

## COMMERCIAL ANTIRABIES VACCINE USED AS ANTIGEN IN A MODIFIED ENZYME-LINKED IMMUNOSORBENT ASSAY

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*Summary.* — A modification of enzyme-linked immunosorbent assay (ELISA) for rabies serology was elaborated. Commercial rabies vaccine was bound to horse anti-rabies immunoglobulin. This ELISA modification proved to be equally highly specific and sensitive as the neutralization test or ELISA with the use of concentrated and purified rabies virus.

*Key words:* rabies; serology; enzyme-linked immunosorbent assay

### Introduction

The ELISA used so far for rabies serology has been time consuming and laborious, requiring concentration and purification of antigen (Atanasiu *et al.*, 1977, 1978; Thraenhart and Kuwert, 1977; Savy and Atanasiu, 1978; Atanasiu and Perrin, 1979). We modified the ELISA by using antirabies vaccine as virus antigen.

### Materials and Methods

*ELISA procedure.* To each well of a Linbro S-MRC-98 microplate was added:

— 100  $\mu$ l of antirabies immunoglobulin (diluted with 0.1 M bicarbonate buffer, pH 9.6) for 18 hr at 4 °C; we prepared the immunoglobulin by ammonium sulphate precipitation from horse antirabies serum (Institut Pasteur, Paris, lot 11521 45);

— 100  $\mu$ l of phosphate buffered saline (PBS) containing 0.5% Tween 20 and 1% bovine albumin fraction V (Serva, Heidelberg) (PBSTA) for 1 hr at 37 °C, to block nonspecific reactions of sera, conjugate or substrate with uncoated portions of the plate;

— 100  $\mu$ l of inactivated rabies vaccine (Mérieux lot P0186, prepared on human diploid cells), diluted with PBSTA, for 1 hr at 37 °C;

— 100  $\mu$ l of test (control) serum (serial twofold dilutions starting with 1 : 50) for 1 hr at 37 °C;

— 100  $\mu$ l of peroxidase-labelled antihuman immunoglobulin, diluted with PBSTA, for 1 hr at 37 °C; we prepared the conjugate according to Nakane and Kawaoi (1974) from rabbit anti-human IgG (Fc fragment, Dako, Denmark, lot 015);

each of the above steps was followed by 3 washings with PBS containing 0.5% Tween 20;

— 100  $\mu$ l substrate (o-phenylene diamine; Sigma) for 15 min at room temperature in a dark room. The enzyme reaction was stopped by the addition of 0.5 M citric acid.

Each sample was diluted sixfold with PBS and its optical density (OD) at 460 nm was measured in a conventional spectrophotometer.

For comparison, we carried out ELISA according to Atanasiu (Atanasiu *et al.*, 1977, 1978); Atanasiu and Perrin, 1979) on a commercial plate coated with rabies virus antigen (lot 91254), kindly supplied by prof. P. Atanasiu, Paris.

The titres were expressed as reciprocals of the highest serum dilution at which the OD was twice that obtained with standard negative serum diluted 1 : 50.

*Sera.* The following were used: a) sera from 21 patients vaccinated with Semple's type vaccine or with a 5% lyophilized vaccine from rabbit brain inactivated by beta-propiolactone; the vaccination schedule consisted of 14 daily injections and 2 booster doses once every 10 days; b) sera from 12 persons not vaccinated against rabies and not bitten by any animal; and c) standard positive and negative control human serum.

The virus neutralization (VN) test was carried out with CVS virus IP 11 on 11–13 g Swiss albino mice by the routine procedure (Atanasiu, 1973). The test sera were diluted serially five-fold from 1 : 5 to 1 : 625. The relation between ELISA and VN test results was subjected to linear regression analysis (Oktaba, 1966, pp. 88–94).

### Results

According to cross-titration, the optimal dilution of antirabies gamma-globulin for plate coating was 1 : 6400, and that of virus antigen (vaccine) 1 : 32. Higher concentrations of gamma-globulin gave nonspecific results.

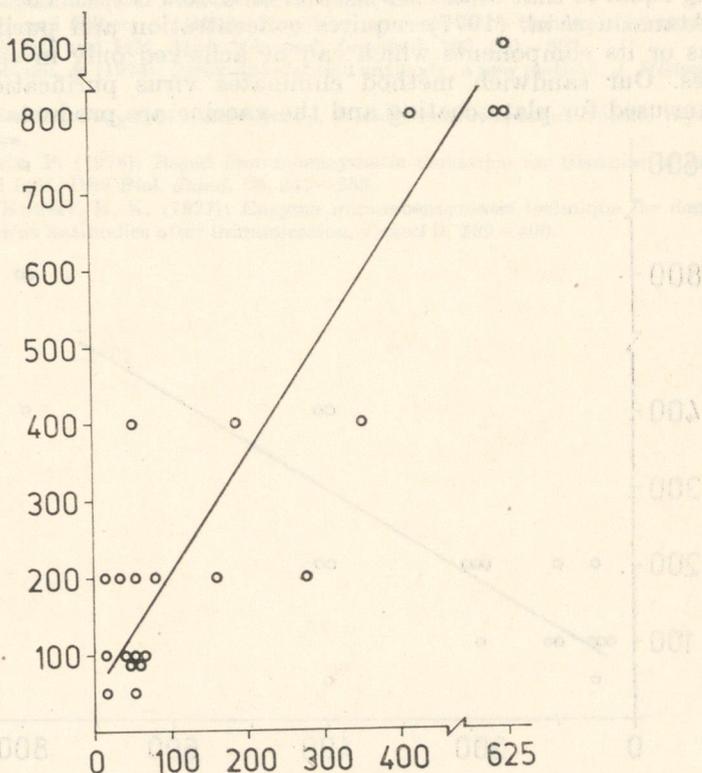


Fig. 1.

