Magnetic Affinity Enzyme-Linked Immunoassay for Diagnosis of Schistosomiasis Japonicum in Persons with Low-Intensity Infection

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Abstract. Most schistosome-endemic areas in China are characterized by low-intensity infections that are independent of prevalence. To establish an effective diagnostic method, we developed a magnetic affinity enzyme-linked immunoassay based on soluble egg antigens (SEA-MEIA) for diagnosing schistosomiasis in persons with low-intensity infection with *Schistosoma japonicum* by comparing it with a conventional enzyme-linked immunosorbent assay (ELISA). Our results showed that the SEA-MEIA had a higher sensitivity and greater precision in the diagnosis of low-intensity *S. japonicum* infections than the ELISA. In addition, when we used Pearson's correlation in associating SEA-MEIA with ELISA, a significant correlation existed between the two assays ($r = 0.845$, $P < 0.001$). Our data indicated that SEA-MEIA, with a higher sensitivity and greater ease of performance, would be valuable for diagnosis of schistosomiasis japonicum in persons with low-intensity infections.

INTRODUCTION

In China, number of *Schistosoma japonicum* infections has been reduced from an estimated 11.6 million to well below 1 million since 1950.^{1,2} However, schistosomiasis remains one of the most prevalent parasitic infections and has significant public health consequences.³ Currently, there is a tendency to alter the epidemiology of schistosomiasis in an era of preventive chemotherapy in China. Lower rates of excreted eggs per gram of feces in the new endemic situation characterized by widespread, low-intensity infections negatively influence accuracy of prevalence assessments.⁴ Remaining pockets of high transmission further complicate the situation. However, advances in schistosomiasis control critically depend on diagnostic technical progress.

Current diagnosis methods can be divided into direct parasitologic techniques (parasite eggs detection) and indirect immunodiagnostic techniques (detection of antigens or antibodies).⁵ Parasitologic diagnoses have advantages of high specificity and require relatively unsophisticated equipment and, in areas of high endemicity, personnel with only basic training. However, in areas of low-intensity infection, it has been recognized that parasitologic detection methods lack sensitivity.⁶ Many lightly infected persons are probably missed. Thus, more sensitive immunodiagnostic assay have been extensively used.

In the schistosome-endemic areas of China, enzyme-linked immunosorbent assay (ELISA) is currently the most common method for schistosomiasis diagnosis.^{7–9} However, the traditional ELISA has relatively low sensitivity and a high rate of false-negative results, especially in patients with light infections. Therefore, more sensitive screening methods are needed for decreasing false-negative results and evaluating the effects of chemotherapy.

Magnetic affinity enzyme-linked immunoassay (MEIA) is commonly used to isolate cells, organelles, nucleic acids, proteins, and other molecules and for parasitologic diagnosis.¹⁰ Teixeira and others reported that detection of *Schistosoma mansoni* eggs in feces through their interaction with para-

magnetic beads might significantly improve diagnosis of low-intensity infections.¹¹ Recently, it was suggested that a magnetic bead-based IgY antigen-capture ELISA appeared to be a sensitive and feasible assay for diagnosis of murine schistosomiasis.¹² These studies indicated that MEIA might be an effective method in diagnosis of schistosomiasis japonicum and assessment of chemotherapy.

We have developed an MEIA based on soluble egg antigens (SEA-MEIA) and demonstrated that this assay was feasible and sensitive for diagnosis of schistosomiasis japonicum. Furthermore, it was suggested that the assay had higher sensitivity and a lower negative predictive value than the indirect hemagglutination assay and the dipstick dye immunoassay in field tests.¹³ It indicated that the assay might be valuable for diagnosis of low-intensity *S. japonicum* infections. Therefore, this study focused on use of SEA-MEIA for the diagnosis of low-intensity *S. japonicum* infections.

MATERIALS AND METHODS

Preparation of SEA. *Schistosoma japonicum* SEA was used as antigen in this study and was prepared as described.¹⁴ In brief, SEA was prepared from homogenized eggs collected and isolated from infected rabbit livers. Eggs were placed in 0.9% NaCl, homogenized on ice for 1 hour, and centrifuged for 1 hour at $20,000 \times g$ at 4°C. The soluble-phase supernatant was collected and distributed in aliquots. The protein concentration was determined by using the bicinchoninic acid protein assay and stored at –80°C until use.

Mice and serum samples. Eight male BALB/c mice 6 weeks of age were provided by the Experimental Animal Facility of Tongji Medical College (Wuhan, China). Each anesthetized mouse was infected percutaneously with 40 *S. japonicum* cercariae. Serum samples from mice before infection were used as controls. Blood was collected at six weeks post-infection. All serum samples collected were stored at –20°C until use. Mouse livers were analyzed to determine total worm burden. All experiments conformed to local government regulations that comply with Chinese national laws for animal ethics.

