ELISA test for rabies

Dong-Kun Yang/OIE expert for rabies and JE
Advantage and disadvantage of ELISA tests

- ELISA test is easy to use and rapid.
- It do not require virus and cell culture handling.
- ELISA is less dependent on serum quality.
- It is proper to apply large amount of sero-surveillance.
- Problems of cytotoxicity encountered with hemolysed samples can be avoided.
- The FAVN and RFFIT take at least 48 h to get results.
- It is difficult to implement in laboratories from developing countries.
- The price of ELISA kit is expensive and the kit should be proven by official Authority.
A quantitative indirect ELISA to monitor the effectiveness of rabies vaccination in domestic and wild carnivores

A. Servat a,* M. Feyssaguet b, I. Blanchard b, J.L. Morize b, J.L. Schereffer a, F. Boue a, F. Cliquet a

a Affi Nancy, Laboratoire d’Études et de Recherches sur la Rage et la Pathologie des Animaux Sauvages, Domaine de Pissérécourt, B.P. 9, 54220 Molsheim, France
b Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-La-Coquette, France

Received 31 March 2006; received in revised form 7 June 2006; accepted 16 July 2006
Available online 2 November 2006

- Commercial indirect ELISA kits are available that allow detection of rabies antibodies in individual dog and cat sera following vaccination.

- In 2007, the International committee adopted such methods as prescribed tests for evaluating vaccine responses in dogs and cats prior to international movement provided a kit as used that has been validated and adopted on the OIE register as fit for such specific purposes.

- Other ELISA methods or kit should not be regarded as proscribed but may be useful for monitoring of vaccination campaign.
Preparation of antigen for ELSA

• A PV strain of rabies virus grown in a BSR cell line was collected and inactivated with β-propiolactone (2.5% vol/vol).
• The antigen was concentrated (amicon DC2) and purified by ultracentrifugation at +4 °C on a 15–45% (w/v) discontinuous sucrose gradient at 25,000 rpm.
• Rabies virus specific fractions were collected at the interphase, diluted with 0.01 M Tris/0.15 M NaCl buffer (pH 8.0) and pelleted at 29,000 rpm for 90 min at +4 °C.
• The glycoprotein was extracted from the membrane with detergent treatment by a zwitterion (n-octyl-D-glucopyranoside 2%) after resolubilisation of the pellet, followed by ultracentrifugation at 400,000 rpm for 100 min at +4 °C.
• The protein concentration of the supernatant containing the glycoprotein was estimated by using the Bio-Rad protein assay. The fractions were collected and conserved at −20 °C.
• Purified G glycoprotein was diluted in 0.1 M sodium carbonate buffer (pH 9.5) and used to coat 96-well microdilution plates (0.5–1 μg/ml).
• Residual adsorption sites on the plates were saturated at room temperature by incubation with 300 μl of 0.1 M phosphate buffered saline (pH 7.4) supplemented with 2.5% skim milk powder.
Sensitivity and correlation with FAVN titer

An ELISA (Platelia™ Rabies II kit) was developed for domestic carnivores and wildlife. This ELISA is the only one certified and prescribed by the OIE.
Evaluation of a rabies ELISA as an alternative method to seroneutralisation tests in the context of international trade of domestic carnivores

Currently the kit is not included in the Register of diagnostic kit certified by the OIE as “fit for purpose” validated.
Many discordant results were observed when comparing the titres obtained by the Platelia Rabies II kit and those obtained with the FAVN test, regardless of the kind of study undertaken (internal or collaborative).

Compared to the FAVN test, this ELISA was not sensitive enough for rabies antibody detection with the current threshold of 0.5 EU/mL.

The Platelia Rabies II kit could possibly be used for research purposes with a threshold set at 0.3 EU/mL instead of 0.5 EU/mL in order to increase sensitivity.
The RABV combined with gold-conjugated Mab is trapped at the test line where the unconjugated mab is fixed, and unbounded gold labeled mab is trapped at the control line.
Diagram of rapid test strip (G detection kit) for the detection of neutralizing antibody. The sample is applied to the test strip at the sample hole and allowed to travel along the nitrocellulose membrane and from an antigen-antibody complex with gold-conjugated MAb in the presence of RABV G in the sample. As the antigen-antibody complex or unbound antibody move further, they are trapped in the test or control line by RABV G-specific or goat anti-mouse polyclonal antibody, respectively, to produce a red band.
Sensitivity and specificity of RAPINA test

<table>
<thead>
<tr>
<th></th>
<th>Virus-neutralizing antibody</th>
<th>Total samples tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.5 IU/ml</td>
<td>≥0.5 IU/ml</td>
</tr>
<tr>
<td>Band formation</td>
<td>+</td>
<td>57</td>
</tr>
<tr>
<td>In RAPINA test</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Total samples tested</td>
<td></td>
<td>62</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>88.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>91.9%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>90.4%</td>
</tr>
</tbody>
</table>

Positive predictive value 90.5%: ratio of neutralizing antibody < 0.5 IU/ml among positive band formation. Negative predictive value 90.4%: ratio of neutralizing antibody ≥ 0.5 IU/ml among negative band formation.

* Ratio of negative band formation among total of neutralizing antibody ≥ 0.5 IU/ml.

* Ratio of positive band formation among total of neutralizing antibody < 0.5 IU/ml.

* Positive band formation among neutralizing antibody < 0.5 IU/ml + negative band formation among neutralizing antibody ≥ 0.5 IU/ml)/total of subjects.
Conclusion

• The ELISA kit, Platelia Rabies II kit could possibly be used for research purposes.
• After the rapid test strip (G detection kit) is evaluated and adopted as the detection method of neutralizing antibody by the International committee first, and then the kit will be used for evaluating vaccine responses in dogs and cats.