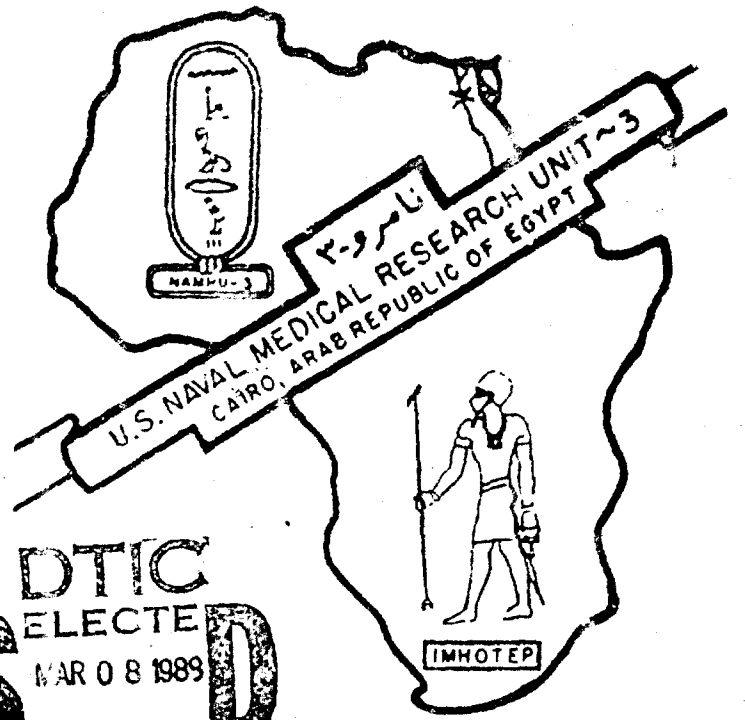

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FOR HUMAN SCHISTOSOMIASIS MANSONI

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SIMPLIFICATION AND STANDARDIZATION OF DOT-ELISA FOR HUMAN SCHISTOSOMIASIS MANSONI*

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ABSTRACT: Dot-ELISA, a techique that shares the same principles as the enzyme immunoassay, is useful for detection of anti-*Schistosoma mansoni* antibodies in the sera of patients with *Schistosoma mansoni* infections. The antigens were fixed to the nitrocellulose strips, blocked with 1% bovine serum albumin in 0.05% Tween 20. Patient sera (40) and normal laboratory personnel sera (9) were applied to the sheet directly, without cutting the strips into small discs. The nitrocellulose sheets are kept in a humid chamber for 30 min and then washed. After incubation with peroxidase-conjugated goat anti-human antibody, washing, and addition of substrate, positive reactions appear as brown dots against the white background. The room temperature assay takes about 2 hr. The optimum antigen concentration is 20–80 ng per dot and the optimum serum dilution is 1:100–1:400. The sensitivity and specificity of the assay are 90–95% and 90%, respectively. The level of positivity of the dot-ELISA by an arbitrary scale compares with standard micro-ELISA. The single positive reaction in a normal serum sample in dot-ELISA is also positive in micro-ELISA. Cross-reactivity between the *S. mansoni* antigen and human fascioliasis sera was noticed in 2 out of 8 patient sera. Good correlation between the arbitrary level of dot-ELISA and the absorbance of standardized micro-ELISA shows that the dot-ELISA is useful both for laboratory and field studies.

Human schistosomiasis is a worldwide parasitic disease that affects more than 200 million persons. Development of rapid and sensitive serological assays for diagnosis is expected to replace the more cumbersome microscopic examination of stool and urine. Enzyme-linked immunosorbent assay (ELISA) is a simple, sensitive, and rapid serodiagnostic technique, which has been widely used for detection of the circulating antibodies and antigens related to many parasitic diseases (Voller et al., 1976). The standard ELISA, however, has a limitation as a tool for field studies. Dot-ELISA, a modification of the standard ELISA assay, offers a practical tool for field studies. The nitrocellulose dot technique was first developed for screening large numbers of hybridoma antibodies using ^{125}I reagents (Sternberg and Jeppesen, 1983) by covalently binding protein antigens to nitrocellulose paper. Recently, a technique for detecting anti-*Leishmania* antibodies, by using nitrocellulose discs to which *Leishmania* antigen was fixed, has been described (Pappas et al., 1983). In the present report, we used *S. mansoni*-derived antigens fixed to nitrocellulose strips for detecting anti-*Schistosoma* antibodies. The use of such strips greatly facilitates the technique, its reproducibility, and field applicability.

MATERIALS AND METHODS

Antigen preparations

Schistosoma mansoni soluble worm antigen (SWAP) and soluble egg antigen (SEA) were prepared from the Egyptian strain of *S. mansoni*. Worms and eggs were obtained from infected hamsters according to standardized procedures. The SWAP and SEA were prepared by homogenizing the worm or egg preparations in 0.01 M phosphate-buffered saline (PBS), pH 7.2 (Boros and Warren, 1970; Boctor and Shaheen, 1986). The respective homogenates were centrifuged at 40,000 *g* and the supernatants extensively dialyzed against PBS. These antigen preparations were then measured for protein content (Lowry et al., 1951) and stored at −70 C until used.

