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# EVALUATION OF THE ENZYME-LINKED-IMMUNO-ELECTRO-DIFFUSION-ASSAY (ELIEDA) FOR THE DIAGNOSIS OF *SCHISTOSOMA MANSONI* INFECTION WITH LOW WORM BURDEN

**Grace Mary LEAL-BACELAR (1), Hermínia Y. KANAMURA (1), Sumie HOSHINO-SHIMIZU (2), Dirce Mary C. LIMA (3), Clarice P. ABRANTES-LEMOS (3) & Luiz Caetano da SILVA (3).**

## SUMMARY

An immunoprecipitation technique, ELIEDA (enzyme-linked-immuno-electro-diffusion assay), was evaluated for the diagnosis of *Schistosoma mansoni* infection with low worm burden. One hundred of serum samples from patients excreting less than 600 eggs per gram of feces (epg), with unrelated diseases and clinically healthy subjects were studied. In patients with egg counts higher than 200 epg, the sensitivities of IgM and IgG ELIEDA were 1.000 and 0.923, respectively, not differing from other serologic techniques, such as indirect hemaglutination (IHAT), immunofluorescence (IFT) tests and immuno-electrodiffusion assay (IEDA). However in patients with low egg counts (< 100 epg), the IgG ELIEDA provided better results (0.821) than IgM ELIEDA (0.679), showing sensitivity that did not differ from that of IgG IFT (0.929), but lower than that of IgM IFT (0.964). However, its sensivity was higher than that found with IHAT (0.607) and IEDA (0.536). The specificity of IgG ELIEDA was comparable to that of other techniques. The data indicate that IgG ELIEDA might be useful for the diagnosis of slight *S. mansoni* infections, and the cellulose acetate membrane strips can be stored for further retrospective studies.

**KEYWORDS:** *Schistosoma mansoni*; Immunodiagnosis; ELIEDA.

## INTRODUCTION

The diagnosis of schistosomiasis mansoni has been made mainly by direct detection of *Schistosoma mansoni* eggs in patient fecal samples or occasionally in rectal biopsies [1]. However in endemic areas in which the schistosomiasis mansoni control programs has been established, the intensity of infection lowered to an extent that parasitologic methods became unsensitive. So, the utilization of immunologic techniques in conjunction with those methods is now being emphasized for the diagnosis of low infections [1, 5, 18, 27].

To date many serologic assays have been proposed [3, 4, 6, 10, 12, 13, 17, 18] for the diagnosis of schistosomiasis mansoni, including the enzyme-linked-immuno-electro-diffusion-assay (ELIEDA) described by PINON & DROPSY [19, 20, 21]. This microassay derives from the immuno-electrodiffusion assay (IEDA) to which the use of cellulose acetate membrane strips [9] and immuno-enzymatic assays were introduced.

The ELIEDA was shown to give more precipita-

(1) Department of Clinical and Toxicological Analyses, Faculty of Pharmaceutical Science, University of São Paulo, São Paulo, Brazil.
(2) Institute Adolfo Lutz, São Paulo State Health Department, São Paulo, Brazil.
(3) Institute of Tropical Medicine of São Paulo, São Paulo, Brazil, (LIM/HCFMUSP-06)
**Correspondence to:** Sumie Hoshino-Shimizu, Instituto Adolfo Lutz - Immunology Section. Av. Dr. Arnaldo, 355 - 11. andar, 01246-902 São Paulo, SP, Brazil.

ting lines than IEDA in *S. mansoni* and in other antigenic systems [19-25]. Nevertheless, the data available about the diagnostic performance of ELIEDA for schistosomiasis mansoni are very scarce.

In the present work we tried to evaluate this assay in schistosomiasis mansoni patients with low egg counts in comparison with other serologic techniques such as, indirect hemagglutination (IHAT), immunofluorescence (IFT) tests and immuno-electro-diffusion-assay (IEDA).

## MATERIALS AND METHODS

### *Serologic Techniques*

**IFT**: *S. mansoni* worm cryostat sections were utilized in the IFT as described [12] for the detection of IgM and IgG antibodies.

