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Evaluation of Eight Serological Tests for Diagnosis of Imported Schistosomiasis

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The diagnosis of schistosomiasis in individuals from countries where the disease is not endemic is challenging, and few data are available on the accuracy of serological diagnosis in those patients. We evaluated the performance of eight serological assays, including four commercial kits, in the diagnosis of imported schistosomiasis in individuals from areas where the disease is not endemic, including six enzyme-linked immunosorbent assays using three different antigens, an indirect hemagglutination assay, and an indirect immunofluorescent-antibody test. To analyze the assays, we used a total of 141 serum samples, with 121 derived from patients with various parasitic infections (among which were 37 cases of schistosomiasis) and 20 taken from healthy volunteers. The sensitivity values for detection of schistosomiasis cases ranged from 41% to 78% and were higher for *Schistosoma mansoni* than for *S. haematobium* infections. Specificity values ranged from 76% to 100%; false-positive results were most frequent for samples from patients with cestode infections. By combining two or more tests, sensitivity improved markedly and specificity decreased only moderately. Serological tests are useful instruments for diagnosing imported schistosomiasis in countries where the disease is not endemic, but due to limitations in test sensitivities, we recommend the use of two or more assays in parallel.

Schistosomiasis is one of the world's most prevalent parasitic infections, with at least 200 million people infected and about 700 million at risk in Africa, Asia, and South America (46). Although five species of waterborne trematodes in the genus *Schistosoma* are capable of causing human infection, the most important are *Schistosoma mansoni* and *S. haematobium*, and the majority of cases occur in sub-Saharan Africa (18). Due to the geographical distribution of schistosomiasis and the affected populations, schistosomiasis is listed as a neglected tropical disease and a neglected infection of poverty. Apart from this, imported schistosomiasis has been recognized as an emerging clinical problem in countries where the disease is not endemic. The infection affects expatriates and immigrants but also travelers, especially in association with adventure and ecotourism (9, 17, 23, 35).

During early stages, schistosome infections might cause severe manifestations, such as Katayama fever, schistosomal myeloradiculopathy, and pneumonitis (6, 7, 25, 28, 37). However, up to 50% of newly infected patients remain asymptomatic (29, 35, 45). Independent of the initial presentation, untreated schistosomiasis might lead to complications such as obstructive uropathies, hepatic fibrosis, or granulomatous cerebral lesions (7, 29, 35). To prevent those late manifestations, any case of schistosomiasis should be detected and treated (35).

Like the case for other parasitic infections, the diagnostic approach to schistosomiasis depends on the epidemiological situation. In endemic settings, parasitological examinations are the mainstay of diagnosis. Serological examinations, such as screening for antischistosomal antibodies, are of limited use for the diagnosis of active infection, as large parts of the population may carry antibodies due to past infections. The diagnosis of imported schistosomiasis in individuals from countries where the disease is not endemic bears other challenges. First, those patients seem to be more prone to acute manifestations, which occur during early stages of infection and sometimes during the prepatent period (18). Furthermore, exposure to cercarial larvae is usually limited, resulting in infections with low parasite loads (11, 25, 28). Therefore, direct parasitological methods often fail (11). For this patient group, serological tests detecting antischistosomal antibodies are an important diagnostic tool (26, 38, 44), although the "seronegative window" has to be considered in very early infections (9, 25, 43).

Over the last decades, various serological methods have been developed to detect antibodies against *Schistosoma* antigens. Different techniques have been applied, including indirect immunofluorescent-antibody tests (IFATs), indirect hemagglutination assays (IHAs), and enzyme-linked immunosorbent assays (ELISAs) using different antigens, such as crude or purified adult worm antigen (AWA), soluble egg antigen (SEA), and cercarial antigen (CA) preparations (3–5, 8, 10–12, 14, 15, 20, 21, 30, 33, 34, 38, 41, 42, 44). Nevertheless, very few studies have addressed the value of serological assays for diagnosis of schistosomiasis in individuals from areas where the disease is not endemic and who are carrying light and/or recently acquired infections; most of those describe single in-house assays and are limited by small sample numbers (14, 39, 41). Only one study analyzed a commercial test together

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with an in-house assay (42), and to the best of our knowledge, comparative studies of multiple serological tests have not yet been published.

