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POLYVINYL ALCOHOL-GLUTARALDEHYDE AS SOLID-PHASE IN ELISA FOR SCHISTOSOMIASIS

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SUMMARY

Soluble adult *Schistosoma mansoni* antigen preparation (SWAP) was covalently fixed onto polyvinyl alcohol-glutaraldehyde discs and an enzyme linked-immunosorbent assay (ELISA) was set up. The best conditions for the assay were established and it was found that small amount of antigen such as 1.5 µg was required. A comparison between this procedure and the conventional ELISA was proceeded. A reliable method of antigen immobilization was achieved and the low prices of the employed reagents are economically attractive.

KEYWORDS: Polyvinyl alcohol; Glutaraldehyde; ELISA; *Schistosoma mansoni.*

INTRODUCTION

Since it was accidentally discovered in 1924[9] polyvinyl alcohol (PVA) has become one of the most studied polymer in the literature. Applications ranging from water insoluble textile fiber[17] to support for biomaterial immobilization such as heparin[10, 16], catalase [19] and drugs [20] have been proposed. The reason for that it seems to be the fact that PVA can be industrially produced rather cheaply, it is not toxic and can form membranes with large surface areas suitable for enzyme reaction[11, 13].

In our laboratory, a semi interpenetrating polymer network (IPN) based on PVA-glutaraldehyde network was prepared[6]. The infrared analysis showed that when the samples were prepared off the stoichiometric relations some carbonyl groups remained free and it increased as the glutaraldehyde concentration increased. From these results high swelling discs with free carbonyl groups to be covalent bounded to amine groups from biomaterials were synthesized.

Recently, this PVA-glutaraldehyde network was also synthesized in bead forms and used for enzyme immobilization. Xanthine oxidase, a-amylase and amylo-glucosidase were covalently fixed on the beads yielding preparations with specific activities retention of 72.3%, 1.6% and 1.4%, respectively. Km of xanthine oxidase PVA-glutaraldehyde beads (24 ± 4 µM) was slightly higher than the estimated for the soluble enzyme (16 ± 2µM)[2].

The antigen F1 purified from *Yersinia pestis* was covalently linked to this modified polymer and an enzyme-linked immunosorbent assay (ELISA) was established for the diagnosis of plague[1].

This same antigen preparation was also incubated with fluorescein labeled antibody against F1-antigen and excited at 4880 Å by either an argon laser or a dye laser and the fluorescence detected at 5200 Å[6]. Therefore, PVA-glutaraldehyde antigen coated discs were used for laser induced fluorescence detection of plague.

In this work, an antigenic preparation obtained from the adult *Schistosoma mansoni* (soluble fraction) was fixed onto PVA-glutaraldehyde discs and an enzyme linked-immunosorbent assay (ELISA) was established to test the use of this modified polymer as a solid-phase for Schistosomiasis diagnosis.

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MATERIALS AND METHODS

PVA-glutaraldehyde discs synthesis and Antigen immobilization: according to ARAUJO et al.[1], except that 100 µl of diluted (1.25 µg/well) Soluble Proteins of Adult Worms (SWAP) were used.

Antigen and sera: SWAP of *S. mansoni*, São Lourenço da Mata (SLM) strain, were obtained [15] at the Immunology Sector of the Laboratório de Imunopatologia Keizo Asami. Positive sera were collected from individuals who were accidentally infected by *S. mansoni*, 40 days after to be in contact with cercariae. The diagnosis was estalished clinically and by conventional laboratory findings, whereas the control sera were from those proved being not having schistosomiasis according to these procedures. Protein content was established according to LOWRY et al. [14].

Enzyme linked immunosorbent assay (ELISA) and blocking conditions were also carried out as described by ARAUJO et al.[1]. Here, rabbit anti-human IgG (Sigma) conjugated to peroxidase (100 µl) diluted, 2,500 times in 3% w/v skim milk was used as the second antibody.

