed in detail in the report of the previous collaborative study (*5*). In the present study the apparently low specificity is judged to be acceptable. The validity of an immunodiagnostic test for *S. japonicum* infection must be established in a public health context. To be of use in a control programme, a valid immunodiagnostic test must be at least as sensitive as the routine parasitological technique, i.e., it must give a positive result in all sera from persons reported to have *S. japonicum* eggs in the stool. On the other hand, both the single MIFC and the Kato examination will not detect low levels of faecal egg counts; thus individuals in endemic areas may be infected even if the routine stool examination is reported to be negative and prevalence underestimated (*11*). In this respect a sensitive diagnostic technique becomes useful in the surveillance phase of control when egg counts are low (*12*).

Serological results in any *S. japonicum* endemic area must be interpreted in relation to seroreactivity of the antigen/test system to other helminthic and trematode infections. The seroreactivity of the adult *S. japonicum* antigens in this study in sera from individuals infected with *Ascaris* was higher than that of egg antigens. The specificities of the crude egg antigens and the monoclonal antibody were 100%. In another study cross-reactivity between *S. japonicum* egg antigen and *Ascaris* was not observed (*13*). The low cross-reactivity in sera from individuals with *Paragonimus* infection, also described elsewhere (*13–15*), is important since the clinical differential diagnosis of CNS involvement in these endemic areas includes both *Paragonimus* and *S. japonicum* infection.

It was observed that the seroreactivity of two antigens (a crude *S. japonicum* egg antigen and the target antigen in adult worms of the monoclonal antibody I.134) was higher in the group with hepatosplenomegaly than in the group without organomegaly, in spite of similarity in age and egg counts. In the previous study it was noted that sera from persons with hepatosplenomegaly reacted differently in some test systems. These observations support a hypothesis that the morbidity status affects seroreactivity and further investigations are warranted.

All *S. japonicum* antigens cross-reacted to a low degree with sera from *S. mansoni*-infected persons from endemic areas. These findings are in contrast with the high degree of cross-reactivity between *S. japonicum* cercarial and egg antigens including the COPT and sera from Chinese workers whose *S. mansoni* infections were of less than two and a half years' duration (*16*). In the previous study it was shown for the first time that seroreactivity of *S. mansoni* antigens in *S. japonicum*-infected sera was high. These observations have been confirmed independently using SDS-PAGE two-dimensional gel electrophoresis (*8*) and Western blot (*15*) assays. This study has further shown that the cross-reactivity of

S. japonicum antigens in *S. mansoni*-infected sera does not generally correlate with the intensity of *S. mansoni* infection. Previously the cross-reactivity of *S. mansoni* antigens to *S. japonicum*-infected sera correlated positively with the faecal egg count but at a lower level than with the homologous infection (*5* and unpublished data).

The COPT has long been recognized by experienced investigators to be a highly reliable immunodiagnostic technique for diagnosis of *S. japonicum* infection. Its robustness has been confirmed under rigorous field conditions (*17*). However, the question of its reproducibility and reliability remains to be further investigated. In this study the results of the COPT in paired and triplicate specimens were the same in the same laboratory. However, there were differences in results between laboratories using slightly different techniques. Large-scale egg production, standardization and adequate stabilization of the eggs may obviate these problems. A large-scale evaluation of a standard antigen and a standard technique in a multicentre collaborative study is now warranted.

This study included the assessment of the reproducibility of the test results and confirmed the validity of including both duplicate and triplicate sera. The range of differences in quantitative results can be compared in duplicate sera. Triplicate sera provide the possibility to establish mean values and statistical measures of dispersion for comparative purposes.

This is the first comparative evaluation of a monoclonal anti-*S. japonicum* antibody for diagnosis. Its high sensitivity and specificity were confirmed in the RIA test system (*18*). As research progresses in this area, other monoclonal antibodies of immunodiagnostic potential may be defined. Molecular engineering techniques may be applied to produce this type of antigen for large-scale use.

Future activities

This collaborative study was the second in a series to identify immunodiagnostic techniques for public health use. Standard preparations of crude antigens and test system protocols could be evaluated in a well characterized serum bank in a multicentre trial. This present study will aid the selection of an antigen or antigens to be tested in a national scheme.

Research on other aspects of the immunodiagnosis of schistosomiasis may include the change in seroreactivity to different types of antigens after treatment, the factors that influence the correlation between quantitative seroreactivity and intensity of infection, i.e., age, sex, duration of infection, HLA, etc., the usefulness of new techniques, and the characterization of potential *S. mansoni* antigens for immunodiagnosis of *S. japonicum* infection.

The results of the present collaborative study augur well for the eventual application of an immunodiagnostic test for *S. japonicum* infection in public health laboratories in endemic countries. Such tests will facilitate diagnosis and maintenance of control of schistosomiasis.