Sera

Sera were obtained from 40 Egyptian males with parasitologically defined chronic *S. mansoni* infection. Pooled serum from 9 individuals were used for some of the experiments, and others used serum from a single sample. Normal control sera were obtained from age- and gender-matched healthy volunteers. The sera were serially diluted in PBS with 0.05% Tween 20. Sera were also obtained from 8 patients with fever and eosinophilia, who were excreting viable *Fasciola gigantica* eggs in their stools.

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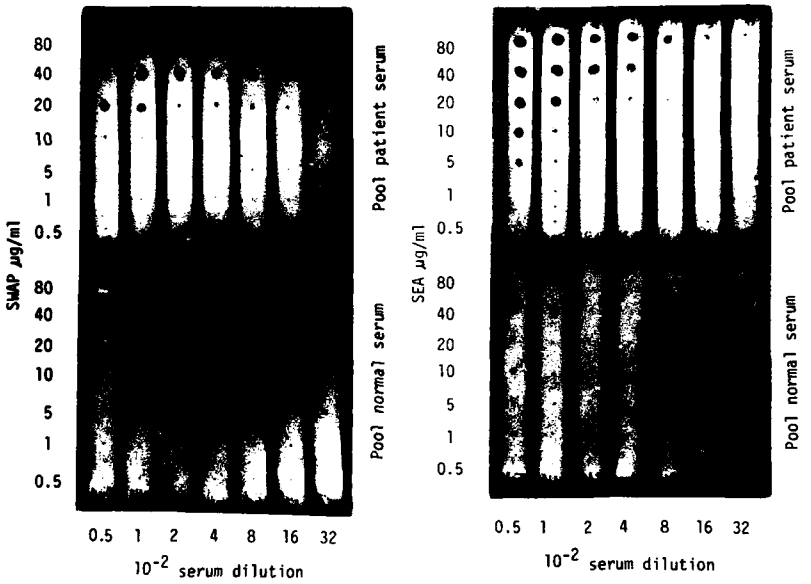


FIGURE 1. Dot-ELISA checkerboard strips tested using (A) SWAP and (B) SEA as antigen and pooled sera from 10 patients with stools positive for *S. mansoni* (50–100 eggs/g stool).

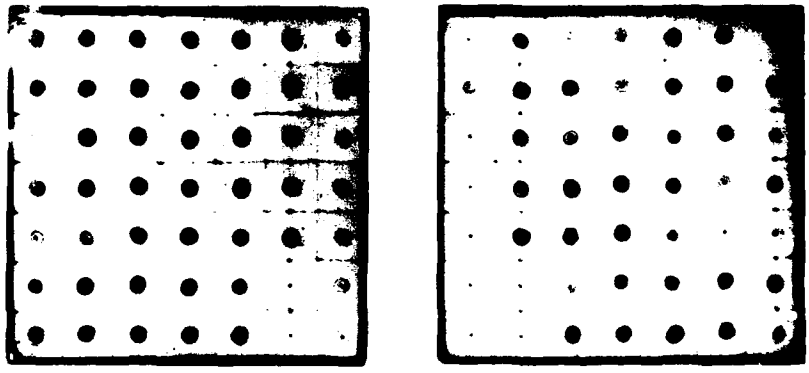
Dot-ELISA procedure

Serial dilutions of SEA and SWAP (80—0.5 µg/ml) were prepared in PBS. The nitrocellulose paper strips were divided into small 1 × 1-cm squares using a hard lead pencil. Two microliters of several different antigen concentrations were dotted on separate squares. The strips were air dried at room temperature for 30 min, then soaked in a solution consisting of PBS–1% bovine serum albumin (BSA)–0.05% Tween 20 (PBS-BSA-Tween) for 1 hr and air dried for 5 min. The strips were either processed immediately or stored at −20 C for later analysis. In either case, the strips were then placed in a humid chamber containing a sponge saturated with water, and on its top, a pad of filter paper wetted with the PBS-BSA-Tween solution. Subsequently, 2 µl of diluted serum were pipetted onto the nitrocellulose squares that had been previously dotted with antigen and left in the humid chamber. In addition to control sera, antigen and serum blanks were run in parallel. After 30 min incubation, the strips were washed 3–5 times in the PBS-BSA-Tween solution, soaked in 1:500–1:1,000 dilution of peroxidase-conjugated (Sigma Chemical Co.) anti-human IgG (γ-chain specific), and reacted at room temperature for 30 min. The strips were washed and soaked in freshly prepared substrate solution, which consisted of 25 µg of dimethoxybenzidine/ml in 0.1 M Tris-HCl buffer, pH 7.4, with 0.01% H_2O_2 (Towbin et al., 1979). Color developed within 15 min and the strips were then washed with water and left to dry. The intensity of the color was judged by the naked eye, and numbers were on an arbitrary scale of 0, +; + +, + + +; or + + + + in reference to the negative control (0). Micro-ELISA was performed as described previously (Boctor and Shaheen, 1986), and optical densities were measured using Titertek reader (Flow Company, Alexandria, Virginia).

