

Serodiagnosis of Imported Schistosomiasis by a Combination of a Commercial Indirect Hemagglutination Test with *Schistosoma mansoni* Adult Worm Antigens and an Enzyme-Linked Immunosorbent Assay with *S. mansoni* Egg Antigens

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A commercial indirect hemagglutination (IHA) test using erythrocytes coated with *Schistosoma mansoni* adult worm antigens (WA) and an enzyme-linked immunosorbent assay (ELISA) with *S. mansoni* egg antigens (SEA) were assessed for their use in serodiagnosis of imported schistosomiasis (hereafter these tests are designated WA/IHA and SEA/ELISA, respectively). The sensitivity of the tests was evaluated with sera from 75 patients with proven *S. mansoni* infection, 25 with proven *S. haematobium* infection, and 10 with clinical Katayama fever. The specificity was assessed with sera from 283 patients with various parasitic, bacterial, viral, and fungal infections and sera containing autoimmune antibodies. Sensitivities of the WA/IHA with a cutoff titer of 1:160 (WA/IHA¹⁶⁰) in detecting *S. mansoni*, *S. haematobium*, *S. mansoni* and *S. haematobium* combined, and clinical Katayama fever were 88.0, 80.0, 86.0, and 70.0%, respectively, with a specificity of 98.9%. The WA/IHA with a cutoff of 1:80 (WA/IHA⁸⁰) showed sensitivities of 94.7, 92.0, 94.0, and 90.0%, respectively, with a specificity of 94.7%. The comparable values of SEA/ELISA were 93.3, 92.0, 93.0, and 50.0%, respectively, with a specificity of 98.2%. Combined use of ELISA and WA/IHA⁸⁰ gave sensitivities of 100% for *S. mansoni*, *S. haematobium*, and *S. mansoni* and *S. haematobium* combined and 90% for Katayama fever. The specificity of this combination in detecting schistosomiasis was 92.9%. Combination of SEA/ELISA with WA/IHA¹⁶⁰ gave sensitivities of 98.7, 96.0, 98.0, and 80% with a specificity of 97.2%. Our findings suggest that WA/IHA and SEA/ELISA are each sensitive and specific serological tests that are easy to use for the diagnosis of imported schistosomiasis. The combined use of these two tests enabled the serological diagnosis of schistosomiasis to be achieved with very high degrees of both sensitivity and specificity.

Schistosomiasis is a major cause of morbidity and mortality and has been estimated to infect over 200 million people. An estimated 500- to 600 million people worldwide are still at risk of infection. The disease occurs mostly in the tropical regions, particularly in Africa, South America, and the Far East, and is endemic in 74 developing nations (25).

Schistosomiasis is frequently imported into nonendemic areas by immigrants and travelers returning from the tropics (30). Cases of imported schistosomiasis are on the increase due to changes in travel destinations and habits of travelers while abroad (7).

Most of these patients are asymptomatic, but recent infection can cause serious disease, such as Katayama fever or severe neurological complications involving the spinal cord (22, 28).

Diagnosis of schistosomiasis by detection of specific antibodies is likely to be more sensitive than the traditional method of diagnosis by detection of eggs in stool or urine (18). In im-

ported infections, with only a few or no eggs being excreted, antibody detection may be the only means to diagnose schistosomiasis.

In order to incorporate serodiagnostics in routine clinical laboratory practice, an easy to use, sensitive, and specific serological test is needed. Unfortunately only a few serological tests for schistosomiasis are commercially available, and still fewer have been evaluated for their diagnostic use. These and other difficulties (e.g., those inherent in antigen preparation for such tests) tend to restrict serodiagnosis in general to larger research centers. Serological tests which could, however, be used in routine clinical laboratories are an indirect hemagglutination (IHA) assay with adult *Schistosoma mansoni* worm antigens (WA) produced by Fumouze Laboratories (Levallois-Perret, France) (hereafter this assay is referred to as WA/IHA) and an enzyme-linked immunosorbent assay (ELISA) with *S. mansoni* soluble egg antigens (SEA) (hereafter referred to as SEA/ELISA).