ACKNOWLEDGEMENTS

The study was aided by Mr G. Brighouse, Blood Transfusion Centre, Cantonal Hospital, University of Geneva, in preparing the serum bank; Mr E. Labbé, Mr E. Dayer, and Mr M. Cerdan in packing and shipping all the material to the participating laboratories; and Mrs Lynn Hollies who prepared the final manuscript. The participation of Mrs Gulnar Mendonça in the analysis of the data and the bibliographic review is gratefully acknowledged. Dr N. R. Bergquist and Dr A. Davis reviewed the manuscript.

The serum bank was contributed by: Department of Parasitology, School of Public Health, University of the Philippines System, Manila, Philippines (E. G. Garcia); Centro de Pesquisas "René Rachou", Fundação Oswaldo Cruz, Belo Horizonte, Brazil (G. Gazzinelli and N. Katz); Nucleo de Medicina Tropical, Universidade de Brasília, Brasília, Brazil (A. Prata); the Tana River Project, Ministry of Health, Nairobi, Kenya (D. H. Smith); Institute of Tropical Medicine, Yonsei University, Seoul, Republic of Korea (C. T. Soh); and the Blood Transfusion Centre, Cantonal Hospital, University of Geneva, Geneva, Switzerland (P.-H. Lambert).

RÉSUMÉ

ETUDE COLLECTIVE SUR LES ANTIGÈNES UTILISÉS DANS L'IMMUNODIAGNOSTIC DES INFESTATIONS À *SCHISTOSOMA JAPONICUM*

L'endiguement de la schistosomiase à *Schistosoma japonicum* progresse rapidement en Chine et aux Philippines, et la transmission a déjà cessé au Japon. Dans les régions où la prévalence et l'intensité de cette infestation ont déjà diminué, on a besoin d'une technique de diagnostic simple, fiable et donnant des résultats reproductibles. Lors

de la présente étude collective, on a évalué la sensibilité et la spécificité des antigènes de *S. japonicum* actuellement utilisables pour l'immunodiagnostic.

Six laboratoires de recherche situés en Australie, au Japon, aux Philippines et aux Etats-Unis d'Amérique ont participé à l'étude; ils ont utilisé une banque de sérums consistant en 385 sérums dûment documentés en provenance du Brésil, du Kenya, des Philippines, de la République de Corée et d'Europe, dans 12 systèmes d'épreuve utilisant un antigène de *S. japonicum*. Avec les sérums en provenance d'Europe, tous les systèmes avaient une spécificité de 100%, sauf dans le cas d'un antigène hautement purifié d'œuf de *S. japonicum*. On a observé une réactivité croisée, le plus fréquemment avec les sérums de sujets infestés par *Ascaris* et le plus rarement avec les sérums de sujets porteurs de *Paragonimus*.

D'une façon générale, les antigènes bruts de *S. japonicum* étaient aussi sensibles et spécifiques que les antigènes purifiés utilisés dans cette étude. Tous les systèmes avaient une sensibilité de 100% lorsqu'ils étaient appliqués à des sérums de sujets excrétant plus de 100 œufs par gramme de selles. Dans les quelques sérums de sujets traités examinés, la spécificité était faible. Il est intéressant de noter que les résultats quantitatifs obtenus chez les sujets n'excrétant plus d'œufs après le traitement étaient régulièrement et significativement plus élevés que chez les sujets continuant à excréter des œufs après traitement. On a observé un faible degré de réactivité croisée avec les sérums de sujets porteurs de *S. mansoni*.

Bien que ni la sensibilité ni la spécificité des antigènes testés ne soient liées à la morbidité, la densité optique moyenne en ELISA et les titres en épreuves radio-immunologiques présentaient une corrélation significative avec l'hépatosplénomégalie. Tous les systèmes donnant des données quantitatives montraient une corrélation significative avec la numération d'œufs dans les selles ($\log x+1$). Dans un même système d'épreuve, les coefficients de corrélation obtenus avec les antigènes bruts étaient analogues à ceux fournis par les antigènes purifiés.

On n'a observé aucune corrélation significative entre les résultats quantitatifs et l'âge ou le sexe du sujet. Dans un même système d'épreuve, la reproductibilité des résultats était bonne.

La conclusion principale de cette étude collective est que ce sont les antigènes bruts d'œufs de *S. japonicum* qui possèdent la meilleure sensibilité et la meilleure spécificité. Les antigènes purifiés éprouvés n'étaient pas supérieurs aux antigènes bruts. La forte sensibilité et spécificité d'un anticorps monoclonal anti-*S. japonicum* ouvre la voie à des développements prometteurs.