Human serum samples. Informed consent was obtained from all participants before this part of the study was conducted. Serum samples were obtained from 73 persons with low-intensity infections of *S. japonicum*. All patients

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were from regions along the Yangzi River in Hunan Province, which are regions in China to which schistosomiasis is endemic and were identified by finding eggs in feces by using the Kato-Katz technique. *Schistosoma japonicum* egg excretion was < 100 eggs/gram of feces. Of these persons, 15 were treated for six months with praziquantel (PZQ, 40 mg/kg of body weight) and found to be egg negative by using the Kato-Katz technique. Serum samples were also collected from 30 healthy adults from a non-endemic area for comparative purposes. Six serum samples from patients with paragonimiasis confirmed parasitologically and serologically diagnosed were also assayed. Samples from these patients did not have *S. japonicum* eggs in feces.

Coupling SEA to magnetic beads and SEA-MEIA. The coupling procedure is shown in Figure 1. Carboxy-terminated magnetic beads (Beijing Bio Biology Company, Beijing, China) were coupled with SEA according to the following methods. In brief, magnetic particles (diameter = 2 μ m) were suspended in 0.1 M 2-morpholineethanesulfonic acid hydrate buffer, pH 6.0 (Sigma, St. Louis, MO). 1-ethyl-3-(dimethylaminopropyl)-

carbodiimide hydrochloride (Sigma) and N-hydroxysuccinimide (Sigma) were added and the mixture was mixed and incubated for 15 minutes at 25°C. The beads were then washed three times with 0.01 M phosphate-buffered saline, pH 7.4. The SEA was mixed and incubated with active magnetic beads for 2 hours at 37°C. After the second incubation with antigens, 1.5% (w/v) bovine serum albumin was added to block any residual binding sites on the coated beads. The beads were washed with Tris-buffer solution containing 0.05% Tween 20, pH 7.4 (TBST), and re-suspended in TBST containing bovine serum albumin and 0.01% sodium azide. The SEA-conjugated magnetic beads were stored at 4°C until use.

The SEA-MEIA method was conducted in small flat-bottom tubes fitted to a magnet rack. Sixty microliters of magnetic beads (0.2 mg) were used per tube, and 30 μ L of serum sample diluted 1:200 in 0.1 M TBS (pH 7.4) was added to each tube. The entire mixture was incubated with rotation for 20 minutes at 37°C. Washing buffer (TBST) was added and beads were washed three times to remove unbound antibody. Subsequently,

FIGURE 1. Schematic diagram of the procedure for magnetic affinity enzyme-linked immunoassay based on soluble egg antigens (SEA-MEIA).

60 μ L of alkaline phosphatase-conjugated anti-IgG (Proteintech, Wuhan, China) diluted 1:2,000 was added and beads were incubated with rotation for 20 minutes at 37°C. After washing, 100 μ L of phenolphthalein monophosphate was added and incubated with rotation for 20 minutes at 37°C. The reaction was terminated by addition of 300 μ L of stopping reagent (0.05 M Na_2CO_3), and absorbance at 550 nm was measured by using a Serozyme I Instrument (Merck Serono, Geneva, Switzerland). Each serum sample was tested three times, and arithmetic means of results were used in subsequent data analysis. Cut-off values for assays were at least two times higher than mean absorbance for the healthy group.

The ELISA was performed as described.¹³ Ninety-six well flat-bottomed plates were coated with SEA. Absorbance was measured at 550 nm in an ELISA reader (Bio-Rad, Zurich, Switzerland). Cut-off values for assays were determined by using the mean absorbance of the healthy group plus 3 SD.

Statistical analysis. The χ^2 test or Fisher's exact test was used to compare differences between groups. The correlation between SEA-MEIA and ELISA was analyzed by using the nonparametric Pearson's correlation coefficient. Statistical significance was considered at a *P* value < 0.05.

RESULTS

Assessment of SEA-MEIA for diagnosis of *S. japonicum* infection in mice. To determine whether SEA-MEIA could be a feasible and sensitive method for diagnosis of schistosomiasis japonicum in infected mice, this assay was used to detect specific IgG in serum of mice infected with *S. japonicum*. All serum samples from infected mice had specific antibody against *S. japonicum*. The control group showed no responses. All positive serum samples from mice infected with *S. japonicum* were detected by the SEA-MEIA and the ELISA (Figure 2). Moreover, the ratio of mean positive to negative values in the SEA-MEIA was higher than that in the ELISA (5.74 versus 4.13) at the same dilution ratio. Therefore, the SEA-MEIA was feasible and sensitive for diagnosis of murine schistosomiasis.

