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SHORT COMMUNICATION

ALKALINE SOLUBLE *TRYPANOSOMA CRUZI* EPIMASTIGOTE ANTIGEN (ASEA) APPLIED TO DOT-ELISA

Ana Maria LISSALDO (1), Sumie HOSHINO-SHIMIZU (3), Eufrosina Setsu UMEZAWA (2) & Anna Maria Simonsen STOLF (1)

SUMMARY

The alkaline soluble *Trypanosoma cruzi* epimastigote antigen (ASEA) was assessed in dot-ELISA for the diagnosis of Chagas'disease. Serum samples (355) from chagasic and non-chagasic patients were studied, and IgG antibodies to ASEA were found in all patients with chronic Chagas'disease. In non-chagasic patients 95.6% were negative, except for those with leishmaniasis (visceral and mucocutaneous), and some patients from control group reacted in low titers. The data indicate that dot-ELISA using ASEA is suitable for seroepidemiologic surveys to be employed in endemic areas for Chagas'disease.

KEYWORDS: Chagas' disease; Epimastigote; Dot-ELISA; Serodiagnosis.

INTRODUCTION

The process to obtain soluble *Trypanosoma cruzi* antigen is one of the factors which influences the outcome features of serologic assays in the diagnosis of Chagas'disease.

The alkaline solubilization process of epimastigotes described by one of us [8] was first utilized to prepare the reagents for the indirect hemagglutination test (IHA). This alkaline soluble epimastigote antigen (ASEA) had the advantage to give higher reagent yielding with longer stability than the usual saline epimastigote extract. In addition a further study confirmed that ASEA was more sensitive than other epimastigote extracts obtained by different procedures [17].

The sensitivity and specificity of IHA [16] and ELISA[7] using ASEA were seen to be high.

In developing countries, the seroepidemiologic surveys require practical and economic assays with no need of special equipment and qualified personnel. In this context, the dot-ELISA has been suggested [1, 10] as one of the simplest and yet the most practical assay. Also in some instances, the dot-ELISA seems to be more sensitive than other serologic assays, because of nitrocellulose strips binding a wider range of proteins, and in larger amounts, in relation to the red cell or plastic plate surfaces.

To date the ASEA was not tried in the dot-ELISA although some other types of epimastigote antigens [10] had been utilized.

In the present article the results obtained in the dot-ELISA using the ASEA were assessed comparatively to

(1) Instituto de Ciências Biomédicas, USP, São Paulo, Brasil.
(2) Instituto de Medicina Tropical de São Paulo, São Paulo, Brasil.
(3) Instituto Adolfo Lutz, São Paulo, Brasil.
Correspondence to: Dr. Eufrosina Setsu Umezawa. Laboratório de Protozoologia - IMT. Av. Dr. Enéas de Carvalho Aguiar, 470; 05403-000 - São Paulo, SP, Brazil.

those given by the conventional immunofluorescence test (IFT), in the study of sera from patients with and without Chagas'disease.

MATERIAL AND METHODS

Alkaline soluble antigen: *T. cruzi* epimastigotes (Y strain) obtained from liver infusion tryptose (LIT) liquid medium [3] were treated with 0.3M NaOH, kept at 4°C for 18 hr, neutralized with 0.3M HCl (pH 7.4). The protein content (2-8mg/ml) was determined by Lowry method [11], and ASEA was stored at -20°C in small aliquots as described previously [8].

Sera: A total of 355 serum samples were studied, of these 80 were from patients with clinical, epidemiological and serologic evidences of chronic Chagas' disease and they belonged to the serum bank of the Laboratório de Seroepidemiologia, Instituto de Medicina Tropical de São Paulo. The remaining 275 serum samples corresponded to the group control, in which 170 sera belonged to the Laboratório de Soroepidemiologia, mostly from patients with non-related diseases as defined by their respective clinical and laboratory diagnosis as follows: cysticercosis (8); schistosomiasis mansoni (7); toxoplasmosis (5); malaria (8); visceral leishmaniasis (11); muco-cutaneous leishmaniasis (2); amebiasis (11); South American blastomycosis (9); histoplasmosis (6); syphilis (10); leptospirosis (7); brucellosis (6); *Streptococcus pyrogenes* infections with high levels of anti-streptolysine antibodies (6); mononucleosis (8); rheumatoid arthritis with high level of rheumatoid factor (7); *Lupus erythematosus* with high levels of anti-DNA antibodies (7), and from clinically healthy individuals (52). In the control group also were included 105 serum from clinically healthy blood donors from Hospital das Clínicas de São Paulo and Hospital Municipal Dr. Arthur Ribeiro de Sabará (S. P.), all of them showing negative serology for Chagas'disease.

