RESEARCH NOTE

Rapid Elisa for Plague

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Recently, an enzyme linked immunosorbent assay (ELISA) for plague was proposed in our laboratories using a modified polymer, polyvinil alcohol (PVA) - glutaraldehyde, as an alternative solid phase (AM Araujo et al. 1996 *Mem Inst Oswaldo Cruz 91:* 195-198). An antigen (F1) obtained from *Yersinia pestis* was covalently fixed onto PVA-glutaraldehyde discs.

The synthesis of these discs is simple and the low prices of the employed reagents are economically attractive. The present study describes a modification of this method, aiming to reduce the time of procedure from 36 hr to 3 hr.

The discs were introduced into flat bottomed microplates covered with 100 ml of diluted F1 antigen (1.3 mg/well) and left at 28°C for 1 hr (instead of overnight as in the original method). These treated discs were washed twice with PBS, containing 0.05% Tween 20 (Labsynth); blocked with skimmed milk (Molico, Nestlé) for 1 hr (instead of overnight as in the original method) at 28°C and washed with PBS/Tween once.

Diluted serum (100 ml of a 1:200 dilution in PBS) was incubated with the antigen-disc into clean microplates at 37°C for 30 min (instead of 1 hr as previously). After washing the antigen-antibody-disc complex five times with PBS/Tween, 100 ml of goat anti-human IgG (Sigma) conjugated to peroxidase diluted 1,500 times in 3% w/v skimmed milk were added and incubated at 37°C for 30 min (instead of 1 hr as previously). Afterwards, five washings with PBS/Tween were carried out. Then, the substrate solution (100 ml), 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 ml), the discs removed and the plates read in ELISA reader (Bio-rad) at 492 nm.

Tables I and II show the results obtained using this rapid ELISA in human sera from patients and health individuals living at different plague foci in the northeast Brazil, respectively.

TABLE I

Serological analysis of human sera from individuals living at different plague foci in the northeast of Brazil

Patient number	ELISA[a]
1	1,257
2	1,986
3	1,128
4	1,420
5	1,308
6	1,315
7	0,811
8	1,688
9	1,221
10	1,031
11	0,896
12	1,134
13	0,990
14	1,810
15	1,113
16	1,467
17	1,347
Mean ± SD[b]	1,289 ± 0,315

a: OD; *b*: standard deviation.

TABLE II

Serological analysis of human sera from health individuals living at northeast of Brazil

Sample number	ELISA[a]
1	0,046
2	0,040
3	0,061
4	0,067
5	0,060
6	0,012
7	0,005
8	0,010
Mean ± SD[b]	0,037 ± 0,025

a: OD; *b*: standard deviation.

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These subjects were clinically selected by the Centro de Pesquisas Aggeu Magalhães-Fiocruz, Research Institute responsible to monitor plague in the northeast Brazil. The mean value of the optical densities (OD) in Table I was equal to 1.289. From the results shown in Table II one can establish a "cut-off" value of 0.113. This method also gave OD equal to 0.004 for the blank controls. Furthermore, the ELISA OD positive values obtained using the 3 hr procedure plotted against those from the 36 hr presented a linear relationship (r and p values equal to 0.87 and 0.01, respectively).

Therefore, the ELISA procedure for plague, employing PVA alcohol glutaraldehyde as solid-phase, was successively shorted from 36 hr to 3 hr.