Titration of conjugate: The human anti-IgG produced in rabbit conjugated to horseradish peroxidase (Sigma) was diluted to 1:1,000; 1:2,000 and 1:3,000 in skim milk 3% w/v. These diluted conjugates were used to develop the ELISA using 5 µg of SWAP/pellet and positive and negative sera diluted to 1:100. Other experimental conditions were elsewhere [1].

Antigen concentration: Samples (100 µl) of SWAP containing protein at concentrations ranging from 0.039 to 5 µg were introduced into flat bottomed microplates containing the discs and left overnight at 4°C. These antigen-discs were then proceeded as described above in antigen immobilization and ELISA.

Titration sera: Positive sera were diluted from 1:50 to 1:6,400 in skim milk 3% w/v and ELISA was carried out following the best conditions for blocking conjugate dilution and antigen concentration.

RESULTS

The blockage using 3% w/v skim milk prepared in PBS and incubation overnight at 4°C was found to be the best conditions as proved by O.D. close to zero for the blanks. Furthermore, infrared spectroscopy of the blocked discs demonstrated that the absorption at wavenumber of 1,723 cm^{-1}, corresponding to the aldehyde carbonyl groups, disappeared after skim milk treatment. A value of 1:2,500 was established as the optimal dilution for the human anti-IgG produced in rabbit conjugated to horseradish peroxidase. This concentration yielded O.D. values equal to 0.052-0.005, 0.047-0.020 and 0.854-0.459 for the blank, negative and positive sera, respectively. Fig. 1 displays the relationship between the amount of fixed antigen onto the PVA-glutaraldehyde discs and the O.D. achieved by carrying out the ELISA. The described hyperbolic curve reveals that the minimum amount of fixed antigen (optimum fixed antigen) to produce the maximum O.D. is equal to 1.5 µg of protein. A typical antibody titration is presented in Fig. 2. Table 1 shows the comparison between ELISA using PVA-glutaraldehyde as support and conventional ELISA and the results for health individuals as controls. The independent t-test on this and conventional ELISA showed a $p = 0.79$, namely, at the 0.05 level their two means are not significantly different. From the control results a cut-off equal to 0.059 was established.

DISCUSSION

ELISA has been widely employed in schistosomiasis and soluble worm antigen preparation, known as SWAP, is one of the most used antigen for this method. ELISA has also been set up using other preparations such as crude and purified egg antigen[7, 8], *S. mansoni* tegument antigen[12], schistosomule tegument, cercaria antigen and gut-associated circulating anodic antigen, abbreviated as CAA[3, 5, 18, 21].

In this work, ELISA was based on the covalent fixation of SWAP on the proposed matrix, PVA-glutaraldehyde, and small amount of antigen was required (only 1.5 µg). Conventional ELISA[22] uses 5 µg of SWAP adsorbed to a NUNC immunoplate using carbonate buffer pH 9.0 overnight. Probably, the spline nature of the hyperbolic curve attained in Figure 1 was due to

TABLE 1
Serological analysis of human sera from schistosomotic patients and healthy individuals using conventional and the present ELISA procedure

	Controls	Patients with schistosomiasis	
Individual number	ELISA PVA-glutaraldehyde	ELISA PVA-glutaraldehyde	ELISA conventional
1	0.042	0.422	0.402
2	0.026	0.326	0.253
3	0.005	0.387	0.191
4	0.005	0.551	0.669
5	0.015	0.409	0.401
6	0.015	0.251	0.344
7	0.037	0.389	0.289
8	0.000	0.298	0.372
9	0.017	0.286	0.203
10	0.011	0.462	0.509
Mean ± SD	0.017±0.014	0.378±0.090	0.363±0.146

