

## DOT-DYE-IMMUNOASSAY FOR THE DIAGNOSIS OF SCHISTOSOMIASIS MANSONI

ANA LÚCIA TELES RABELLO; EMMANUEL DIAS NETO; MARIA MONICA AGUIAR GARCIA & NAFTALE KATZ

Centro de Pesquisas René Rachou - FIOCRUZ, Av. Augusto de Lima 1715, 30190-002  
Belo Horizonte, MG, Brasil

*A new serological assay dot-dye-immunoassay (dot-DIA) was evaluated for the diagnosis of schistosomiasis mansoni. This method consist of four steps: (a) biding of antigens to a nitrocellulose membrane (NC); (b) blocking of free sites of the NC; (c) incubation in specific primary antibody; (d) detection of primary antibody reactivity by color development using second antibody coupled to textile dyes.*

*Sera from 82 individuals, 61 with Schistosoma mansoni eggs in the stool and 21 stool negative were tested by ELISA, dot-ELISA, and dot-DIA. A high level of agreement between the methods tested was observed for all sera tested: ELISA x dot-ELISA: 95.1%, ELISA x dot-DIA: 92.7% and dot-ELISA x dot-DIA: 97.6%.*

*In this study, dot-DIA proved to be a feasible, sensitive, rapid and practical test for the diagnosis of schistosomiasis.*

Key words: schistosomiasis - immunoassay - dot-ELISA - dot-DIA

Diagnosis of schistosomiasis mansoni is primarily based on microscopic detection of eggs in the excreta or on the presence of specific antibodies or circulating antigens (WHO, 1985; De Jonge et al., 1988).

Although the available technology provides a variety of sensitive, specific and reproducible serological diagnostic tools, the lack of infrastructure in health care systems in most endemic countries challenge their usefulness. Thus, a test to be performed under field condition should present high sensitivity and specificity and be rapid, simple and cheap.

A number of immunological techniques have been developed to identify antiparasite antibodies. Up to now, the ELISA and the dot-ELISA methods have been reported as the most useful serological techniques for schistosomiasis diagnosis (Mott & Dixon, 1982; Xue et al., 1987), even though equipment require-

ment and elevated costs limit their wide application.

In this work, a novel immunoassay employing textile dyes as the reaction indicator (dot-DIA) is described as a rapid, simple, cheap and reliable method for the diagnosis of schistosomiasis mansoni, a promising test for field trials.

### MATERIALS AND METHODS

The presence of anti-soluble egg antigen (SEA) IgG antibodies were simultaneously tested in sera from 82 individuals by three immunoassays: dot-DIA, dot-ELISA and ELISA. Schistosomiasis was determined by demonstration of *Schistosoma mansoni* eggs in one feecal sample using the Kato/Katz method (Katz et al., 1972). Sera from 61 stool positive patients (mean eggs per gram = 406, min = 12, max = 2086) and from 21 individuals without eggs in their stools, who have never visited an endemic area were evaluated.

Figure 1 shows dot-ELISA and dot-DIA protocols schematically represented.