In this study we evaluated the results obtained with WA/IHA and SEA/ELISA and the combined results of both tests for their sensitivity and specificity for patients returning from the tropics with egg-positive *S. mansoni* and *S. haematobium* infections and individuals presenting at the clinic with Katay-

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TABLE 1. Sensitivity and specificity of the WA/IHA, SEA/ELISA, and the combination of both tests in detecting schistosomiasis

Group studied (no.) and criterion assessed	Test results (%)				
	IHA ⁸⁰	IHA ¹⁶⁰	ELISA positive ^a	IHA ⁸⁰ and/or ELISA positive ^a	IHA ¹⁶⁰ and/or ELISA positive ^a
Sensitivity					
<i>S. mansoni</i> (75)	94.7	88.0	93.3	100	98.7
<i>S. haematobium</i> (25)	92.0	80.0	92.0	100	96.0
<i>S. mansoni</i> and <i>S. haematobium</i> (100)	94.0	86.0	93.0	100	98.0
Katayama (10)	90.0	70.0	50.0	90.0	80.0
Specificity					
Controls (283)	94.7	98.9	98.2	92.9	97.2

^a ELISA OD₄₉₂ > 0.222.

ama fever. The specificity of the test was evaluated with patients with various other infections and autoimmune disorders.

MATERIALS AND METHODS

Patients and sera. Patients incorporated into this study attended The Academic Medical Centre, Amsterdam, The Netherlands; The Harbor Hospital and Institute of Tropical Diseases, Rotterdam, The Netherlands; and The Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. A total of 393 patients with the following characteristics were used. (i) One hundred patients had egg-proven schistosomiasis (75 patients with *S. mansoni* and 25 patients with *S. haematobium*). (ii) Ten patients had Katayama fever. All patients had recently visited a schistosoma-endemic African country. Patients presented with eosinophilia, fever, symptoms of myalgia, arthralgia, persistent dry cough, weight loss, or general malaise. In two cases *S. mansoni* eggs were observed in stool samples. (iii) Two hundred-forty patients had other infectious diseases. All patients had proven active infections or were recently infected. Included were patients with fascioliasis hepatica (3), hookworm infection (10), trichuriasis (16), strongyloidiasis (11), filariasis bancrofti (24), onchocerciasis (12), loiasis (10), hepatic amoebiasis (7), visceral leishmaniasis (5), malaria (9), toxoplasmosis (11), syphilis (8), borreliosis (9), human immunodeficiency virus infection (11), cytomegalovirus infection (12), Epstein-Barr infection (12), hepatitis A (22), hepatitis B (13), rubella (11), Coxsackie B virus infection (11), and aspergillosis (11). (iv) Twenty-three patients had autoimmune antibodies (12 patients with rheumatoid factor and 11 with anti-nuclear antibodies). (v) Twenty patients were healthy blood donors originating from The Netherlands.

WA/IHA. The IHA test kit sold by Fumouze Laboratories was used according to the manufacturer's instructions, with a modification that the U-shaped plates provided by the manufacturer were replaced with V-shaped microtiter plates (Greiner Laboratories, Alphen, The Netherlands), as the latter allowed better readability of the results. Absence of agglutination was observed with the V-shaped well as a clear, sharp dark spot instead of the more or less wide ring seen with the original U-shaped well.

Briefly, the test procedure was as follows. Fifty microliters of a 1:20 initial dilution of each serum was subjected to further twofold serial dilutions, and 10 μ l of sheep red blood cells sensitized with *S. mansoni* adult WA was added to each diluted sample. Positive and negative control sera and nonsensitized red blood cells were included in each test as controls for naturally occurring antibodies. After incubation for 2 h at room temperature the titer in the test serum was recorded as one dilution before that which yielded a clear, sharp dark spot similar to those in the negative control wells. Titers were expressed as reciprocal values. All sera were tested in duplicate. The results were evaluated with a cutoff titer of 1:160 as recommended by the manufacturer and also with a cutoff of 1:80.