Our study analyzed the performance of eight serological assays, including four commercial tests, in the diagnosis of imported schistosomiasis in individuals from countries where the disease is not endemic, using sera from patients with parasitologically proven infections with *S. mansoni*, *S. haematobium*, and various other parasites.

MATERIALS AND METHODS

Patients and sera. A total of 141 sera, all stored at −80°C at the serum bank of the Institute of Tropical Medicine and International Health (ITMIH), Berlin, Germany, were used. Samples were obtained from patients visiting the institute's outpatient clinic between 1985 and 2005 and were stored for further research with the written consent of the patients. All parasitic infections were diagnosed by the institute's diagnostic laboratory, which serves as a reference center for parasitic diseases. Parasitological diagnosis of schistosomiasis was reached by microscopic demonstration of viable eggs in stool samples, filtered urine, or rectal biopsy specimens. Other parasites were detected using standard parasitological and serological methods.

Patients were identified using the institute's databases. To avoid false-positive serological results caused by past infections, only individuals from countries where schistosomiasis is not endemic and with parasitologically proven active schistosomiasis who acquired infection within 52 weeks before diagnosis and who did not have a previous travel history to countries where schistosomiasis is endemic were included. Patients with parasitic coinfections were excluded. A total of 37 patients were identified, 12 of whom were female, who met all of the above-mentioned criteria. Most of them had acquired schistosomiasis during long-term travel to sub-Saharan Africa (Table 1). Twenty-one patients were infected with *S. mansoni* and 14 with *S. haematobium*, 1 patient suffered from double infection with both species, and 1 had a triple infection with *S. mansoni*, *S. haematobium*, and *S. intercalatum*. Serum samples were taken at the time of parasitological diagnosis, when egg production was already present. Serum samples from 104 individuals who had never visited areas where schistosomiasis is endemic served as controls. Of those individuals, 84 had other parasitic infections, including ascariasis (15), hookworm infection (3), trichuriasis (9), *Taenia solium* infection (3), *Trichuris trichiura-T. solium* coinfection (1), strongyloidiasis (6), fascioliasis (1), filariasis (1), *Hymenolepis nana* infection (5), *Dicrocoelium dendriticum* infection (1), cysticercosis (4), echinococcosis (4), *Entamoeba histolytica* infection (5), giardiasis (5), *E. histolytica-Giardia lamblia* coinfection (4), visceral leishmaniasis (6), and malaria (11). Patients with multiple parasitic infections were excluded from the control group.

TABLE 1 Characteristics of cases of imported schistosomiasis[a]

Serum no.	Species	Travel destination(s) of patient	Maximal possible time of exposure (wk)	Time between return and serological testing (wk)
1	*S. mansoni*	Africa, Central America	42	20
2	*S. mansoni*	West Africa, Central America	28	24
3	*S. mansoni*	Sudan	20	4
4	*S. mansoni*	Sudan	4	13
5	*S. mansoni*	Morocco	8	2
6	*S. mansoni*	Uganda	10	7
7	*S. mansoni*	East Africa	18	1
8	*S. mansoni*	Ghana, Togo	8	2
9	*S. mansoni*	Ghana	4	4
10	*S. mansoni*	Ghana	4	6
11	*S. mansoni*	Ghana	ND	ND
12	*S. mansoni*	Somalia	16	3
13	*S. mansoni*	Ghana	8	4
14	*S. mansoni*	Ghana	11	37
15	*S. mansoni*	Ghana	3	6
16	*S. mansoni*	West Africa	20	7
17	*S. mansoni*	Ghana	28	17
18	*S. mansoni*	South Africa	8	16
19	*S. mansoni*	Saudi Arabia	8	6
20	*S. mansoni*	West Africa	32	1
21	*S. mansoni*	Egypt	5	4
22	*S. haematobium*	Chad	24	4
23	*S. haematobium*	West Africa	8	8
24	*S. haematobium*	South Africa	5	3
25	*S. haematobium*	East Africa	10	40
26	*S. haematobium*	East Africa	8	24
27	*S. haematobium*	Mali	4	15
28	*S. haematobium*	Mali	4	16
29	*S. haematobium*	South Africa, Botswana	6	24
30	*S. haematobium*	Zambia	8	45
31	*S. haematobium*	South Africa, Kenya	20	0
32	*S. haematobium*	Tanzania	4	36
33	*S. haematobium*	East Africa, southern Africa	50	8
34	*S. haematobium*	South Africa	16	10
35	*S. haematobium*	Cote d'Ivoire	77	12
36	*S. mansoni*, *S. haematobium*	Central America	6	4
37	*S. mansoni*, *S. haematobium*, *S. intercalatum*	Ghana	4	1