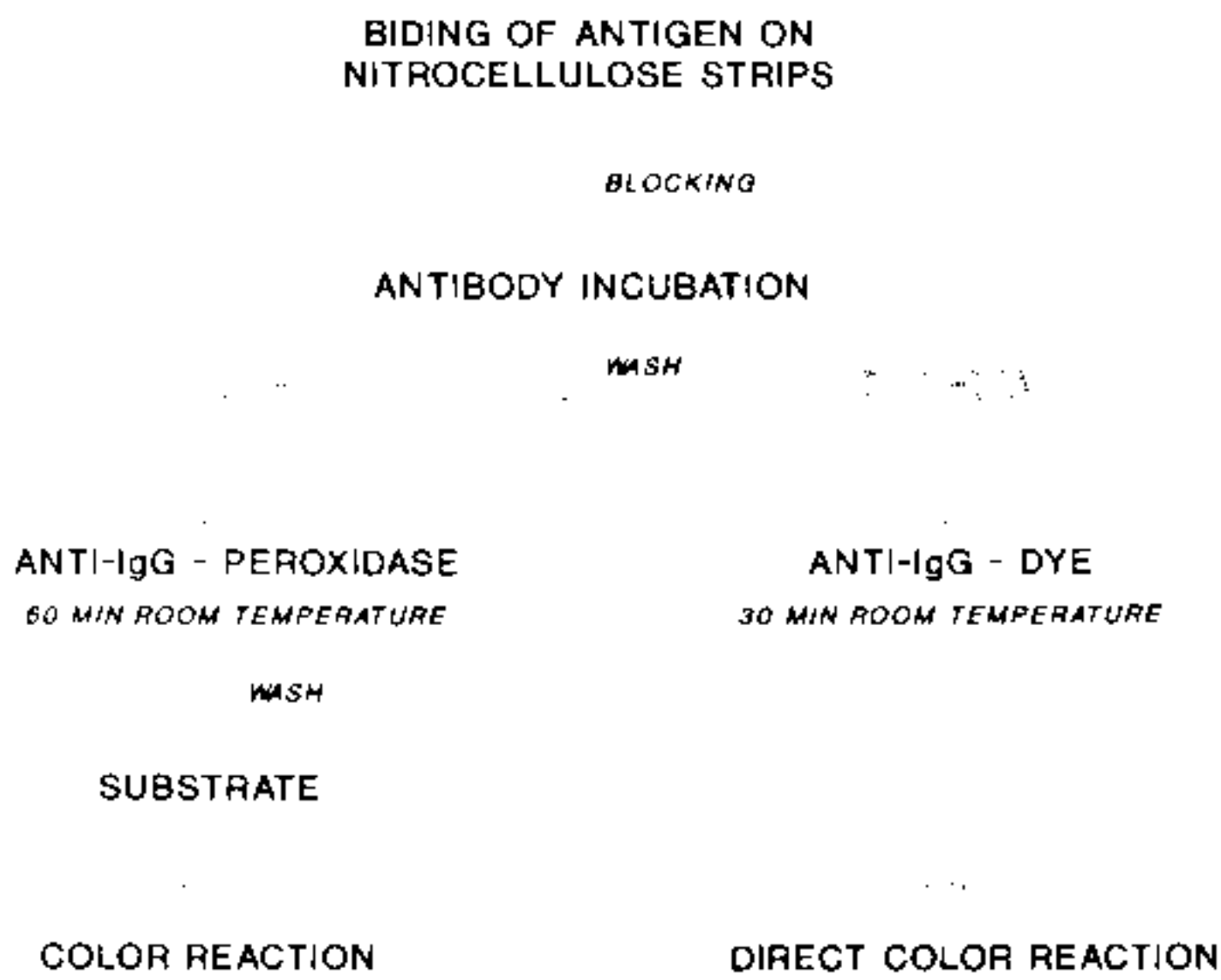


Fig. 1: dot-ELISA and dot-DIA protocols.

*Antigen preparation* – The same antigen preparation was used for the three assays. A stock solution of SEA was prepared according to Carter & Colley (1978) and kept at  $-20^{\circ}\text{C}$  until use.

*Nitrocellulose processing (dot-DIA and dot-ELISA)* – Dot-DIA and dot-ELISA assays were processed after essentially similar nitrocellulose (NC) preparation. NC sheets were marked with a pencil to produce strips 1.5 cm long and 0.7 cm wide. In each strip a line drawn 0.7 cm from the end formed a square of 0.7 x 0.7 cm which was used for the antigen dot. The entire membrane was immersed in PBS immediately prior to use. The membrane was blocked, before cutting the strips. For antigen adsorption each NC strip was dotted with 2  $\mu\text{l}$  of SEA diluted from the stock solution in PBS to a final protein concentration of 250  $\mu\text{g}/\text{ml}$ . The NC was then dried at  $37^{\circ}\text{C}$  for 30 min. For the blocking of the free sites, the NC was dipped in a blocking solution (casein 1%, gelatin 3% and Tween 20 0.05% in TRIS HCl 0.5 M pH 7.5/NaCl 2.5 M) for 90 min at  $37^{\circ}\text{C}$ . Membrane was processed immediately or following overnight storage at  $4^{\circ}\text{C}$ .

*Serum incubation* – Strips were numbered and cut on the premarked lines. Each strip was immersed into 3 ml of a 1:500 diluted test serum and soaked at room temperature for 1 h. The NC was then washed five times in PBS containing 0.05% Tween 20 (PBST).

*Dot-ELISA procedure* – Strips were incubated for 1 h at room temperature in a 1:1000 dilution of anti-human IgG conjugated to peroxidase (Cappel/Cooper Biomedical, Inc.

