

# SENSITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY WITH URINE SAMPLES : A TOOL FOR SURVEILLANCE OF SCHISTOSOMIASIS JAPONICA

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**Abstract.** An enzyme-linked immunosorbent assay (ELISA) to detect antibodies to *Schistosoma japonicum* soluble egg antigens (SEA) in un-concentrated urine was developed. The urine ELISA was applied to samples collected in a schistosomiasis-endemic village in China. The levels of anti-SEA antibodies detected in urine correlated well with those obtained with paired serum samples ( $r = 0.694$ ,  $p < 0.0001$ ). Among 129 serum ELISA positives, 112 (86.8%) were positive by urine ELISA, while all 40 serum ELISA negatives from a non-endemic area were negative. The levels of anti-SEA in urine samples were stable up to 8 weeks of storage at 37°C, with sodium azide as a preservative. Therefore, ELISA with urine samples can be used for the surveillance of schistosomiasis.

## INTRODUCTION

Schistosomiasis is one of the most important infectious diseases in the world. It has been estimated that 200 million people harbor the parasites, while 20 thousand die from the disease annually. To control schistosomiasis japonica, simple diagnostic methods suitable for mass surveys are essential. Serodiagnostic methods, such as the enzyme-linked immunosorbent assay (ELISA) have been used to detect antibodies specific to the parasite antigens in serum samples, since eggs are not always detected in stool samples (Yogore *et al*, 1983; Lewert *et al*, 1984; Yu *et al*, 1998).

Compared with serum samples, the collection of which has a risk of accidental infection with blood-borne diseases, such as human immunodeficiency virus (HIV) and hepatitis viruses, urine samples can be collected safely and easily. Collection of urine samples does not require trained staff, syringes or a centrifuge. Furthermore, compliance is easy to obtain. ELISAs with urine samples used to diagnose lymphatic filariasis and visceral leishmaniasis have shown high

sensitivity and specificity (Itoh *et al*, 2001; Islam *et al*, 2002).

In this study, we investigated anti-*Schistosoma japonicum* specific IgG in urine samples and showed that un-concentrated urine contained enough antibodies to diagnose schistosomiasis japonica.

## MATERIALS AND METHODS

### Serum and urine samples

Paired serum and urine samples were collected from 373 habitants in Rinjiang Village, an endemic area of schistosomiasis japonica, located close to Dongting Lake, 45 km northwest of Yueyang City, Funan Province, People's Republic of China, in 1995 and 1996. Forty in habitants of Yueyang City were used as negative controls.

Serum samples were kept at -20°C until used. NaN<sub>3</sub> (at a final concentration of 0.1%) was added to urine samples just after collection, and kept at 4°C until used.

### Enzyme-linked immunosorbent assay (ELISA)

Preparation of *S. japonicum* soluble egg an-

tigens (SEA) was carried out according to a method described by Tanaka *et al* (1983). Anti-SEA antibodies in serum and urine samples were measured by an ELISA method. A 96-well microtiter plate was coated with 5 µg/ml of SEA overnight. After blocking the plate with 0.05M Tris-HCl buffer, pH 7.6, containing 1% casein for 2 hours at room temperature, 100 µl of 2,000 times diluted serum or un-concentrated urine samples were added to the wells. The plate was incubated at 37°C for one hour for serum and two hours for urine samples. After washing the plate three times with a washing buffer, 0.067M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20, 100 µl of anti-human IgG conjugated with peroxidase (4,000 times diluted, Bio Source International Inc, USA) were added to each well and the plate was incubated at 37°C for an hour. Then the plate was washed three times with the washing buffer and 100 µl/well of ABTS (Kirkegaard and Perry Laboratories, USA) were added as a substrate. The optical density of each well was measured at 415 nm with a reference at 492 nm after one hour incubation at room temperature.

Antibody levels were expressed as units based on a standard curve. To construct the curve, pooled sera from five schistosomiasis japonica patients were threefold serially diluted with the casein buffer (1:1,000 to 1:729,000 for serum ELISA and 1:3,000 to 1:2,187,000 for urine ELISA), and a set of the serially diluted sera was prepared for each microtiter plate. As antibody units, a value of 21,870U was arbitrarily assigned to the 1:1,000 dilution and a value of 10U to the 2,187,000 dilution. Antibody units of serum samples higher than 21,870U and those of urine samples higher than 7,290U were regarded as 21,870U and 7,290U, respectively. Cut-off values for urine and serum ELISA were defined as the average unit of control samples + 3 standard deviations. A geometric mean of (antibody unit +1) was used for the average calculation. The cut-off unit for urine and serum ELISA were 124 and 949 units, respectively.

#### Effect of storage of urine samples on antibody levels

In order to study how long urine samples could be kept without deterioration at ambient

temperatures, urine was kept in an incubator at 37°C for up to 8 weeks and the change in antibody units was examined at 5 and 8 weeks. Twelve samples (4 with high units, 4 with lower units and 4 negatives) were used.

## RESULTS

### Antibodies to SEA in urine samples

As shown in Fig 1, IgG to SEA could be detected in urine samples and the levels correlated well with those obtained with serum samples ( $r=0.694$ ,  $p<0.0001$ ). Out of 129 serum ELISA positives, 112 (86.8%) were positive with urine ELISA. On the other hand, all the 40 urine samples from the non-endemic area were negative.

Positive rates of serum and urine ELISA by age groups are shown in Fig 2. Both positive rates of serum and urine ELISA increased with age and the rates of urine ELISA in all age groups were the same or more than those of serum ELISA. All four urine ELISA positives in the age group  $\leq 10$ , were also serum ELISA positive.

