SHORT COMMUNICATION

Cellulose Acetate as Solid Phase in ELISA for Plague

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Antigen from Yersinia pestis *was adsorbed on cellulose acetate discs (0.5 cm of diameter) which were obtained from dialysis membrane by using a paper punch. ELISA for human plague diagnosis was carried out employing this matrix and was capable to detect amount of 1.3 µg of antigen, 3,200 times diluted positive serum using human anti-IgG conjugate diluted 1:4,000. No relevant antigen lixiviation from the cellulose acetate was observed even after washing the discs 15 times. The discs were impregnated by the coloured products from the ELISA development allowing its use in dot-ELISA. Furthermore, cellulose acetate showed a better performance than the conventional PVC plates.*

Key words: cellulose acetate - *Yersinia pestis* - ELISA

In our laboratories, modified polymers have been proposed as matrices for antigen immobilization such as Dacron; ferromagnetic Dacron (Carneiro Leão et al. 1991) and PVA-glutaraldehyde (Araujo et al. 1996). Here, cellulose acetate is proposed as an alternative support for antigen immobilization.

Dialysis membrane of cellulose acetate (Sigma) was cut in discs by using a paper puncher (0.5 cm of diameter). The discs were then introduced into flat bottomed tissue culture microplates covered with 100 µl of F1 antigen (Baker et al. 1952) containing 130 ng of protein, obtained from *Yersinia pestis*, and left at 4°C overnight. These treated discs were washed twice with 0.1 M sodium carbonate buffer (SCB), pH 9.2, added by 0.05% Tween 20 (Labsynth); blocked with 3% w/v skimmed milk (Molico, Nestlé), prepared in SCB, for 2 h at 4°C and washed with SCB/Tween once.

Diluted human serum (100 µl of a 1:3,200 dilution in SCB) was incubated with the antigen-disc into clean microplates at 37°C for 60 min. After washing the antigen-antibody-disc complex five times with SCB/Tween, 100 µl of goat anti-human IgG (Sigma) conjugated to peroxidase diluted from 1:2,000 to 1:32,000 times in 3% w/v skimmed milk were added and incubated at 37°C for 60 min. Afterwards, five washings with CBB/Tween were carried out. Then, the substrate solution (100 µl), composed of 0.325% w/v orthophenylenediamine dihydrocloride (OPD-Sigma) and 0.085% H_2O_2 prepared in 0.3M Tris-citrate buffer, pH 6.0, was added. After incubation at room temperature (28°C) for 15 min, in the dark, the reaction was stopped with 2.5M H_2SO_4 (25 µl) and the plates read in ELISA reader (Bio-rad) at 492 nm. This procedure was carried out in duplicates as well as all those throughout this work. The results are displayed in Fig. 1. The highest ratio (7.97) between positive and negative optical density (OD) was found at the dilution of 1:4,000 which was selected as the best for the ELISA development.

ELISA was carried out as above except that antigen amount and serum dilution ranged from 78 ng to 20 µg/well and 1:200 to 1:25,600, respectively. Goat anti-human IgG conjugate was diluted 1:4,000 as above estimated. These titrations are presented in Fig. 2. According to this figure the optimal antigen and serum dilutions were determined to be 1:320 (1.3 µg/ml) and 1:3,200, respectively. Hyperbolic curves were almost described since OD decay was observed above a certain antigen concentration value. This can be attributed to no antigen-antibody complex formation due to steric hindrance .

Antigen-discs were washed from twice to 15 times with SCB/Tween previously to ELISA and the results showed OD values for control, nega-

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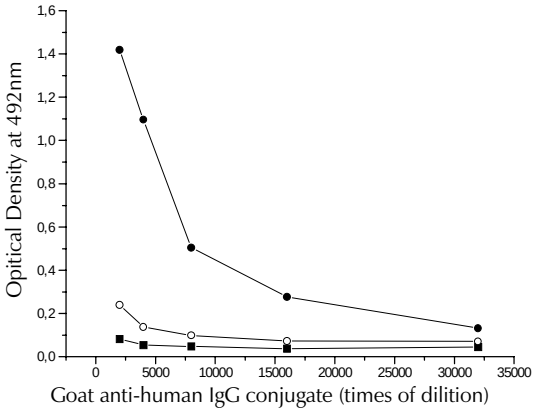

Fig. 1: titration of goat anti-human IgG conjugate. Experimental conditions: antigen-disc (1.3 µg/disc); blocked with 3% w/v skimmed milk prepared in sodium carbonate buffer (SCB); positive (●) and negative (O) human serum (diluted 1:3,200); SCB as control (■); goat anti-human IgG conjugated to peroxidase diluted as indicated and the reaction read at 492 nm.

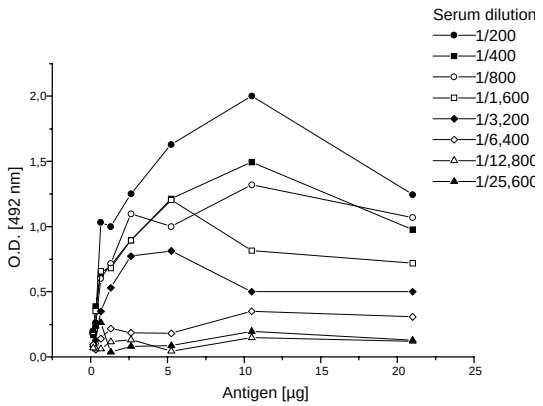

Fig. 2: antigen and serum titration using ELISA based on cellulose acetate discs. Experimental conditions: antigen-disc (blocked with 3% w/v skimmed milk prepared in sodium carbonate buffer) concentrations and human serum dilutions as indicated; goat anti-SCB human IgG conjugated to peroxidase diluted 1:4,000 and the reaction read at 492 nm.

tive and positive serums equal to 0.035 ± 0.008; 0.033 ± 0.007 and 0.310 ± 0.038, respectively. No relevant antigen lixiviation from the cellulose acetate was observed provided that the OD decreased only 12.3% after 15 washings using positive serum, whereas negligible OD variation was detected for the negative serum and control.

Comparison between a commercially available ELISA microplate (Maxsorbent) with the cellulose acetate discs was carried out under the same experimental conditions (Fig. 3). Cellulose acetate, the present solid-phase, showed a better performance than the conventional PVC plates.

Finally, it is worthwhile to register that the cellulose acetate discs were impregnated by the coloured products from the ELISA development (reduced OPD by the peroxidase catalysis). Thus,

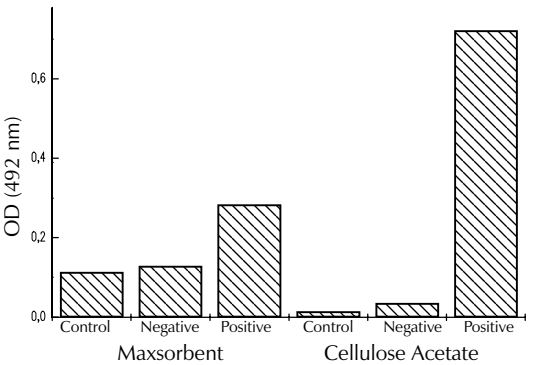

Fig. 3: comparison of ELISA using PVC microplates (Maxsorbent) or cellulose acetate discs.

high OD for the ELISA will give intensive yellow colour disc. This can be easily seen at least for two hours. Therefore, dot-ELISA can also be performed by using these discs obtained from cellulose acetate membrane dialysis.

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