Malver, PA, USA). Reactions were demonstrated by immersion of the strips in a freshly prepared solution of diaminobenzidine (DAB 6 mg, 10 ml Tris 50 mM pH 7.4, 10  $\mu\text{l}$   $\text{H}_2\text{O}_2$ ). Color development occurred in 5 min and was interrupted with distilled water.

*Dot-DIA procedure – Dye-antibody conjugation* – Four samples of textile disperse dyes kindly provided by the following commercial sources were tested: yellow palanil 3 GE 200, red palanil BF and the dark blue palanil 3 RT from BASF and Samaron blue FBL from Hoescht. Dye particle suspensions and antibody-dye conjugates were prepared according to Snowden & Hommel (1991). Briefly, a water suspension with 5% (w/v) of each disperse dye was washed four times by centrifuging 20,000 x g for 30 min and resuspended in the same volume of water. A last centrifugation at 125 x g, 30 min was used to remove the aggregated colloidal particles. A small volume of each dye suspension was solubilized in ethanol for the spectrophotometric scan (Shimadzu UV – 160 A) determination of the optimal wavelength ( $\lambda_{\text{max}}$ ). The concentration of dye in the water solution used for conjugation was then based on the wavelength for each color and expressed as multiples of the dye concentration that presents a  $\lambda_{\text{max}} = 1$ . In this experiment, the dark blue palanil 3 RT ( $\lambda_{\text{max}} = 10$ ) was incubated with 100  $\mu\text{g}/\text{ml}$  goat anti-IgG (WL Imunoquimica/Rio de Janeiro/Brazil) for 1 h at a final concentration of 10 mM  $\text{PO}_4$  2.7 mM NaCl buffer pH 7.4. The solution was then spiked with V/5 vol of bovine serum albumin 30% (BSA) solution in 5 mM NaCl pH 7.4 and incubated for another hour. The dye-Ab reagents were centrifuged at 12,000 x g for 20 min and the pellet resuspended in 33.3 mM phosphate, 0.125 M NaCl solution pH 7.4, containing 5% BSA.

*Reaction indication* – After serum incubation and washing steps the strips were incubated in dye-Ab solution for 30 min shaking at room temperature and the reaction stopped with distilled water.

*ELISA* – Polystyrene plates (Hemobag, São Paulo, Brazil) were coated with 100  $\mu\text{l}$  of SEA (5  $\mu\text{g}/\text{ml}$ ) in a carbonate buffer pH 9.6 overnight at  $4^{\circ}\text{C}$ , washed with PBST and blocked with 150  $\mu\text{l}$  BSA 2% for 1 h at  $37^{\circ}\text{C}$ . After PBST washing, 100  $\mu\text{l}$  of serum were added at a dilution of 1:100 and the plates incubated at  $37^{\circ}\text{C}$  for 1 h. After further PBST washes,

100 µl of 1:1000 anti-IgG conjugated with horse peroxidase were added for another hour at 37 °C. After a further washing step, reactions were detected using ABTS. Serum reaction was considered positive when the optical density was higher than the medium plus two standard deviations of ten negative control sera.

Results were compared for sensitivity, specificity, percentage correctly classified by the test (efficiency) and agreement. Chisquare test was used for statistical analysis (Snedecor & Cochran, 1977).

RESULTS

Figure 2 shows positive and negative patterns obtained in the dot-ELISA and dot-DIA assays. Although spots obtained in dot-ELISA were slightly more intense than in dot-DIA, "ghost spots" in negative sera were more frequently seen with dot-ELISA. All color dyes tested produced stable dye/antibody conjugates, although the RED palanil BF and the dark blue palanil 3 RT from BASF provided the most visible spots.

