ELISA SYSTEMS FOR
RABIES ANTIGEN DETECTION

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1 INTRODUCTION

Several techniques are available for the diagnosis of rabies. These can detect either histologic or cytologic signs of viral replication, or viral antigen or virulence and the pathogenicity of non-inactivated virus.

The basic technique used in experimental diagnosis of rabies is fluorescent antibody test. It is used both directly on smears and to detect viral replication in mice or cells after inoculation tests. Sometimes the interpretation of fluorescent antibody test is not easy, particularly with autolysed samples. The most important point with fluorescent antibody test is the regular training of the persons who work with this technique.

Immunoenzymatic techniques were developed to detect viral nucleocapsid by another way: the antibody is not revealed by fluorescence of FITC but by the transformation of a substrate by an enzyme linked to the specific antibody.

* Immunoperoxidase on smears may be used instead of immunofluorescence. Both have the same sensitivity (Genovese and Andral, 1978), but as the test takes longer and is more expensive, it is not used in routine laboratory diagnosis.

* ELISA test: this paper deals with detection of rabies virus nucleocapsid in brain samples using an ELISA technique commercially available.

2 GENERAL INFORMATION OF THE TECHNIQUE

This technique is used to detect viral antigen whether the virus is inactivated or not.

The technique was developed in 1986 by P. Perrin. The Rapid Rabies Enzyme Immuno-Diagnosis is commercially available. It detects the presence of rabies nucleocapsid in the brain sample.

Antibodies specific for rabies nucleocapsid are adsorbed on the microtiter plate. The conjugate used has the same specificity.

2.1 Equipment needed

The kit itself is distributed by Diagnostic Pasteur (ref number 72201).

Plates may be read directly but the utilisation of a spectrophotometer makes it easier. The estimated investment for the laboratory is indicated in table 1.

2.2 Training of technicians

The interpretation of the test does not need any special training. The positive and negative controls provide clear-cut answers, the threshold is easy to determine and the reaction may be interpreted.

If the technicians do not know any other ELISA technique, a few days training are sufficient.

1 CNEVA, Malzeville, France
2.3 Sensitivity of the technique

The correlation between fluorescent antibody test and RREID is between 96 and 99 %.

2.4 Delay of answer

Diagnosis is achieved in less than 4 hours by RREID. 40 samples may be analysed in duplicate using one single plate.

2.5 Cost of the technique

Table 1: Equipment needed for RREID (price were estimated in 1992)

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometer</td>
<td>7300 US $</td>
</tr>
<tr>
<td>Titerplates washer</td>
<td>3700 US $</td>
</tr>
<tr>
<td>37 °C incubator</td>
<td>1100 US $</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>550 US $</td>
</tr>
<tr>
<td>Multichannel pipette</td>
<td>750 US $</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>750 US $</td>
</tr>
</tbody>
</table>

The cost of one test is 4.5 US $ if it is made in duplicates. It is possible to use one single well per sample.

3 Practical use of RREID

3.1 Composition of the kit

The different solutions and controls needed are included in this kit. The microtiter plate may be divided into 6 trays of 16 wells. The volume of positive and negative controls given in the kit is sufficient for two reactions. If the plate has to be divided in more than 2 parts it will be necessary to prepare some 'home made' controls:

* the positive control is the supernatant of an 10% homogenate of rabid mice brains inactivated by BPL and then aliquoted and freeze dried,
* the negative control is the supernatant of an 10% homogenate of non rabid mice brains inactivated by BPL and then aliquoted and freeze dried.

The other solutions consist of

* washing solution (PBS + 0.05% Tween 20, pH 7.4),
* IgG anti-nucleocapsid conjugated with peroxidase,
* Substrate buffer (citrate - H2O2 ),
* OPD tablets,
* stopping solution (H2SO4, 4N).

The interpretation is made either by eye or using a spectrophotometer (492nm).

3.2 Results obtained with RREID

An evaluation has been made by 6 national or WHO reference laboratories on 1253 specimens from 27 animal species. Using fluorescent antibody test as reference, 97.4% of specimens (651 positive and 569 negative) gave identical results to both tests. 22 specimens (3%) were FAT+ / RREID-.
A second evaluation was made involving 12 field laboratories from Africa and Latin America on 818 samples. The following results were obtained: 96.7% of samples gave the same result (428 positive and 363 negative), 22 specimens (2.7%) were FAT+ / RREID- 5 specimens (0.6%) were FAT- / RREID+.

The test used in the French national reference laboratory for rabies on 2290 routine examinations correlated well with fluorescent antibody test: 99.2% of identical answers (287 positive and 1985 negative), 0.6% (15 specimens) were FAT+ / RREID- and 3 (0.1%) were FAT- / RREID+.

In Malzéville, 2572 specimens were tested by fluorescent antibody test, cell culture test and RREID. FAT and RREID gave the same answer for 98.9% samples (1853 negative and 692 positive ones), 12 specimens were positive only to FAT, 15 only to RREID (cell culture test was negative for these samples).

The estimated correlation between fluorescent antibody test and RREID thus ranges between 96 and 99%.

This kit can detect a concentration of 0.8 to 1ng of nucleocapsid of rabies virus per ml for serotype 1 strains.

This technique has two principal advantages:

1. it does not need any regular training for the interpretation of the test,
2. it is rapid: within 4 hours, 40 diagnosis may be performed in duplicate.

These two points make RREID a good technique for large scale epidemiological studies, but the test is more expensive than fluorescent antibody test: 4.5 US$ (in duplicate) for RREID and 1US$ for fluorescent antibody test.

4 RREID LYSSA

RREID detects a concentration of 1 ng of nucleoprotein per ml of serotype 1 virus. The sensitivity of the test is low for rabies-related viruses, despite the common antigenic properties of the nucleoprotein isolated from different serotypes.

In order to increase the sensitivity of the test for rabies related viruses (Perrin et al., 1992), 3 polyclonal antibodies were prepared against nucleoprotein of serotype 1 (PV strain), serotype 3 (Mokola strain) and EBL stain (not classified in serotype). These polyclonal antibodies were coated on micro-titer plates and conjugated to biotin. The reaction involves the use a classical peroxidase-avidin conjugate.

These changes increase the sensitivity of the detection of nucleoprotein (concentration up to 0.2 ng/ml) and the range (nucleoprotein of the different rabies viruses are detected whatever their serotype).

5 CONCLUSION

The ELISA method is simple and rapid to perform in large scale epidemiological studies. The immunocapture phenomenon allows to perform easy and reliable diagnosis even on autolysed samples.

BIBLIOGRAPHY

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