Performance of SEA-MEIA for diagnosis of low-intensity infections with *S. japonicum* in humans. To evaluate performance of the SEA-MEIA for diagnosis of low-intensity infections in humans, 58 patients from schistosome-endemic areas

FIGURE 2. Detection of anti-*Schistosoma japonicum* IgG using magnetic affinity enzyme-linked immunoassay based on soluble egg antigens (SEA-MEIA) and enzyme-linked immunosorbent assay (ELISA) in serum samples from eight mice infected with *Schistosoma japonicum*. Horizontal lines represent mean values. OD = optical density.

TABLE 1
Performance of SEA-MEIA and ELISA for diagnosis of low-intensity *Schistosoma japonicum* infections in humans, China*

Procedure	ELISA		
	No. positive	No. negative	Total
SEA-MEIA			
No. positive	53	3	56
No. negative	0	2	2
Total	53	5	58

*SEA-MEIA = magnetic affinity enzyme-linked immunoassay based on soluble egg antigens; ELISA = enzyme-linked immunosorbent assay. Positive rates = 56 (96.55%) of 58 and 53 (91.38%) of 58 as evaluated by SEA-MEIA and ELISA, respectively ($\chi^2 = 21.95$, *P* < 0.01).

were tested by using the SEA-MEIA and the ELISA. The SEA-MEIA detected positive serum Samples (positive rate = 96.55%). However, the ELISA showed lower detection capability (positive rate = giving 91.38%), and the difference between the two methods was statistically significant ($\chi^2 = 21.95$, *P* < 0.01) (Table 1).

There were five false-negative results in the ELISA. Of these results, three were for patients who had positive results by the SEA-MEIA. The absorbance value of the two assays for a sample of 58 positive serum samples and 30 negative serum samples is shown in Figure 3. A higher ratio of mean positive to negative values was observed for the SEA-MEIA than for the ELISA (4.42 versus 3.73) at the same dilution ratio. In addition, when we used Pearson's correlation in associating SEA-MEIA with ELISA, a significant correlation was found between the two assays (*r* = 0.845, *P* < 0.001) (Figure 4).

Performance of SEA-MEIA for diagnosis of low-intensity infections with *S. japonicum* in treated humans. Fifteen patients with low-intensity infections were treated with PZQ, and IgG was detected by using the SEA-MEIA and the ELISA for chemotherapy evaluation. The ELISA results showed that six months after treatment, 33.33% of the infected persons became negative, a value that decreased to 26.67% ($\chi^2 = 10.909$, *P* < 0.01) when the SEA-MEIA results were used (Table 2).

Cross-reactivity. Cross-reactivity should be considered as an important factor in immunologic diagnosis. Six serum samples from patients with paragonimiasis were tested by using the SEA-MEIA and the ELISA. The cross-reactivity rate for detection of antibodies against *S. japonicum* in serum samples

FIGURE 3. Detection of anti-*Schistosoma japonicum* IgG using magnetic affinity enzyme-linked immunoassay based on soluble egg antigens (SEA-MEIA) and enzyme-linked immunosorbent assay (ELISA) in serum samples of 58 patients with low-intensity infections of *S. japonicum*. Dotted lines represent cut-off values. OD = optical density.

FIGURE 4. Correlation between optical density (OD) values of serum samples analyzed by magnetic affinity enzyme-linked immunoassay based on soluble egg antigens (SEA-MEIA) and enzyme-linked immunosorbent assay (ELISA) from 58 persons with low-intensity infection infections of *S. japonicum*. A significant correlation was observed ($r = 0.845$, $P < 0.001$).

from patients with paragonimiasis by these two methods was 50% (3 of 6). There was no difference between the detection rates for the two assays.

DISCUSSION

In many schistosomiasis-endemic areas of China, infection intensity and prevalence and morbidity caused by schistosomiasis had significantly decreased because of repeated chemotherapy of humans with PZQ.¹⁵ In this context, conventional parasitologic diagnosis, especially for low-intensity infections, has low sensitivity; therefore, more sensitive immunodiagnostic methods are needed.¹⁶ Schistosomiasis remains a challenge for a sensitive and specific diagnostic method in cases of low-intensity infection. Use of magnetic beads is advantageous because they can be fully automated, resulting in minimum manual labor and providing more precise results. Immunomagnetic beads have been used preferentially in *in vitro* diagnosis because they can provide results rapidly and require simple methods.^{17–19} Our previous study showed that SEA-MEIA is a new tool for the diagnosis of schistosomiasis.¹³

In this study, we evaluated SEA-MEIA as a diagnostic method for schistosomiasis japonicum in persons with low-intensity infection. We determined whether SEA-MEIA could be a feasible and sensitive method for diagnosis of schistosomiasis japonicum in infected mice. Higher precision and higher sensitivity were observed. These results support our previous finding that SEA-MEIA is feasible for the diagnosis of schistosomiasis.