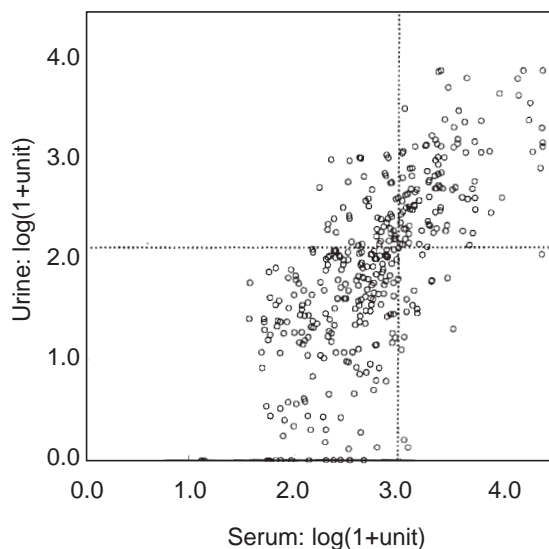


Fig 1—Correlation of anti-SEA IgG detected in urine and serum samples. Anti-SEA IgG levels in urine (without dilution) and serum (1,000 times diluted) samples were measured by ELISA. Antibody levels were indicated as  $\log(1 + \text{unit})$ . Dotted lines indicate cutoff points of both urine and serum ELISAs.

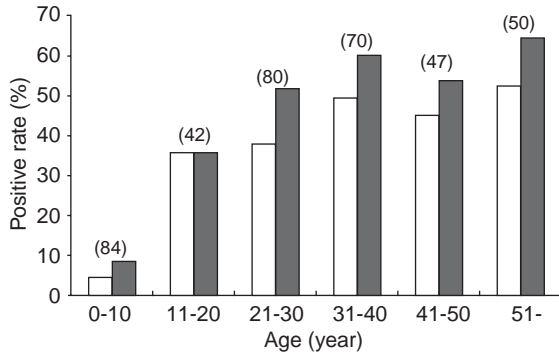


Fig 2—Changes in positive rates of urine and serum samples by age group. Open and dotted bars indicate urine and serum samples, respectively. Sample numbers are in parentheses.

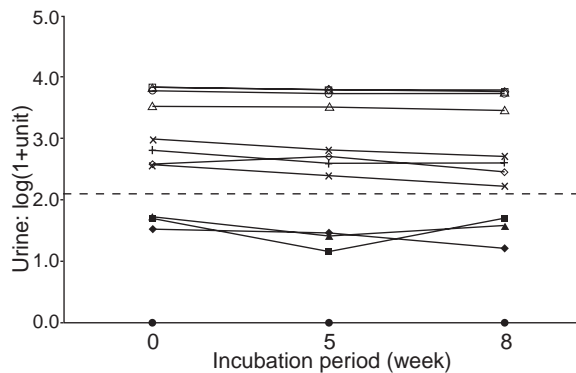


Fig 3—Effect on anti-SEA IgG levels of keeping urine samples at 37°C. Four samples were used for each of the high, low and negative groups.

### Effect of storage of urine samples on their antibody levels

Anti-SEA levels in urine samples stored at 37°C for 5 and 8 weeks are shown in Fig 3. The storage condition affected little on anti-SEA levels of samples with not only high but also low antibody levels. None of the positive samples became negative and vice versa.

## DISCUSSION

ELISA with SEA as antigens has been used for the serodiagnosis of schistosomiasis (Hillyer *et al*, 1979; Ishii and Owhashi, 1982; Tanaka *et al*, 1983). The ELISA method is more sensitive and accurate than stool examination in obtaining prevalence rate (Yogore *et al*, 1983) and incidence

(Lewert *et al*, 1984) of schistosomiasis japonica. A drawback of antibody detection is that it cannot distinguish previous and current infections. However, the ELISA method is still a useful and sensitive diagnostic tool for surveillance, especially in finding new endemic foci. It can also be useful for evaluating a control program; successful control will reduce antibody levels.

Recently, urine was successfully substituted for serum as samples to diagnose lymphatic filariasis and visceral leishmaniasis with ELISA (Itoh *et al*, 2001; Islam *et al*, 2002). Compared with serum samples, urine can be easily and safely collected, especially from children. This can facilitate compliance of people in field activities. This study revealed that urine samples from schistosomiasis patients contained anti-SEA IgG as well, and the levels significantly correlated with those of serum samples. Anti-SEA IgG was detected in 86.8% of urine samples from serum-positives and all the urine samples from a schistosomiasis non-endemic area were negative with the urine ELISA. Leak of IgG into urine caused by nephritis, which may occur in chronic patients (Tada *et al*, 1975) is unlikely, since positive rates of urine and serum ELISA were almost the same in all age groups and protein levels of all the urine samples were judged normal with a reagent strip (data not shown). As a successful control program will stop transmission of the parasite and make young age groups anti-SEA free, anti-SEA levels of the group provide useful information to evaluate control programs. Storage of urine samples for 8 weeks at 37°C had little effect on their anti-SEA levels, making the urine ELISA more practical, especially in remote areas.

The effects of the Three Gorges Dam, currently under construction on the Yangtze River in China, on the transmission of *S. japonicum* have been suggested, and it is submitted that systematic surveillance and preventive strategies against the disease are necessary (Xu *et al*, 2000). This sensitive and safe urine ELISA will be one of the essential tools for the surveillance of schistosomiasis. Application of this urine ELISA to school-based examination will elicit information on the prevalence of the disease and aid in the evaluation of control programs. Combination of the urine ELISA with other simple urine examinations, *eg*

diagnosis for proteinuria by a reagent strip, will contribute to maintain child health. The application of recombinant antigens may improve the sensitivity and specificity of urine ELISA.

In conclusion, the urine ELISA can be a useful tool for the surveillance of schistosomiasis.

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