RESULTS

Antigen concentrations and serum dilutions

Minimum concentrations of 10 µg SWAP/ml and 5 µg SEA/ml (equivalent to 20 ng and 10 ng/dot respectively) detected anti-schistosomal IgG at 1:50 serum dilutions (Fig. 1A, B). However, the optimal concentration of these antigens that can be used to detect specific antibodies in sera diluted as low as 1:3,200 in 80 ng/dot for both SWAP and SEA (Fig. 1A, B). At all antigen concentrations, pooled normal sera gave negative results and nonspecific reactions were not found in either antigen or serum blank squares. The colored strips can be kept in the dark for more than 1 yr at either room temperature or at −20 C without affecting the stability of the developed color.



A. Different serum samples (2 µl of 1:100 dilution) using 40 µg/ml SEA (2 µl per dot).

B. Different serum samples (2 µl of 1:100 dilution) using 40 µg/ml SWAP (2 µl per dot).

FIGURE 2. Dot-ELISA of individual sera obtained from 40 schistosomiasis patients with positive egg counts and 9 normal laboratory personnel with negative egg counts. The A square contains the SEA antigen and the B square contains the SWAP antigen. The 2 squares are mirror images, and the negative controls are the last 9 samples on the A square. Conditions are as described in the text.

Incubation time

The optimum incubation time for both antigen and serum was between 30 and 60 min and was 30–60 min for the conjugate.

Conjugate dilution

Different conjugate preparations gave different optimum dilutions. However, 1:500–1:1,000 dilutions of conjugate were found to be optimal for most preparations.

Reproducibility and cross-reactivity

When 10 individual sera were repeatedly tested, the level of positivity did not significantly change among the experiments. When sera from 8 patients with fascioliasis were tested, 2 gave weakly positive reactions with SWAP, but none cross-reacted with SEA.

Correlation of dot-ELISA with micro-ELISA

Forty individual samples of serum were used at dilutions of 1:100 with a concentration of 40 µg/ml (80 ng/dot) of SWAP or SEA to detect IgG antibodies with dot-ELISA (Fig. 2A, B). In a micro-ELISA test, 10 µg of the antigens/ml with 50 µl/well were compared. The results showed a good correlation between the visual grading of the dot-ELISA and the absorbance of micro-ELISA (Table I).

DISCUSSION

ELISA is a sensitive technique for detecting the antibodies and antigens of many parasites (Voller et al., 1976). However, the procedure has limitations in field studies. Dot-ELISA shares the same principles of ELISA; the major difference is in the binding of antigen to nitrocellulose paper as a solid phase rather than coating of the plastic microwells. The original use of dot-ELISA was for screening hybridoma supernatants using [^{125}I] anti-mouse Ig as detector reagent (Sternberg and Jeppesen, 1983). Pappas et al. (1983) used a small

TABLE I. *Comparison between micro-ELISA absorbance and dot-ELISA positivity.**

Dot-ELISA arbitrary scale	Micro-ELISA absorbance 405 nm	Number of samples tested
+ + + +	>0.90	2
+ + +	0.70–0.90	9
+ +	0.30–0.70	18
+	0.20–0.30	10
−	0.03–0.07	8 (negative controls)

* SWAP is used as an antigen (Fig. 2B).

nitrocellulose disc with bound *Leishmania* antigen for detecting anti-*Leishmania* antibodies; the discs were placed into the microwells and the rest of the assay performed as in ELISA. In this study, we eliminated pipetting the antigen on small discs with subsequent transfer of the discs into wells. Rather, we pipetted the sera directly onto large nitrocellulose strips and were able to run relatively large numbers of samples directly (i.e., without transfer to microwells). The use of a humid chamber and wet filter paper prevented the complete drying of the serum samples; the technique saves time and materials. The schistosomiasis dot-ELISA gave 90–95% positivity, which is similar to that of standard ELISA (Boctor and Shaheen, 1986). One of the normal sera gave a positive reaction in both dot-ELISA and ELISA; this might represent either a false positive in the 2 techniques or this person may have antibody to *Schistosoma* due to antigen exposure or subclinical infection.

The level of positivity for the 2 techniques was comparable (Table I). With the dot-ELISA, both SWAP and SEA could be used for detecting antibodies with a concentration of 20–80 ng antigen/dot. A serum dilution of 1:100–1:400 was shown to be optimal. The known cross-reactivity between *S. mansoni* and *Fasciola* species antigens appears to be resolved by using purified *Schistosoma* antigens (Pelley et al., 1977; Mott and Dixon, 1982; Tsang et al., 1983; Boctor and Shaheen, 1986). We believe that the *S. mansoni* dot-ELISA test will have wide applicability in the field and also for rapid screening in established laboratories.

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