TABLE 2

Performance of SEA-MEIA and ELISA for diagnosis of low-intensity *Schistosoma japonicum* infections in treated humans*

Procedure	ELISA		
	No. positive	No. negative	Total
SEA-MEIA			
No. positive	10	1	11
No. negative	0	4	4
Total	10	5	15

*SEA-MEIA = magnetic affinity enzyme-linked immunoassay based on soluble egg antigens; ELISA = enzyme-linked immunosorbent assay. Negative rates = 4 (26.67%) of 15 and 5 (33.33%) of 15 as evaluated by SEA-MEIA and ELISA respectively ($\chi^2 = 10.909$, $P < 0.01$).

Other studies showed that assays for detection of antigens may not have the expected high sensitivity and specificity when used for diagnosis in areas with low endemicity or light infections.^{20–22} Use of the MEIA for antibody detection had been shown to be more sensitive than antigen detection and might be needed in areas characterized by low levels of transmission, low prevalence, and particularly low infection intensity.^{13,23} In our study, we used SEA-MEIA for antibody detection to diagnose low-intensity infections of humans with *S. japonicum*. The sensitivity of antibody detection was high with the MEIA-SEA for low-intensity infections in schistosomiasis-endemic areas. Results also showed that the SEA-MEIA had a higher positive detection rate than the ELISA. The higher positive rate might be caused by use of magnetic beads as the solid phase, which enlarges the contacting area and makes the assay more sensitive.

Fifteen patients were treated with PZQ and serum samples from these patients were pooled after six months. Chemotherapy evaluation by using the SEA-MEIA also showed a higher detection rate than the ELISA. Because the SEA-MEIA detected more positive samples, it provided a lower false-negative rate than the ELISA. The lower false-negative rate after chemotherapy for the SEA-MEIA might be caused by higher sensitivity of the assay.

Assessment of chemotherapy accurately is influenced by the sensitivity of the parasitologic detection method. The high proportion of antibody-positive egg-negative persons that is often found in samples from schistosome-endemic areas could be largely attributable to the lack of sensitivity of the gold standard (microscopy). In contrast, persons who have no antibodies against schistosomes but show egg excretion might have been treated effectively.²⁴ Therefore, the SEA-MEIA might assess chemotherapy efficiency more accurately because of its high sensitivity. Because of the small number of available patients, evaluation of chemotherapy awaits further confirmation in larger studies.

Cross-reactivity is a major issue in serologic diagnosis. In our study, antibodies against *S. japonicum* in humans with paragonimiasis were detected by the SEA-MEIA. The SEA-MEIA showed cross-reactivity for samples from patients with paragonimiasis, but there was no difference in detection rate between the SEA-MEIA and the ELISA. Thus, when a positive result is obtained for a patient from an area to which schistosomiasis and paragonimiasis are co-endemic, history of infection with a disease or exposure to the disease should also be considered.²⁵

In addition to high sensitivity and specificity, an ideal diagnostic tool should have certain operational characteristics such as being easy to perform, rapid, simple, and convenient.^{26–28} The SEA-MEIA has several advantages for schistosomiasis diagnosis in comparison with a traditional ELISA. One of the main advantages of using the SEA-MEIA is the short interaction time between antibody and antigen coupled to magnetic beads. The kinetics of an antigen-antibody reaction is enhanced when a solid phase-bound element such as antigen-coated beads is not fixed.^{29,30} As a result, the time of incubation for reactions with antibodies in serum, and consequently, non-specific binding are reduced. Moreover, SEA-conjugated magnetic beads were used to further reduce the coating time (2 hours instead of 12–14 hours). Magnetic bead detection with magnetic washing could provide the automation and compact system integration desirable in rapid

diagnostics. Commercial magnetic beads are also available at low cost in China. Therefore, these characteristics showed that the SEA-MEIA is more user-friendly and cost-effective than the conventional ELISA.

On the basis of the results of this study, the SEA-MEIA is a valuable and sensitive method for diagnosis of schistosomiasis japonicum in persons with low-intensity infections. We conclude that this approach provides reliable and time-efficient diagnosis for schistosomiasis detection, especially for screening patients in a large-scale study or for fast clinical diagnosis in the laboratory. Further studies are also required to evaluate its value as a diagnostic method for large-scale applications in field tests.

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