[a] ND, no data available; wk, week.

Serological assays. Eight serological assays were tested in parallel. All tests were performed by experienced technicians at the diagnostic laboratory of the ITMIH, Berlin, Germany. Four tests were commercial products; the other four represented established in-house protocols at the ITMIH. Since patients with suspected imported schistosomiasis usually suffer light and early infections, all indeterminate results were counted as positive.

In-house IFAT. The IFAT was performed on paraffin-embedded sections of adult *S. mansoni* worms (Puerto Rico strain), which were prepared as previously described (33). Sections were first deparaffinized by two washes (30 min at 37°C and 15 min at 20°C) in 100% xylene (Merck, Darmstadt, Germany) and five washes (1 min each at 20°C) in ethanol at decreasing concentrations (100%, 95%, 70%, 50%, and 30%), with a final rinse in sterile water. Serum samples were diluted in phosphate-buffered saline (PBS). Next, 12-μl samples of 1:20 and 1:80 dilutions were applied to the slides with the sections. After incubation (30 min, 37°C) in a humid chamber, slides were washed thrice with PBS and once with sterile water and then dried at room temperature. Each section was covered with 12 μl of 1:200-diluted (PBS) fluorescein isothiocyanate (FITC)-conjugated goat anti-human secondary antibodies (Fluoline; bioMérieux, Marcy l'Etoile, France) against human immunoglobulins (IgG and IgM) and incubated (30 min, 37°C) in a humid chamber. After further washing with PBS (thrice) and sterile water (once), slides were dried, mounted with Fluoprep (bioMérieux, Marcy l'Etoile, France), and covered with a cover slide prior to microscopic evaluation. Ten different worm sections were examined for each patient. A consistent focal gut-associated fluorescence staining pattern for the dilution of 1:80 or higher was considered positive; staining at dilutions of 1:20 and 1:40 was classified as indeterminate. A negative control (1:20 dilution) and a positive control at three dilutions (1:20, 1:80, and 1:320) were included in each test series to ascertain validity.

In-house ELISAs. Three separate assays using different crude antigen preparations were performed: (i) ELISA/CA, with cercarial antigen; (ii)

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TABLE 2 Performances of eight serological tests for diagnosis of schistosomiasis

Test[a]	Sensitivity[d]			Specificity[d] (*n* = 104)	Indeterminate results[c]
	All cases[b] (*n* = 37)	*S. mansoni* (*n* = 21)	*S. haematobium* (*n* = 14)		
IFAT	75.7 (58.4–97.6)	85.7 (62.6–96.2)	64.3 (35.6–86.0)	98.1 (92.5–99.7)	10.7/5.6/22.2
ELISA/CA	40.5 (25.2–57.8)	47.6 (26.4–69.7)	21.4 (5.7–51.2)	95.2 (88.6–98.2)	80.0/70.0/100.0
ELISA/AWA	54.1 (37.1–70.2)	76.2 (52.4–90.9)	21.4 (5.7–51.2)	100 (95.6–100)	28.6/31.3/25.0
ELISA/SEA	75.7 (58.4–97.6)	90.5 (68.2–98.3)	57.1 (29.6–81.2)	97.1 (91.2–99.3)	50.0/52.6/50.0
IHA	73.0 (55.6–85.6)	76.2 (52.4–90.9)	71.4 (42.0–90.4)	99.0 (94.0–100)	22.2/0.0/60.0
ELISA/NovaTec	64.9 (47.4–79.3)	81.0 (57.4–93.7)	35.7 (14.0–64.4)	99.0 (94.0–100)	16.7/11.8/20.0
ELISA/DRG	78.3 (61.3–89.6)	85.7 (62.6–96.2)	71.4 (42.0–90.4)	88.4 (80.3–93.6)	NA
ELISA/Viramed	67.6 (50.1–81.4)	71.4 (47.7–87.8)	64.3 (35.6–86.0)	76.9 (67.4–84.4)	NA