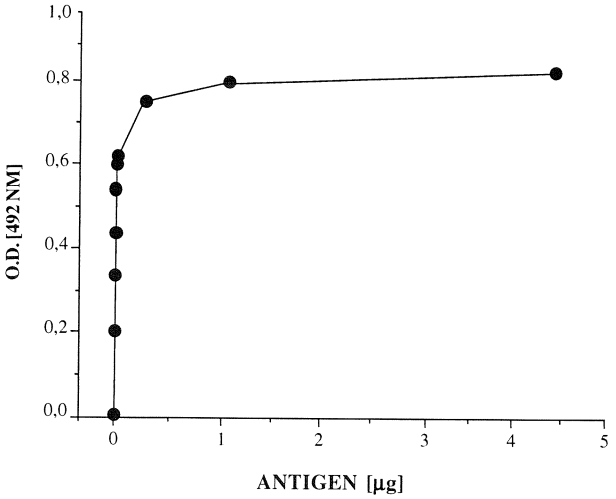

Fig. 1 – Relationship between fixed antigen (SWAP) onto the PVA-Glutaraldehyde discs and the ELISA results. SWAP (100 µl) containing protein at concentrations ranging from 0.039 to 5 µg were introduced into flat bottomed microplates containing the PVA/glutaraldehyde disc and left overnight for 12 h at 4°C. The antigen-discs were washed twice with PBS, containing 0.05% Tween 20; blocked with skim milk overnight at 4°C and washed with PBS/Tween once. The ELISA was carried out.

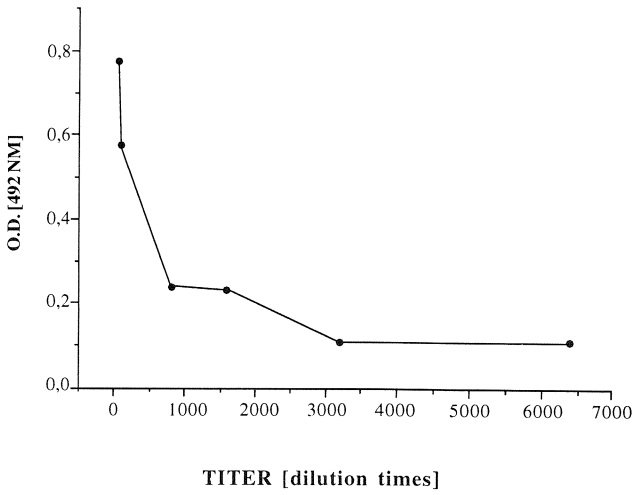

Fig. 2 – Titration of positive sera against schistosomiasis by using PVA-Glutaraldehyde as solid-phase. Positive sera were diluted from 1:50 to 1:6,400 in skim milk 3% w/v and ELISA was carried out following the best conditions for blocking, conjugate dilution and antigen concentration.

the covalent linkage between antigen and the matrix, namely, PVA-glutaraldehyde.

Patients with schistosomiasis presented high O.D compared with the controls as it is shown in Table 1 and higher than the established cut-off. A plot of those O.D. *versus* those estimated by the conventional ELISA yielded to the following equation:

$$O.D_{\text{PVA-glutaraldehyde}} = 0.200 + 0.527 \times O.D_{\text{conventional ELISA}}$$

$$r = 0.978 \text{ and } p = 0.000136$$

The results of the present procedure and those reported for the immunodiagnosis of plague[1,4] indicate that a reliable method of antigen immobilization was achieved which can be applied for other antigen-antibody interactions. The low prices of the employed reagents are also economically attractive. Furthermore, other physical shapes of PVA-glutaraldehyde can be used for immunoadsorvance purpose such as beads. Investigations on the use of PVA-glutaraldehyde to cover surfaces are in progress.

RESUMO

Polivinil álcool-glutaraldeido como fase sólida em ELISA para esquistossomose

Preparações antigênicas solúveis de *Schistosoma mansoni* adulto (SWAP) fixadas covalentemente em discos de polivinil álcool-glutaraldeido foram usadas em teste de ELISA. As melhores condições para o ensaio foram estabelecidas e foi encontrado que uma quantidade mínima de antígeno, em torno de 1,5 µg é necessária. Comparação entre este procedimento e o de ELISA convencional foi estabelecida. Um método seguro de imobilização do antígeno foi alcançado e os baixos preços dos reagentes empregados são economicamente atraentes.

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