SEA/ELISA. SEA was prepared by retrieving eggs from the tissues of mice heavily infected with an *S. mansoni* isolate from Puerto Rico. The origin of the parasites, details of their laboratory maintenance, and methods for isolation of eggs have been described previously (11, 12). The eggs were homogenized by disrupting them for 3 min in an ice-cooled glass homogenizer with a mechanically rotated Teflon plunger and were centrifuged at 20,000 \times g for 3 h, and the supernatant was used as the antigen in the ELISA.

The ELISA for detection of serum immunoglobulin G against SEA was performed as described previously (10, 20, 21), the concentrations of antigen and the dilution of serum samples and anti-human immunoglobulin G peroxidase conjugate having been determined by checkerboard titration (29). However, polystyrene high-binding and flat-bottom enzyme immunoassay/radioimmunoassay

plates (Costar, Corning, N.Y.) with small wells were used. The reaction volume of each reagent at each step in these plates was 30 μ l per well. Wells were sensitized with SEA to make a concentration of 1.3 μ g/ml with 0.05 M carbonate-bicarbonate buffer, pH 9.6. The sensitization was performed overnight at 4°C. After removal of the unbound antigen, the wells were treated with 1% chicken egg white in phosphate-buffered saline (PBS) at 37°C for 30 min. Wells were washed five times with 0.05% Tween 20 in PBS, and serum samples (1:400 dilution) were added to the wells and incubated for 30 min at 37°C. Each serum was tested in duplicate. After washing the wells five times they were filled with a 1:1,500 dilution of horseradish peroxidase-conjugated goat antibody to human immunoglobulin G (Nordic, Tilburg, The Netherlands). Following incubation for 30 min at 37°C the wells were washed three times and substrate solution (0.01% 5-amino-2-salicylic acid in phosphate buffer, pH 5.95, with 0.03% H₂O₂) was applied to the wells for 1 h in the dark at room temperature. The optical density (OD) at 492 nm was read in an ELISA reader designed by Labsystems (Helsinki, Finland). To correct for day-to-day assay variation the results were expressed as ratios between the absorbance value of the sample and that of a well-defined control serum. The cutoff value of 0.222 was defined as the mean OD plus two standard deviations of the serum samples from the 283 controls (240 patients with other infectious diseases, 23 with autoantibodies, and 20 healthy Dutch blood donors).

Definitions. The WA/IHA was considered positive if the titer of the serum was equal to or greater than the cutoff titers of 1:80 or 1:160. The SEA/ELISA was considered positive if the OD value of the serum was equal to or greater than the cutoff OD of 0.222. The combination of both tests was deemed positive if either one or both of the tests gave a positive reaction. Negative reactions in WA/IHA and SEA/ELISA showed lesser values than the cutoff values. A sample was considered negative if both tests had given negative reactions.

The sensitivity of the WA/IHA, the SEA/ELISA, and of the combination of both tests was defined as the number of patients who gave a positive test result as a proportion of the total number of patients who had parasitologically proven schistosomiasis or clinical Katayama fever.

The specificity of the tests and of the combination of the two tests in detecting schistosomiasis was defined as the number of patients who gave a negative test result as a proportion of the total number of control patients (other infections, autoimmune antibodies, and healthy Dutch blood donors).

RESULTS

Sensitivities of WA/IHA, SEA/ELISA, and the combination of both tests in detecting *S. mansoni*, *S. haematobium*, *S. mansoni* and *S. haematobium* combined, and clinical Katayama fever and the specificities are shown in Table 1. Individual titers determined by WA/IHA and OD values determined by SEA/ELISA from schistosomiasis cases, clinical Katayama fever cases, and control cases are shown in Fig. 1 and 2, respectively.