[a] For explanations of test abbreviations, see Materials and Methods.
[b] Includes two cases of infections with multiple *Schistosoma* species.
[c] Percent of indeterminate test results among positive test results for all schistosomiasis cases/*S. mansoni*/*S. haematobium*.
[d] Data are percentages (95% CI). NA, not applicable (test interpretation schedule provided by the manufacturer does not include indeterminate results).

ELISA/AWA, with soluble adult worm antigen; and (iii) ELISA/SEA, with soluble egg antigen. Antigens were derived from *S. mansoni* (Puerto Rico strain). Antigen preparation and testing were performed according to the method of Feldmeier and Büttner (15). Antigens were stored at −80°C until use. Microtiter plates were pretreated with a mixture of acetone and methanol (1.5:8.5) to improve antigen coating. Plates were freshly coated with antigen prior to every test run. Crude antigen preparations were used at the following concentrations: AWA, 0.49 μg/ml; SEA, 0.18 μg/ml; and CA, 0.18 μg/ml (diluted in 50 mM carbonate buffer, pH 9.6). One hundred microliters of serum (diluted 1:100 in PBS-0.05% Tween 20 with 1% bovine serum albumin [BSA]) was added and incubated for 45 min at room temperature. After three washes, 100 μl of alkaline phosphatase-conjugated rabbit anti-human IgG (Dako, Glostrup, Denmark) diluted 1:1,500 (in PBS-0.05% Tween 20 with 1% BSA) was added and incubated for 45 min at room temperature. After three washes, 100 μl of *p*-nitrophenylphosphate (Sigma, Munich, Germany) was added and incubated for up to 50 min at room temperature. The optical density at 405 nm (OD_{405}) was determined, with a reference at 490 nm. Every test series included negative and positive controls, in duplicate. A test was defined as positive, indeterminate, or negative if the respective OD_{405} was ≥0.1, ≥0.05 and <0.1, or <0.05, respectively.

Commercial tests. Four commercial serological assays were tested, including an IHA and three ELISAs: (i) Bilharziose Fumouze IHA (Fumouze Diagnostics, Levallois-Perret, France), (ii) *Schistosoma mansoni* IgG-ELISA (ELISA/NovaTec; NovaTec Immundiagnostica, Dietzenbach, Germany), (iii) *Schistosoma mansoni* IgG ELISA (ELISA/DRG; DRG Instruments, Marburg, Germany), and (iv) *Schistosoma* serology microwell ELISA (ELISA/Viramed; Viramed Biotech, Planegg, Germany). Tests were performed according to the manufacturers' instructions. For the detection of serum antibodies, the IHA used sensitized sheep erythrocytes coated with adult worm antigen (42), ELISA/NovaTec utilized a soluble worm antigen preparation (male and female; Puerto Rico strain) (H. Duchman, NovaTec Immundiagnostica, personal communication), and ELISA/DRG as well as ELISA/Viramed used an unspecified soluble egg antigen preparation. IHA results were expressed as the last serum dilution that did not result in a clear spot of sedimented erythrocytes at the bottom of the microwell. Titers of ≥1:160 were considered significant, and a titer of 1:80 was considered indeterminate. For ELISAs, ODs were determined at 450 nm, with a reference at 620 nm, within 30 min after addition of stop solution. Samples were classified as positive, negative, or indeterminate according to the manufacturers' cutoff values.