**IHAT**: The reagent was prepared with an alkaline-solubilized adult worm antigen as previously reported [13].

**IEDA**: Some modifications were introduced to the originally described IEDA [9] as follows. The antigen was obtained by grinding 1,000 fresh adult worms in a pyrex Brand tissue homogenizer containing 10 ml of 0.15 NaCl solution. After stirring at 4°C for 24 hrs., the antigenic suspension was centrifuged (11,000 g) at 4°C for 30 min. The protein content of the supernatant was determined [16] and its concentration adjusted to 1mg/ml. This assay was performed in cellulose acetate membrane strips, applying on them 10 µl of antigen and, at a distance of 1.5 cm, 5 µl of undiluted serum sample. Afterwards, they were submitted to an electrophorectic running by using a Tris-glycine-phosphate buffer solution, pH 8.6, for 90 min. (110 V, 1mA/cm).

**ELIEDA**: Modifications were also introduced to the previous described assay [19, 20]. ELIEDA was performed in two steps being the first step similar to that of IEDA, using the same *S. mansoni* worm antigen under the same electrophoretic conditions. To obtain more specific results, the cellulose acetate membranes were treated with 5% skim milk in 0.01M phosphate buffered saline solution, pH 7.2 (PBS), containing 0.1% Tween 20, for 30 min. Goat anti-human IgM (1:200) and anti-human IgG (1:1,000) peroxidase conjugates (Biolab Diag., Brazil) were diluted in 0.1M Tris-HCl buffer, pH 7.6. The positive serum samples gave clear well defined brown stained precipitation lines and the absence of precipitting lines or the presence only of diffuse or broad ill defined lines were considered as negative results.

**Parasitologic methods** - All the patients were submitted to Kato - Katz [15] quantitative method (3 slides per fecal sample) and in those giving negative results but positive in at least one of the serologic tests (IFT and IHAT), the Ritchie formol-ether method [27] was carried out. Some patients had 5 to 6 stool examinations repeated to confirm the seropositivity.

**Serum samples** - Hundred serum samples were collected, 50 of these from schistosomiasis mansoni patients yielding positive stool examination. Thirty two of these patients were migrants from different states of Brazil and residing presently in the City of São Paulo (São Paulo, Brazil); and 18 remainders always lived in endemic areas for schistosomiasis mansoni. To better evaluate the diagnostic parameters of IgM and IgG ELIEDA, the serum samples were separated into 3 groups according to egg counts: the first group comprised 28 sera from patients excreting 8 to 99 epg; the second group had 9 sera from those excreting 100 to 199 epg; and the third group had 13 sera from excreting with more than 199 epg. The control serum samples were obtained from 20 clinically healthy subjects and from 30 patients with unrelated disease to schistosomiasis: acute toxoplasmosis (5); syphilis (6); *Streptococcus pyogenes* infections with high anti-streptolysin O antibody titers (4); rubella (2); cytomegalovirus infection (2); and *Mycoplasma pneumoniae* infection (2); and serum samples from patients with autoimmune diseases were also included: connective tissue disease with high anti-nuclear antibody titers (7); and thyroiditis with high titered anti-thyroglobulin antibodies (2).

**Statistical analysis** - The serologic techniques were evaluated in terms of sensitivity and specificity [8], determining the 95% confidence intervals [26].

## RESULTS

Schistosomiasis mansoni patients had their egg counts ranging from 8 to 580 epg (geometric mean = 65.2 + 3.7 epg).

The values of sensitivity and specificity found for IgM and IgG ELIEDA, IEDA, IHAT and IgM and IgG IFT with their 95% confidence intervals are presented in Figure 1, ordered from low to high values and according to egg counts.