Sensitivity of WA/IHA with a cutoff titer of 1:80 (WA/IHA⁸⁰) for individuals excreting eggs of *S. mansoni*, *S. haematobium*, both infections combined, and with clinical Katayama fever ranged from 90.0 to 94.7%. Sensitivity of WA/IHA with a cutoff titer of 1:160 (WA/IHA¹⁶⁰) for detection of egg-posi-

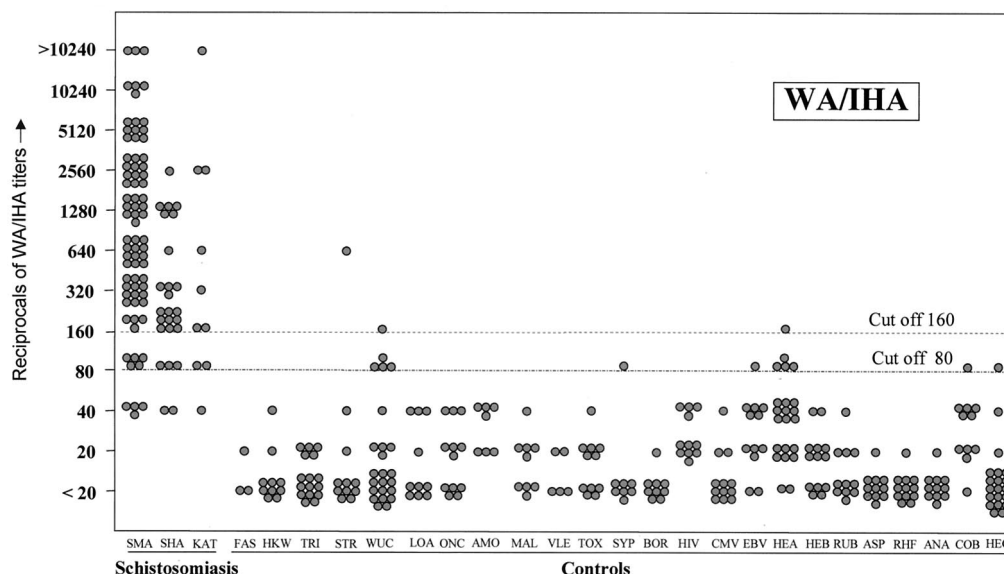


FIG. 1. Reciprocals of WA/IHA titers in sera of individuals with *S. mansoni* (SMA), *S. haematobium* (SHA), Katayama fever (KAT), *Fasciola hepatica* (FAS), hookworm infection (HKW), trichuriasis (TRI), strongyloidiasis (STR), filariasis bancrofti (WUC), loiasis (LOA), onchocerciasis (ONC), hepatic amoebiasis (AMO), malaria (MAL), visceral leishmaniasis (VLE), toxoplasmosis (TOX), syphilis (SYP), borreliosis (BOR), human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis A (HEA), hepatitis B (HEB), rubella (RUB), aspergillosis (ASP), rheumatoid factor (RHF), anti-nuclear factor (ANA), and Cocksackie B virus (COB) and in sera of healthy Dutch blood donors (HEC).

tive cases and cases with clinical Katayama fever was lower and ranged from 70.0 to 88.0%. WA/IHA gave higher sensitivity in detecting *S. mansoni* than in detecting *S. haematobium*. The specificity in detecting combined *S. mansoni* and *S. haematobium* by WA/IHA⁸⁰ was 94.7%. Cross-reactive antibodies were observed in some of the putatively negative control sera: most cross-reactive reactions had a titer of 1:80 and were observed in the filariasis bancrofti and hepatitis A control groups. However, with a dilution of 1:160 as the endpoint in WA/IHA, cross-reactive antibodies were rare and WA/IHA¹⁶⁰ had a specificity of 98.9%.