Dot-ELISA: The dot-ELISA was performed as described by PAPPAS [14] and modified according to BENNET & YEOMAN [2]. Briefly, nitrocellulose paper was cut like 96-well microtiter plate, without the bottom, as a mold, then 1 µl of ASEA (2 µg/ml) was spotted as a dot on the center of each well. After blocking the nitrocellulose sheet with 5% skimmed milk, it was placed downwards in a way that each antigen dot faced plastic plate well

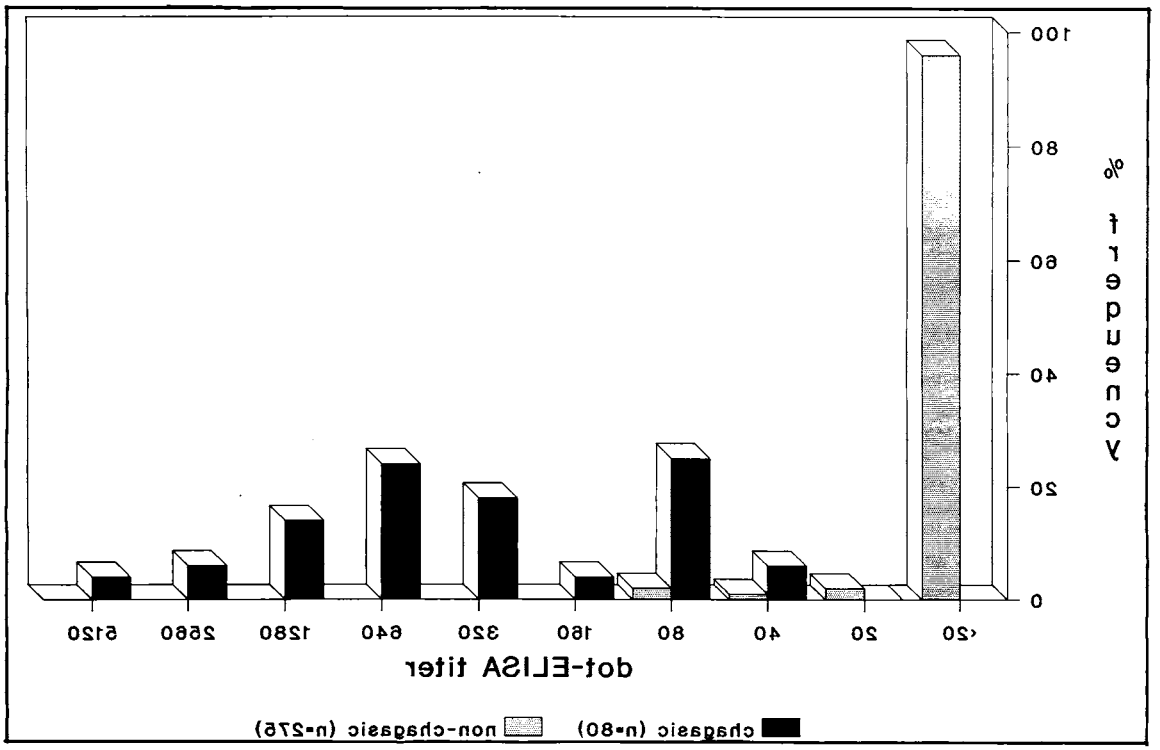

Fig. 1 - Frequency (%) of IgG antibody titer distribution, by dot-ELISA of chagasic and non-chagasic patients.

containing 50 µl serum dilutions. This system was sealed with a layer of Parafilm (American Can. Co., Greenwich, CT). Three sheets of Whatmann 3 MM paper were added and the lid was hold down with 4 spring type of clamps. The plate was then inverted and incubated for 60 min at room temperature, on a rotatory shaker. After washings, the sheet was incubated with anti-human IgG peroxidase conjugate, in the same conditions as above, and revealed with Diaminobenzidine/H_2O_2.