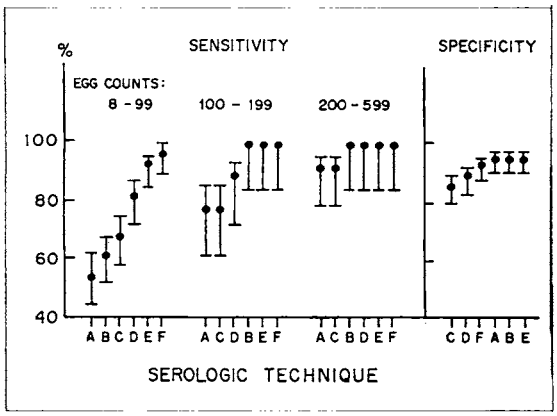


Fig. 1 - Serologic techniques ranked according to the sensivity and specificity with 95% confidence intervals, in the groups of patients with different *S. mansoni* egg counts: A - IEDA; B - IHAT; C - IgM ELIEDA; D - IgG ELIEDA; E - IgG IFT; and F - IgM IFT.

The sensitivities of all serologic techniques were similar when applied to patients excreting more than 200 epg, as can be verified through their 95% confidence intervals which overlap (Fig. 1). However in patients with lower egg excretion rate, a variation in the sensitivities for the studied techniques was verified. The IgM ELIEDA showed a similar sensitivity to the IgG ELIEDA, IEDA and IHAT; and the IgG ELIEDA, in turn, presented a similar sensitivity to IgG IFT but lower than that of IgM IFT.

The specificity of IgM ELIEDA is close to that of IgG ELIEDA and the latter technique has the specificity which do not differ from those of other serologic techniques.

The positivity found in the study of 50 serum samples from patients with schistosomiasis mansoni, and 50 without Schistosomiasis (30 unrelated diseases and 20 clinically healthy subjects), by different serologic techniques, is presented in Table 1.

Observing the 95% interval confidences calculated for the data as presented in Table 1, the IgG ELIEDA which gave positivity of 88% (82.0% - 91.3%) did not differ from 96% (91.2% - 97.5%) of the IgG IFT and also from 78% (71.2% - 82.7%) of IHAT; but, it was lower than 98% (93.7% - 98.7%) of the IgM IFT. The 10% positivity observed for IgG ELIEDA, in the group of non schistosomiasis patients, indicating 90% (84.2% - 92.9%) of negative results did not differ from the 94% (88.8% - 96.9%) of negative results observed for IgM IFT.

## DISCUSSION

The data indicated that the sensitivity of the studied serologic techniques is high and do not significantly differ from the previously reported data [11-14] when we deal with patients excreting more than 100 epg. The sensitivity however is prone to decrease in patients with lower than this egg excretion rate, mainly by using serologic techniques based on secondary antigen-antibody interactions, such as IEDA and IHAT. On the other hand, techniques based on primary

**TABLE 1**

Positivity found in the study of 50 patients with schistosomiasis, and 50 without schistosomiasis, by different serologic techniques.

| Diagnosis | Nº of Sera | IEDA | IHAT | ELIEDA | | IFT | |
|---|---|---|---|---|---|---|---|
| | | | | IgG | IgM | IgG | IgM |
| Schistosomiasis | 50 | 33 | 39 | 44 | 38 | 48 | 49 |
| Positivity for schistosomiasis | | 66% | 78% | 88% | 76% | 96% | 98% |
| Non schistosomiasis | | | | | | | |
| Acute toxoplasmosis | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Syphilis | 6 | 0 | 1 | 0 | 1 | 0 | 2 |
| Streptococcus | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rubella | 2 | 0 | 0 | 1 | 1 | 0 | 0 |
| Cytomegalovirus | 2 | 0 | 1 | 2 | 1 | 0 | 1 |
| Mycoplasma | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thyroiditis | 2 | 2 | 0 | 1 | 2 | 0 | 0 |
| Connective tissue disease | 7 | 0 | 0 | 1 | 2 | 0 | 0 |
| Healthy subjects | 20 | 0 | 0 | 0 | 0 | 2 | 0 |
| Positivity for non-schistosomiasis | | 4% | 4% | 10% | 14% | 4% | 6% |

antigen-antibody interactions, here represen-ted by IFT, seem to have the sensitivity not affected by *S. mansoni* low infections.