Statistics. Sensitivity was defined as the proportion of patients with a positive test result among those with proven infection. Specificity was calculated as the proportion of patients with a negative test result among samples of the control group. The 95% confidence interval (95% CI) according to Wilson was determined using VassarStats' Clinical Calculator 1 (http://faculty.vassar.edu/lowry/clin1.html). To compare tests and test combinations, the 95% CI was used. A significant difference was assumed if the 95% CI ranges did not overlap. Since our serum panel did not represent a clinical setting or given population, other criteria of test accuracy, such as positive and negative predictive values or likelihood ratios, were not calculated. For species-specific values, only samples with monoinfections were included.

RESULTS

Of the 37 samples from patients with schistosomiasis, 35 (94.6%) were detected by one or more serological tests. The two sera (serum numbers 31 and 33) (Table 1) which were negative by all tests were derived from patients with *S. haematobium* infection: one had just returned from a 20-week stay in South Africa and Kenya and suffered from symptoms compatible with acute early infection (fever and skin manifestations), and the other was diagnosed with schistosomiasis 2 months after returning from a 1-year journey through eastern and southern Africa and was asymptomatic.

In total, we recognized 197 true-positive, 99 false-negative, 784 true-negative, and 48 false-positive test results. Performance parameters of each test are summarized in Table 2. Sensitivity values for detection of *S. mansoni* and *S. haematobium* infections ranged from 47.6% to 90.5% and 21.4% to 71.4%, respectively. All evaluated tests detected *S. mansoni* infections at higher rates than those for *S. haematobium* infections. Sensitivities for detection of any *Schistosoma* infection ranged from 40.5% to 78.3%. The sensitivity of ELISA/CA was significantly lower than those of IFAT, ELISA/SEA, and ELISA/DRG. Indeterminate results occurred more frequently with in-house tests and with samples from patients with *S. haematobium* infections (Table 2). The specificities of the tests ranged from 76.9% to 100% (Table 2). All tests except for ELISA/DRG and ELISA/Viramed exhibited high specificity values (>95%). ELISA/Viramed showed marked reactivity to samples from patients with cestode and trematode infections.

To estimate the usefulness of test combinations, we calculated the performance of selected combinations with the aim of minimizing false-negative results, i.e., a case with a positive or indeterminate result of any test within the combination was counted as positive. For test combinations, we chose the current diagnostic panel of the ITMIH (four in-house assays), which has been in routine use for many years, and compared it to different combinations of commercial tests (Table 3). In general, combinations of two or more tests increased the sensitivity of detection of cases of schistosomiasis. The ITMIH diagnostic panel had a sensitivity and

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TABLE 3 Performances of selected test combinations for diagnosis of schistosomiasis

Test combination[a]	Sensitivity[c] All cases[b] (n = 37)	Sensitivity[c] *S. mansoni* (n = 21)	Sensitivity[c] *S. haematobium* (n = 14)	Specificity[c] (n = 104)
All in-house tests (ELISA/CA + ELISA/AWA + ELISA/SEA + IFAT)	89.2 (73.6–96.5)	95.2 (74.1–99.8)	78.6 (48.8– 94.3)	93.3 (86.1–97.0)
All commercial tests (IHA + ELISA/NovaTec + ELISA/DRG + ELISA/Viramed)	91.9 (77.0–97.9)	100 (80.8–100)	78.6 (48.8– 94.3)	75.0 (65.4–82.7)
IHA + ELISA/NovaTec	89.2 (73.6–96.5)	95.2 (74.1–99.8)	78.6 (48.8– 94.3)	98.1 (92.5–99.7)
IHA + ELISA/DRG	89.2 (73.6–96.5)	95.2 (74.1–99.8)	78.6 (48.8– 94.3)	87.5 (79.2–92.9)
ELISA/NovaTec + ELISA/DRG	86.5 (70.4–94.9)	95.2 (74.1–99.8)	71.4 (42.0–90.4)	87.5 (79.2–92.9)

[a] For explanations of test abbreviations, see Materials and Methods.
[b] Includes two cases of infections with multiple *Schistosoma* species.
[c] Data are percentages (95% CI).

specificity of 89.2% and 93.3%, respectively, for detection of any schistosomiasis case. A similar combination of four commercial tests showed a lower specificity, caused mainly by ELISA/Viramed. Different combinations of two commercial tests, however, showed sensitivities and specificities that were comparable to those of the ITMIH standard panel.