REFERENCES

1. MAO, S. P. & SHAO, B. R. Schistosomiasis control in the People's Republic of China. *American journal of tropical medicine and hygiene*, **31**: 92-99 (1982).
2. SANTOS, A. T. The present status of schistosomiasis in the Philippines. *Southeast Asian journal of tropical medicine and public health*, **15**: 439-445 (1984).
3. HUNTER, G. W. & YOKOGAWA, M. Control of schistosomiasis japonica in Japan. *Japanese journal of parasitology*, **33**: 341-351 (1984).
4. MOTT, K. E. Diagnostic tools for *Schistosoma japonicum* in control programmes. *Arzneimittelforschung*, **34**(9B): 1217-1220 (1984).
5. MOTT, K. E. & DIXON, H. Collaborative study on antigens for immunodiagnosis of schistosomiasis. *Bulletin of the World Health Organization*, **60**: 729-753 (1982).
6. LONG, G. W. ET AL. Efficacy of purified *Schistosoma japonicum* egg antigens for ELISA serodiagnosis of human schistosomiasis japonica: specificity and sensitivity. *American journal of tropical medicine and hygiene*, **31**: 1006-1014 (1982).
7. BARRAL-NETTO, M. ET AL. Specificity of antibody and cellular immune responses in human schistosomiasis. *American journal of tropical medicine and hygiene*, **32**: 106-113 (1983).
8. NORDEN, A. P. & STRAND, M. *Schistosoma mansoni*, *S. haematobium* and *S. japonicum* identification of genus and species-specific antigenic egg glycoproteins. *Experimental parasitology*, **58**: 333-344 (1984).
9. KOJIMA, S. ET AL. Associations between HLA and immune responses in individuals with chronic schistosomiasis japonica. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78**: 325-329 (1984).
10. CHEN, M. G. ET AL. Immunologic function in schistosomiasis japonica patients before and after treatment. *Chinese medical journal*, **96**: 917-920 (1983).
11. YOGORE, M. G. JR ET AL. Seroepidemiology of schistosomiasis japonica by ELISA in the Philippines. I. Underestimation by stool examination of the prevalence of infection in school children. *American journal of tropical medicine and hygiene*, **32**: 1322-1324 (1983).
12. HIRATA, M. ET AL. Prevalence of antibodies to *Schistosoma japonicum* in the Chikugo River basin. *Kurume medical journal*, **28**: 227-232 (1981).
13. ISHII, A. & OWHASHI, M. Enzyme-linked immunosorbent assay with egg antigens of *S. japonicum*. *Zeitschrift für Parasitenkunde*, **67**: 279-287 (1982).
14. TANAKA, H. ET AL. Evaluation of micro-ELISA for schistosomiasis japonica using crude egg antigen. *Japanese journal of experimental medicine*, **53**: 147-154 (1983).
15. TSANG, V. C. W. ET AL. Demonstration of species-specific and cross-reactive components of adult microsomal antigens from *Schistosoma mansoni* and *S. japonicum* (MAMA and JAMA). *Journal of immunology*, **132**: 2607-2613 (1984).
16. YU, S. H. ET AL. A study of some laboratory procedures for diagnosing schistosomiasis mansoni. *Acta Academiae Medicinae Sinicae*, **1**: 131-135 (1979).
17. KAMIYA, H. Circumoval precipitin (COP) test using air-dried eggs of *Schistosoma japonicum* and *S. mansoni*. *Southeast Asian journal of tropical medicine and public health*, **14**: 451-455 (1983).

18. MITCHELL, G. F. ET AL. Hybridoma-derived antibody with immunodiagnostic potential for schistosomiasis japonica. *Proceedings of the National Academy of Sciences, USA*, **78**: 3165–3169 (1981).
19. MATSUDA, H. ET AL. Comparative studies on reading criteria of circumoval precipitin reaction of *Schistosoma japonicum* for field survey in highly endemic areas. *Japanese journal of experimental medicine*, **47**: 369–375 (1977).
20. GARCIA, E. ET AL. Evaluation of some laboratory procedures for the diagnosis of schistosomiasis japonica. *Journal of the Philippine Medical Association*, **45**: 86–95 (1969).
21. CARTER, C. & COLLEY, D. *Schistosoma japonicum* soluble egg antigens separation by con A chromatography and immunoaffinity purification. *Journal of immunology*, **18**: 219–225 (1981).
22. NAGATA, T. Evaluation of antigen preparations derived from *Schistosoma japonicum* eggs for enzyme-linked immunosorbent assay (ELISA). *Japanese journal of parasitology*, **31**: 303–312 (1982).
23. OWHASHI, M. & ISHII, A. Fractionation and characterization of allergens extracted from eggs of *Schistosoma japonicum*. *International archives of allergy and applied immunology*, **64**: 146–156 (1981).
24. TSANG, V. C. W. ET AL. *Schistosoma mansoni* adult microsomal antigens, a serologic reagent. I. Systematic fractionation, quantification and characterization of antigenic components. *Journal of immunology*, **130**: 1359–1365 (1983).
25. TSANG, V. C. ET AL. The quantitative single-tube kinetic-dependent enzyme-linked immunosorbent assay (K-ELISA). *Methods in enzymology*, **92**: 391–403 (1983).
26. GARCIA, E. ET AL. Attempts to standardize the circumoval precipitin test (COPT) for schistosomiasis japonica. *Southeast Asian journal of tropical medicine and public health*, **12**: 384–395 (1981).
27. CRUISE, K. M. ET AL. Sj23, the target antigen in *Schistosoma japonicum* adult worms of an immunodiagnostic hybridoma antibody. *Parasite immunology*, **5**: 37–46 (1983).