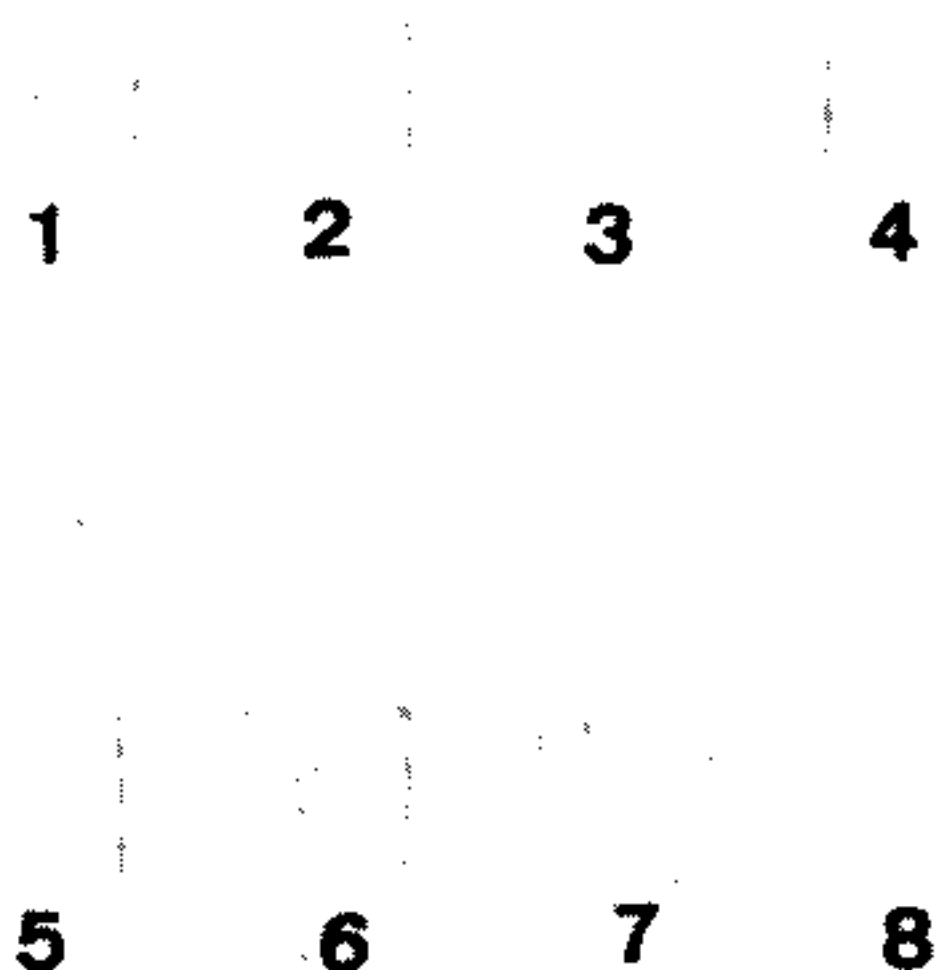


Fig. 2: 1 and 2 – dot-DIA positive; 3 and 4 – dot-DIA negative; 5 and 6 dot-ELISA positive; 7 and 8 – dot-ELISA negative.

Table shows comparative results on the efficacy of the three methods as regards the diagnosis of *S. mansoni* infection. No statistically significant difference in sensitivity, specificity or efficacy was found between the methods. A high level of agreement between the methods was observed for all sera tested: ELISA x dot-ELISA: 95.1%, ELISA x dot-DIA: 92.7% and dot-ELISA x dot-DIA: 97.6%.

TABLE

Diagnosis of *Schistosoma mansoni* infection by ELISA, dot-ELISA and dot-DIA

	Sensitivity (%) (n = 61)	Specificity (%) (n = 21)	Efficacy (%) (n = 82)
ELISA	100.0	90.5	97.6
dot-ELISA	96.7	100.0	97.6
dot-DIA	93.4	100.0	95.1

$\chi^2 = 0.79; p = 0.94.$

DISCUSSION

Demonstration of serum-reactivity using a textile dye linked to an antibody in a simple procedure as described above provides a new tool for diagnosis of schistosomiasis. With the dot-DIA it is possible to take to the field strips pre-prepared in the laboratory, sensitized with antigen and blocked. In the field, only two steps are required: 60 min for the serum incubation and 30 min for the dye-anti human IgG incubation, both at room temperature. Spots are easily seen with naked eye. No special tubes, flasks or equipment are necessary. Interpretation of results is essentially facilitated by comparing strips of positive and negative control serum. Dye textiles are inexpensive and the cost for the assay is low since they are produced in bulk. Moreover, in contrast with the traditional enzyme-substrate reactions, dye-antibody reagent avoids the inconvenient health care staff contact with irritant or carcinogens substances.

In this preliminary study, the dot-DIA method substituted with advantages the dot-ELISA, for the serological diagnosis of schistosomiasis mansoni. A protocol is being set up to ascertain its actual role as a feasible test for field evaluation of schistosomiasis.

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