DISCUSSION

Our study highlights the usefulness but also the limitations of serological assays for diagnosis of imported schistosomiasis in travelers. Within this patient group, serology has two aims: (i) to diagnose symptomatic infections and (ii) to screen asymptomatic individuals with reported freshwater exposure in areas where schistosomiasis is endemic. For both circumstances, a high test sensitivity is crucial. A known obstacle to serodiagnosis of acute or recent schistosome infection is the prolonged seronegative window period. Antibody production in newly infected individuals usually starts 4 to 7 weeks after infection, and although the majority of patients exhibit seroconversion within 3 months (24, 43, 45), prolonged seronegative window periods of up to 6 months have been described (16, 27). Among our serum samples, only 2 (5.4%) of 37 samples were not detected by any serological test. This suggests that almost all patients had seroconverted at the time that the samples were taken. Still, none of the single tests reached a sensitivity of >80% for all cases of schistosomiasis. For cases of *S. mansoni* infection, sensitivities were higher, but only one test reached a level of >90%. For *S. haematobium* infections, all eight tests had lower sensitivities, and weak (indeterminate) reactions were more frequent. These lower sensitivity values were not caused by a selection bias toward earlier infections, since the median return time from areas of endemicity was even longer for *S. haematobium* cases than for *S. mansoni* cases (13.5 weeks versus 5 weeks). Most likely, species-specific heterogeneities of the utilized antigens were responsible for the observed differences. Although it has been shown that *S. mansoni* proteins used for serological testing have sufficient similarity to allow serodiagnosis of *S. haematobium* infections (32), some species-specific differences remain, favoring test systems using homologous antigens (2, 19, 22, 30, 36, 39, 42). Nevertheless, species-specific performance differences were not determined only by the utilized antigens. AWA, for example, exhibited a smaller species-specific difference in the commercial assay (IHA) than in the in-house test (ELISA/AWA). Similar effects were observed for SEA, which suggests an additional effect of the antigen preparation and/or test technique.

The limitations in sensitivity are discordant with the results of a Dutch study that evaluated the identical IHA (using AWA) produced by Fumouze Diagnostics together with an in-house ELISA (using SEA), using a panel of sera from 100 patients with imported schistosomiasis (42). In that study, the sensitivities of both tests were higher and species-specific differences were less pronounced than in the present study: for example, the commercial IHA showed sensitivities of detection of *S. mansoni*, *S. haematobium*, and all schistosomiasis cases of 94.7%, 92.0%, and 94.0%, respectively. Our values were clearly lower, although we used the same cutoff level (1:80). These differences might have been caused by differences in the serum panel that was used in the Dutch study, e.g., a higher rate of migrants and expatriates with later, chronic, or more severe infections and/or a lower rate of asymptomatic or oligosymptomatic travelers. This highlights the variability of test sensitivity in different clinical settings and the difficulty in comparing diagnostic test performances obtained in different studies.

For a systematic comparison of the different test techniques and antigens, the number of positive samples in our study was too limited. In-house tests and commercial tests performed comparably, and no antigen was clearly superior to others. Still, ELISA/CA, the only test utilizing a cercarial antigen, exhibited the lowest sensitivity values. Theoretically, CA is an appropriate antigen to trace early infection, as it is one of the first antigens to which the immune system is exposed. But it has also been proposed that exposure of the dermal immune system to cercariae is of short duration and that the resulting immune response is quickly downregulated (31). Clinical data are controversial: for a group of 10 recently infected Dutch tourists, a CA-based ELISA exhibited high sensitivity (10), and a recent publication supports this finding (8). Still, other studies have come to other conclusions (12). Most probably, differences in antibody binding capacity of the antigens due to preparation techniques are responsible for variances in the accuracy of different tests using the same antigens.