The sensitivities of SEA/ELISA ranged from 50.0 to 93.3%. The sensitivities in detecting *S. mansoni* and *S. haematobium* were comparable. Specificity in detecting schistosomiasis was 98.2%.

The sensitivities of the results for WA/IHA⁸⁰ and SEA/ELISA combined ranged from 90.0 to 100%, while the sensitivities for the WA/IHA¹⁶⁰ and SEA/ELISA combination were 80.0 to 98.7%. The combination of WA/IHA⁸⁰ and SEA/ELISA gave a specificity of 92.9%, whereas WA/IHA¹⁶⁰ and SEA/ELISA combined had a specificity of 97.2%.

DISCUSSION

This study showed that both a commercially available IHA test using *S. mansoni* adult WA and an ELISA with SEA are sensitive and specific tests for the serodiagnosis of schistosomiasis in travelers from the tropics. However, a combination of these tests is recommended, because the pooled results gave higher sensitivity than either test alone while maintaining high specificity.

IHA tests using adult WA have been evaluated previously for serodiagnosis of schistosomiasis. All but one of these IHA

tests were homemade systems that are not readily available for use outside the laboratories that developed them (13–15, 17, 19), the exception being that sold by Dade (Cellognost, Schistosomiasis; Behringwerke AG, Marburg, Germany). In these IHA tests the sensitivity ranged from 71 to 80% and the specificity ranged from 80 to 100%. The IHA test that was produced by Fumouze until 1996 used soluble egg antigen for coating of the erythrocytes instead of the adult WA as in the test kit used for the present study (personal communication). With the former IHA test a sensitivity of 75.3% and a specificity of 96.9% was obtained (3). The present IHA test of Fumouze (i.e., the WA/IHA that was used here) has not been studied before in a clinical setting.

Although earlier studies suggested that IHA gave high specificity, these studies incorporated only a few control serum samples. Because schistosomiasis is often observed in conjunction with other infectious diseases (16, 24, 26) and autoimmune antibodies (1, 4, 5) and because cross-reactive antibodies are frequently observed with other serological tests for schistosomiasis (2, 6, 27), we included a large series of controls in the present study. Taking the size and variety of our control groups into account, the specificities observed with the WA/IHA of 98.9 and 94.7% for cutoff titers of 1:160 and 1:80, respectively, were impressive.

The sensitivities of the WA/IHA with the cutoff titer suggested by the manufacturer (1:160) were relatively low. For diagnosis of imported schistosomiasis a high sensitivity without a concomitant loss of specificity is, however, generally required. By lowering the cutoff from 1:160 to 1:80 the sensitivities of the WA/IHA increased strongly, with only a slight drop in specificity. On the basis of these observations we suggest that the cutoff titer could be lowered to 1:80. It is in any case

