ELISA SYSTEMS FOR RABIES ANTIBODY DETECTION

J. Esterhuysen¹ and J. Barrat²

The reference method to determine the antibody level of a serum sample is seroneutralisation. The principle of this technique is to incubate a constant known amount of rabies virus with a constant volume of the serum to be tested.

The virus that has not been neutralised by the antibodies is quantified using an inoculation test either in vivo or in vitro.

This method is sensitive but time-consuming. Results are obtained within 1 to 5 days (with cells) and up to 14 days with mice. Contaminated or putrefied serum samples are most often impossible to titrate because the bacteria (or their toxins) kill the mice or cells.

The immunocapture phenomenon used in ELISA tests is not susceptible to the contamination risk or to the toxin hazard as the techniques using a "living" final step. A second important point is that ELISA tests give their answer more rapidly than the classical seroneutralisation.

The antibody level may be determined in two different ways with ELISA techniques:

- Antibodies are directly quantified using microplates coated with the corresponding antigen
- Antibodies first react with a known amount of antigen. The remaining antigen is then quantified using a plate coated with the corresponding antibody.

One example of each procedure will be detailed here for rabies antibody titration using the ELISA technique.

1 ANTIBODY TITRATION USING A DIRECT ELISA

The example detailed here corresponds to the use of a commercially available kit: "Platelia" distributed by Diagnostic Pasteur (ref no. 72200).

In this test, the plate is coated with the rabies virus glycoprotein. It allows to determine the amount of serum anti-glycoprotein antibodies, which have neutralising activity.

Positive and negative control serum samples of human origin, are included.

The conjugate used is a proteinA - peroxidase one and the colour agent is OPD.

1.1 Preparation of the reference curve

Dilutions of both positive and negative controls are dispensed in duplicate into the wells.

The mean OD corresponding to the antibodies for every dilution is reported on the Y axis of a graph. The X one has a double scale: one is the inverse of the dilution and the other is the corresponding titre in IU/ml.

¹ Foot and Mouth Disease Laboratory, Onderstepoort
² CNEVA, Malzeville, France
1.2 Titration of sera

Every serum is diluted once at a predetermined working dilution. The corresponding OD is then reported on the reference curve and the titre determined.

If the measured OD is out of range of the reference curve, serum is diluted more or less so that the OD measured is within the confidence limits of the reference curve. The extra dilution is then taken into account for the calculation of the precise titre.

1.3 Advantages / disadvantages

This technique titrates the anti-glycoprotein antibodies which are the neutralising ones.

For species other than human, some problems may arise from the detection system that uses a protein-A peroxidase conjugate:

The affinity of proteinA for antibodies is species dependant and it is then necessary to prepare positive and negative controls for every species to be tested. It is also necessary to determine the correct working dilution.

ProteinA does not react at all with cattle immunoglobulins and ProteinA has then to be replaced by a proteinG conjugate.

2 Antibody determination using a liquid phase blocking system.

The example cited here is a summary of J. Esterhuysen's work adapting a routine technique used for FMD serology to rabies.

In large scale epidemiological studies, the major problem is the diversity of species to be tested. In such a study, the former method supposes the use of controls from the different analysed species.

2.1 Principle of the method

A previously determined constant amount of virus is incubated with the diluted serum. After this step, the result of the reaction is transferred in a microtiter plate coated with rabies antibodies. A predetermined quantity of guinea pig anti-rabies serum is then added to every well. The fixation of this guinea pig serum is revealed with an anti-guinea pig immunoglobulin conjugate.

2.2 Advantages / disadvantages

The test described here is more "seroneutralisation like" than the previous one, but it is longer to standardise.

The main advantage of it is that it may be used on any species.

The correlation between the two techniques described here is close to 79%.

3 Conclusion

Although seroneutralisation is the recommended method to determine the antibody level for rabies, ELISA techniques are an interesting alternative, principally for large scale epidemiological studies.

Either of the two techniques described here may be used. Both correlate well, so the choice of which technique to use depends mainly upon the availability of species controls and upon the methods already used routinely in the laboratory.