Correlation between the results obtained in VN tests and ELISA.

Abcissa: rabies antibody titres in VN tests; ordinate: rabies antibody titres in ELISA

The OD values for control negative serum varied from 0.04—0.08 and for control positive serum from 0.9—1.0, at serum dilutions of 1 : 50. The OD of negative test sera varied from 0.01—0.08.

Antibodies were detected by both ELISA and VN test in all sera from patients vaccinated against rabies, the ELISA titres having been higher than those detected by the VN test (Fig. 1). The geometric mean antibody titres were 213.6 (ELISA) and 88.4 (VN test). The correlation coefficient for values determined by the two tests was 0.87.

The relation between the results obtained by both types of ELISA is illustrated in Fig. 2. The correlation coefficient was 0.89.

### Discussion

As shown by other authors, ELISA proved to be useful for the detection of antibodies against rabies virus, the specificity and sensitivity of ELISA being equal to that of the *in vivo* and *in vitro* VN test. The ELISA according to Atanasiu *et al.* (1977) requires concentration and purification of rabies virus or its components which can be achieved only in specialized laboratories. Our sandwich method eliminates virus purification; the immune serum used for plate coating and the vaccine are products readily available

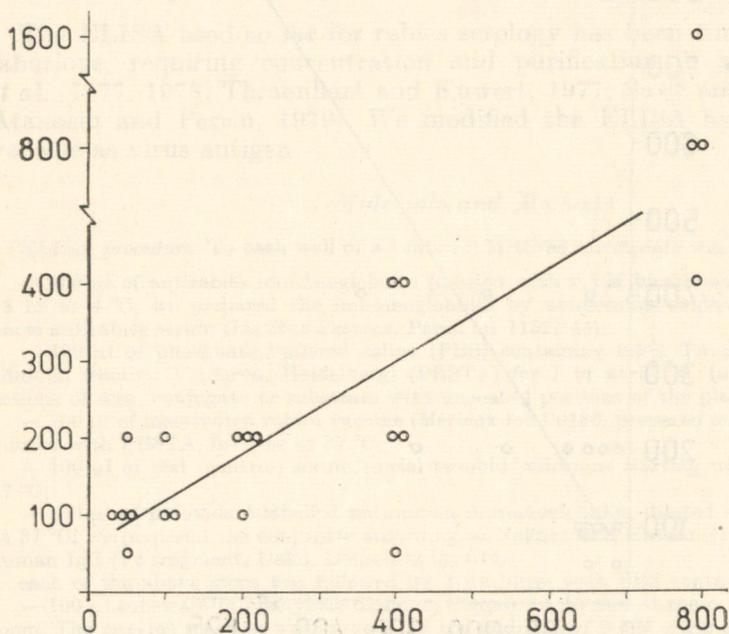


Fig. 2.

Correlation between the results obtained in two types of ELISA  
Rabies antibody titres determined by ELISA according to Atanasiu (abscissa) or our modification (ordinate)

for every laboratory. The ELISA "sandwich" method yielded results comparable with those of the ELISA used so far and was highly specific. Our method offers a simpler and equally sensitive way to detect rabies antibodies.

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#### References

- Atanasiu, P. (1973): Quantitative assay and potency test of antirabies serum and immunoglobulin, pp. 314–318. In M. M. Kaplan and H. Koprowski (Eds): *Laboratory techniques in rabies*, WHO, Geneva.
- Atanasiu, P., and Perrin, P. (1979): Microméthode immunoenzymatique de titrage des anticorps antirabiques. *Ann. Microbiol.* **130A**, 257–268.
- Atanasiu, P., Savy, V., and Perrin, P. (1977): Epreuve immunoenzymatique pour la detection rapide des anticorps antirabiques. *Ann. Microbiol.* **128A**, 489–498.
- Atanasiu, P., Savy, V., and Gilbert, C. (1978): Rapid immunoenzymatic technique for titration of rabies antibodies IgG and IgM. *Med. Microbiol. Immunol.* **166**, 201–209.
- Nakane, P. K., and Kawaoi, A. (1974): Peroxidase-labeled antibody, a new method. *J. Histochem.* **22**, 1084–1091.
- Oktaba, W. (1966): *Elementy Statystyki matematycznej, Metodyka doswiadczalna*. Polskie Wydaw. Naukowe, Warszawa.
- Savy, V., and Atanasiu, P. (1978): Rapid immunoenzymatic technique for titration of rabies antibodies IgG and IgM. *Dev. Biol. Stand.* **40**, 247–253.
- Thraenhart, O., and Kuwert, E. K. (1977): Enzyme immunoenzymatic technique for demonstration of rabies-virus antibodies after immunization. *Lancet* **ii**, 399–400.