To analyze test specificity, we used a panel of 104 samples from individuals without a history of travel to areas where schistosomiasis is endemic. Of those, 84 were taken while patients were suffering from parasitic infections, for study of possible cross-reactions. Six of the eight tests evaluated in the study showed specificity values of >95% and had only a few false-positive results, occurring mostly as indeterminate results for patients with different parasitic infections. The other two tests, ELISA/DRG and especially ELISA/Viramed, exhibited specificity problems, mainly with larval and adult cestode infections but also with some sera from patients with nematode and trematode infections (Table 4). Both tests utilized SEA as an antigen. In contrast, in-house ELISA/

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TABLE 4 False-positive test results for control samples with or without parasitic infections

Infection	No. of false-positive results/no. of samples tested								
	IFAT	ELISA/CA	ELISA/AWA	ELISA/SEA	IHA	ELISA/NovaTec	ELISA/DRG	ELISA/Viramed	Total
Nematodes	0/35	1/35	0/35	0/35	0/35	0/35	4/35	6/35	11/280
Cestodes	0/16	0/16	0/16	0/16	1/16	0/16	4/16	13/16	18/128
Trematodes	0/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2	2/16
Intestinal protozoa	0/14	2/14	0/14	1/14	0/14	0/14	1/14	1/14	5/112
Leishmania	1/6	1/6	0/6	1/6	0/6	0/6	1/6	1/6	5/48
Malaria	1/11	1/11	0/11	1/11	0/11	1/11	1/11	1/11	6/88
No infection	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20	1/160
Total	2/104	5/104	0/104	3/104	1/104	1/104	12/104	24/104	48/832

SEA had similar sensitivities but did not show such cross-reactions, which proves that these differences were not caused just by different cutoff levels. Cross-reactivity of SEA with cestode and nematode antigens was recently reported (8). In another study, false-positive reactions of SEA with samples from hookworm infections were reduced by treatment of SEA with sodium metaperiodate, a reducing substance (1), which again highlights the importance of antigen preparation for immunogenic characteristics of a test antigen.

In areas of endemicity, where past schistosome infections, high schistosome loads, and polyparasitism are frequent, serological testing requires high specificity to avoid false-positive results. In such settings, a conjunctive combination of serological tests (screening followed by confirmation) has been suggested (40). For the diagnosis of imported schistosomiasis, however, a high sensitivity is important, and a disjunctive combination of two or more serological tests seems more useful (26). We evaluated this strategy and demonstrated that the performance of test combinations was clearly superior to that of single tests. With the combination of two commercial assays, sensitivity values of almost 90% were reached. Although our study was too small for a systematic analysis of combination testing, we believe that at least two different serological assays should be used in parallel to achieve sufficient sensitivity for diagnosis of patients from settings where schistosomiasis is not endemic, such as travelers. Combining two or more tests disjunctively might diminish specificity, so test combinations should be chosen carefully.

Some limitations of the present study are that it was done retrospectively and that it included sera only from patients with egg-proven schistosomiasis. Since the prepatent period of schistosomiasis is 4 to 6 weeks, our gold standard had a negative bias for very early infections and a probable positive bias toward larger parasite burdens, since parasitological methods are more sensitive to larger worm loads (13). Still, parasitological proof is the accepted diagnostic gold standard (9) and also allows analysis of species-specific differences. The study was also limited by the number of available positive samples and did not include samples of *S. japonicum* and other *Schistosoma* species. A prospective multicenter study is needed to overcome most of the above-mentioned limitations.

In conclusion, all eight examined serological tests were useful tools for the diagnosis of imported schistosomiasis. Still, sensitivity values were rather low and showed significant species-specific differences. Specificity was high, with the exception of two commercial tests using SEA as antigen. For the screening and diagnosis of imported schistosomiasis, test combinations of two or more serological tests should be used to improve sensitivity.

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