Immunofluorescence test (IFT): The IFT was carried out according to CAMARGO [4].

Statistical analysis: The correlation of antibody titers from dot-ELISA and IFT was verified as described [12], after transforming the titers to $\log_2$. The reproducibility intra and inter assays was verified by a control chart method [9]. For this study, a panel of 26 positive and 26 negative serum samples was assayed.

RESULTS AND DISCUSSION

The results obtained in the dot-ELISA are presented in figure 1, according to the distribution of frequency of antibody titers. The chagasic patients showed antibody titers ranging from 40 to 5120, whereas those from non-chagasic individuals the titers were lower, varying from 5 to 80.

The curves of the Fig. 1 indicate that the dot-ELISA has the ability to discriminate Chagas' disease from non-related diseases, similarly to other serologic assays utilized in the diagnosis of this disease [6].

The selected cutoff titer for the detection of IgG antibodies was 40, which gave higher diagnostic efficiency (0.980) in relation to those provided by titer 20 (0.966) and 80 (0.972). The sensitivity and the specificity correspond to 1.000 and 0.975, respectively. These findings are close to the here included conventional IFT, as well as to those reported [10] dot-ELISA using cytoplasmic and integral epimastigote antigens. Also in relation to other serologic assays these data do not differ significantly [7, 16].

TABLE 1

Detection of IgG antibodies by dot-ELISA and IFT, in the study of 355 serum samples from patients with and without Chagas' disease.

Diagnosis	nº sera	dot-ELISA nº (%)	IFT nº (%)
Chagas' disease	80	80 (100)	80 (100)
Controls			
Healthy	157	3 (1.9)	3 (1.9)
Other disease	118	9 (7.6)	8 (6.8)

The positivity found by dot-ELISA and IFT for the detection of IgG antibodies is shown in Table 1.

A cross-reactivity was seen with 12 sera, although in low titers (<80), they were from patients with visceral leishmaniasis (6); muco-cutaneous leishmaniasis (2); *Streptococcus pyogenes* infection (1), and healthy individuals (3).

In the IFT the results were similar, except for streptococcus infection which gave negative result. This high cross-reactivity with visceral and mucocutaneous leishmaniasis was expected, and confirm the previous findings [5, 13, 15].

The geometric mean titers (GMT) found for dot-ELISA, [6, 16] ($\log_2$) which was about the reciprocal of serum dilution 1/320, close to the GMT given by IFT, 6.24 ($\log_2$). Thus the antibody titers detected by dot-ELISA and IFT showed a high positive correlation coefficient (p=0.88), and the regression equation, y=.26 + .97x allows to obtain the corresponding titers from one assay to the other. The reproducibility of the results obtained in dot-ELISA inter and intra assays was acceptable, as the variability expressed in terms of standard deviations (s. d.) were lower than the stipulated limit (s. d. = 1.00) for the studied system in the control chart method.

The date here obtained indicate the good performance of dot-ELISA using ASEA, and this assay is helpful for the studies to be conducted in populations from endemic areas for Chagas' disease, having in mind to the limitation in areas in which leishmaniasis overlaps.

RESUMO

Antígeno de Epimastigota de *Trypanosoma cruzi*, solubilizado em meio alcalino (ASEA) aplicado em dot-ELISA

O antígeno de epimastigota do *T. cruzi* solubilizado em meio alcalino (ASEA) foi avaliado em dot-ELISA para o diagnóstico da doença de Chagas. Amostras de soros (355) de chagásicos e não chagásicos foram estudadas e anticorpos IgG contra ASEA foram encontrados

em todos os pacientes com a doença de Chagas crônica. Em pacientes não chagásicos, os resultados foram negativos (95,6%), exceto para aqueles com leishmaniose visceral e mucocutânea, e para alguns do grupo controle que reagiram em títulos baixos. Os dados indicam que o ensaio de dot-ELISA, utilizando o ASEA é apropriado para estudos soroepidemiológicos a serem conduzidos em áreas endêmicas da doença de Chagas, tendo em mente a limitação do teste em áreas onde Doença de Chagas e leishmaniose estão presentes.

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