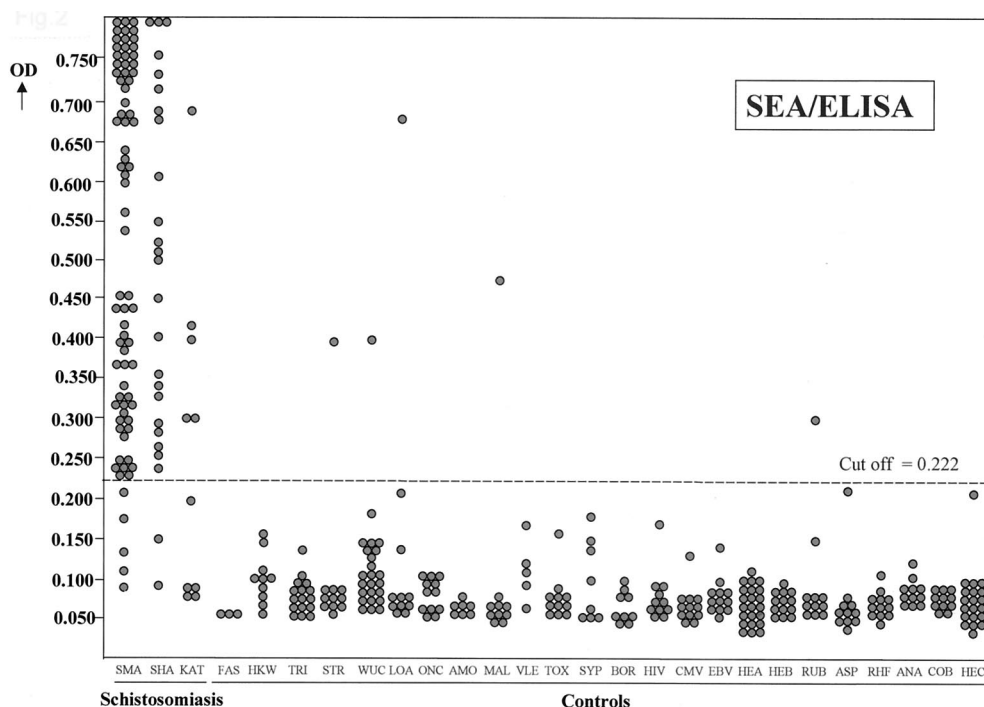


FIG. 2. OD values at 492 nm by SEA/ELISA in sera of individuals with *S. mansoni* (SMA), *S. haematobium* (SHA), Katayama fever (KAT), fascioliasis hepatica (FAS), hookworm infection (HKW), trichuriasis (TRI), strongyloidiasis (STR), filariasis bancrofti (WUC), loiasis (LOA), onchocerciasis (ONC), hepatic amoebiasis (AMO), malaria (MAL), visceral leishmaniasis (VLE), toxoplasmosis (TOX), syphilis (SYP), borreliosis (BOR), human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis A (HEA), hepatitis B (HEB), rubella (RUB), aspergillosis (ASP), rheumatoid factor (RHF), anti-nuclear factor (ANA), and Coxsackie B virus (COB) and in sera of healthy Dutch blood donors (HEC).

questionable as to whether all 15 control samples with titers greater than 1:80 were truly false positives. Most of these patients had visited schistosoma-endemic countries, and although parasitological examinations in these cases were negative, coinfection with *Schistosoma* spp. could not be excluded.

Because severe symptomatology is observed in some cases of Katayama fever, accurate diagnosis in this early stage of a schistosoma infection is important. *Schistosoma* egg production in patients with Katayama fever is often just commencing, and detection of eggs in stool or urine is mostly unsuccessful. Serodiagnosis, therefore, could be important. Our findings suggested that the WA/IHA⁸⁰ correctly diagnosed 90.0% of the patients with early infections associated with Katayama fever. These findings are in contrast to those of two earlier studies with IHA containing adult WA, a homemade IHA (9) and a commercially available IHA (Dade, Cellgnost, Schistosomiasis, Behringwerke AG) (23). These studies reported that the IHA was insensitive for detection of cases of early schistosomiasis associated with Katayama fever. In both studies IHA was compared with an indirect immunofluorescent test (IFAT) on gut-associated antigens of adult *S. mansoni* worms which appeared more sensitive than IHA in early schistosomiasis. Although the precise composition of the antigens used for erythrocyte coating in the commercially available WA/IHA used in our study is unknown, it is possible that the gut-associated antigens of the adult worm have been incorporated in the present IHA coating.

The negative IHA results (1:40) for one of the Katayama

patients studied is possibly a consequence of the short period between infection and clinical presentation. The IFAT also has a window period of approximately 30 days after infection before becoming positive (28). The WA/IHA titer of this patient rose to 1:640 3 months after the initial examination. Because of this delay in antibody response, patients clinically suspected of having Katayama fever but with an initially negative serological test result should be reexamined serologically a few weeks later.