In this context, ELIEDA which is an association of IEDA and an immunoenzymatic assay demonstrated intermediate features. The IgG ELIEDA showed better diagnostic efficiency than IgM ELIEDA showing a sensitivity which did not differ from that of IgG IFT, and although being lower than that of IgM IFT, it was higher than those techniques requiring secondary immunologic interactions, as IHAT and IEDA.

In further studies on low schistosomiasis infections (data presented in the IV International Symposium on Schistosomiasis, R. Janeiro, 1993), another immunoenzymatic assay, ELISA, also showed that IgG antibodies were better detected than IgM antibodies. Probably, the crude *S. mansoni* worm extract gives different features in detecting IgM and IgG antibodies when compared to the frozen whole worm sections utilized as antigen in the IFT.

In our previous study [12], the IgM antibodies were seen to react with worm gut-associated antigens in the IFT. If we take into account that these antigens are present in small amounts in the solubilized total worm antigen used in ELIEDA, the low sensitivity of IgM ELIEDA in relation to IFT can be understood.

Thus, the use of an anti-human gamma globulin peroxidase conjugate rather than anti-IgG or anti-IgM conjugates may enhance the sensitivity of ELIEDA to the level of that found with IgM IFT.

Undoubtedly, ELIEDA disclosed more precipitating lines than IEDA and our data corroborate its higher sensitivity found in previous investigations done in schistosomiasis [18, 21, 23] as well as in other unrelated parasitic and mycotic infections [20, 22, 24, 25].

The specificities of IgG and IgM ELIEDA were somewhat lower than those of other serologic techniques, although these differences can not be considered significant when observed their 95% confidence intervals (Fig. 1). Diffuse and nonspecific precipitating lines were observed in both IgG and IgM ELIEDA, and it might be due to the presence of phospholipids in the *S. mansoni* worm antigen which react with some sera from control group of patients, as observed for hydatic cyst antigen [2]. Thus, trials with different *S. mansoni* antigens including those purified or semipurified are of great interest to improve the specificity of ELIEDA.

The main advantage of ELIEDA is the possibility of storing the acetate cellulose membrane strips allowing further checkings or prospective study. Moreover, this technique seems to be helpful to elucidate some immunopathogenetic aspects of schistosomiasis mansoni in which different circulating immunoglobulin isotypes are involved.

## RESUMO

**Avaliação da técnica de ELIEDA (enzyme-linked-immuno-electro-diffusion-assay) para o diagnóstico da infecção pelo *Schistosoma mansoni* de baixa carga.**

A técnica de imunoprecipitação ELIEDA (enzyme-linked-immuno-electro-diffusion-assay) foi avaliada para fins diagnósticos da esquistossomose mansoni em pacientes com baixa carga parasitária. Amostras de soros de 50 pacientes com exame de fezes positivo para *S. mansoni* (carga parasitária < 600 ovos por grama de fezes = opg) e 50 não esquistossomóticos (30 com outras afecções e 20 normais) foram estudadas. Em pacientes com carga parasitária acima de 200 opg, a sensibilidade da técnica de ELIEDA, tanto para anticorpos IgG como IgM, respectivamente 1,000 e 0,923, não diferiu da observada para outras reações sorológicas, como a de hemaglutinação (RHA), imunofluorescênca (RIF) e imunoeletrodifusão (IED). Entretanto, naqueles com baixa carga (< 100 opg), a ELIEDA-IgG mostrou resultados mais satisfatórios (0,821) que a ELIEDA-IgM (0,679), apresentando sensibilidade que não diferiu à da RIF-IgG (0,929); apesar de inferior à da RIF-IgM (0,964), a sensibilidade da ELIEDA-IgG foi superior à da RHA (0,607) e à da IED (0,536). Quanto à especificidade, esta foi comparável à dos demais testes estudados. Os dados indicam que a ELIEDA-IgG pode ser útil para diagnóstico da esquistossomose, mesmo em pacientes com pequena carga parasitária, com a vantagem de permitir estudos retrospectivos através da análise posterior das fitas de acetato de celulose que podem ser armazenadas e arquivadas.

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