The data from this study suggest that the WA/IHA performs slightly better for detection of *S. mansoni* than for detection of *S. haematobium*. This result agrees with those of others who used IHA tests with adult WA of *S. mansoni* (13, 14, 16). In these studies it was demonstrated that the sensitivity of the IHA improved by using homologous rather than heterologous antigens. To overcome the relatively limited sensitivity of the WA/IHA for *S. haematobium* infections the antigen composition of the IHA could be changed by incorporating antigens of *S. haematobium*.

ELISA with SEA was evaluated in this study as well. Because large amounts of SEA are difficult to obtain, we developed a more economic ELISA method with microtiter plates with smaller wells than the standard plates (i.e., 30 µl as opposed to 300 µl). In this form our ELISA had a sensitivity of 92.0% in detecting egg-proven cases of imported schistosomiasis.

This high sensitivity agreed with the results of several earlier studies carried out with standard microtiter plates in endemic

and nonendemic areas (10, 14, 20, 26), although in one recent hospital study in the United Kingdom a lower sensitivity of 72% was found (30). There was no difference with our ELISA in detecting *S. mansoni* and *S. haematobium*, which confirmed the results of other studies (14, 26).

Although the ELISA showed a high sensitivity in detecting egg-proven cases, the sensitivity in confirming clinical Katayama fever was low because only 5 of the 10 patients (50%) showed positive reactions. In this context it has previously been noted that ELISA against egg antigen is unlikely to detect an infection in which schistosoma egg production has just started but in which anti-egg antibody synthesis has not yet commenced. In this stage of infection signs and symptoms of Katayama fever are, however, often already present (28). Thus, the probability that cases of Katayama fever can be confirmed by ELISA by using egg antigens is relatively low.

Both high and low specificity with ELISA have previously been reported (10, 14, 26). Because of these variable results specificity in the present study was determined by using control sera, including those from patients with parasitic, fungal, bacterial, and viral infections, as well as from people with autoimmune antibodies. Despite this variety of control groups the specificity of the SEA/ELISA in detecting schistosomiasis was 98.2%, which was considered excellent. Cross-reactivity that was earlier observed with, e.g., filariasis and hepatitis, was not observed in our study. Our results thus suggest that ELISA carried out on plates containing small wells is a very economic, sensitive, and specific test to detect schistosomiasis after the onset of egg production.

Although this study suggests that WA/IHA and SEA/ELISA are good diagnostic tools, improvement of diagnosis might be accomplished by combining the results of the two tests. In our study sensitivity and specificity were determined after combination of the results of either the WA/IHA⁸⁰ or the WA/IHA¹⁶⁰ with those of the SEA/ELISA. It showed that these combinations had the highest sensitivities of this study. Thus, the combination of WA/IHA⁸⁰ and SEA/ELISA detected 100% of the schistosomiasis cases and 90% of the Katayama cases and had a specificity of 92.9%. The sensitivity of the combination of WA/IHA¹⁶⁰ and SEA/ELISA was lower but still gave 98.0% detection of egg-proven cases and 80% confirmation of Katayama fever with a specificity of 97.2%. Combining the results of both WA/IHA and SEA/ELISA thus improved the sensitivity with maintenance of high specificity. In an earlier study a combination of both IFAT and ELISA has also been claimed to give a higher reliability in diagnosis of schistosomiasis, but the sensitivity and specificity of this combination were not reported (8).

In conclusion, we suggest that a commercially available IHA is a good diagnostic tool for detection of imported schistosomiasis. The test is easily applicable and specific. Both infections with *S. mansoni* and *S. haematobium* can be diagnosed, and the test is sensitive for confirmation of Katayama fever. Our SEA/ELISA is also an economic, sensitive, and specific test for detection of schistosomiasis after the onset of egg production. Pooling the results of both the WA/IHA and the SEA/ELISA gave the most reliable outcome because it increased considerably the sensitivity in all stages of infection with maintenance of high specificity, particularly when the

results of the WA/IHA¹⁶⁰ were used in the combination with those of the SEA/ELISA.

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