Assessment of a new indirect ELISA for the detection of rabies specific antibodies in vaccinated dogs and cats

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New Kit Introduction

Synbiotics Europe developed a new tool for rabies antibody titration in collaboration with:

AFSSA, Nancy, France
Agence Française de Sécurité Sanitaire des Aliments

VLA, UK
Veterinary Laboratories Agency

SERELISA™ Rabies
Ab Mono Indirect
Exclusivity

ELISA technique is now

OIE prescribed as a screening test for international trade

Since May 2003

ELISA technique is fully described in

5th manual of standards for diagnostics and vaccines

Since May 2004

Protocol SERELISA™ Rabies Ab Mono Indirect

Specific antibodies binding

Positive serum

Step 1:
Add 90 µl of sample diluent + 10 µl of individual sera in each well

Incubation:
1h, +37°C

Wash 4x

Negative serum

Wash 4x
Protocol SERELISA™ Rabies Ab Mono Indirect

Conjugate binding

Step 2:
Add 100 µl of 1:10 diluted conjugate in each well

Incubation:
1 h, +37°C

Wash 4x

Enzyme reaction

Step 3:
Add 100 µl of substrate in each well

Incubation:
30 min, +20°C, in the dark

Add 50 µl of stop solution in each well
**Protocol**

**Preliminary step**

Preparation of a range of 7 dilutions from the OIE reference serum

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
<td>S 8</td>
</tr>
<tr>
<td>TP</td>
<td>S 1</td>
</tr>
<tr>
<td>OIE 1:100</td>
<td>S 2</td>
</tr>
<tr>
<td>OIE 1:300</td>
<td>S 3</td>
</tr>
<tr>
<td>OIE 1:1000</td>
<td>S 4</td>
</tr>
<tr>
<td>OIE 1:1500</td>
<td>S 5</td>
</tr>
<tr>
<td>OIE 1:3000</td>
<td>S 6</td>
</tr>
<tr>
<td>OIE 1:10000</td>
<td>S 7</td>
</tr>
</tbody>
</table>

Test always in duplicate

**Protocol SERELISA™ Rabies Ab Mono Indirect**

**Reading**

Optical density reader
450/630 nm

**Interpretation**

1. Perform a linear regression curve between ln Rabies Ab concentrations (expressed in EU/ml) and ln (OD)
   
   \[ \ln \text{Rabies Ab concentration (EU/ml)} = a + b \times \ln \text{OD} \]

2. Calculate rabies Ab concentration of the sample expressed as «equivalent units per ml» (EU/ml)

   \[ \text{Sample Rabies Ab concentration (EU/ml)} = e^{(a + b' \times \ln \text{OD})} \]

   
   0.6 EU/ml

   
   Non protected | protected

**Validation:**

- \( P > 0.300 \)
- \( N < 0.5 \times P \)

And \( r > 0.95 \)
Interpretation Principle

Result of the OIE serum in dilutions

\[ \text{OD} \]

Titer (IU/mL)

Interpretation Principle

Result of the OIE serum in dilutions

\[ \ln(\text{OD}) \]

\[ \ln(\text{Titer (IU/mL)}) \]

\[ \ln([\text{Ab titer}]) = a + b \ln(\text{OD}) \]
**Interpretation Example**

![Graph showing OD vs In Titer with a threshold of S > 0.6 IU/mL for positive results.]

**Material & methods Samples used**

- OIE reference standard (0.667 IU/mL) at different dilution for analytical sensitivity
- OIE reference standard (0.667 IU/mL) at the last positive dilution for repeatability studies
- 5 samples tested 4 times in 4 laboratories (1 neg, 4 pos) for reproducibility studies
- 253 Naive animals (not vaccinated) from UK, New Caledonia, Hawai… for specificity
- 303 Vaccinated animals for sensitivity
- 1440 samples compared in FAVN and ELISA in 2 labs for correlation
**Material & methods**

Samples were tested in the following labs:

**Internal trials**
- Synbiotics Europe R&D lab
- Synbiotics Europe Quality control lab

**Field trials**
- AFSSA, Nancy, France
- VLA, UK
- European lab A
- European lab B

Results interpreted thanks to:
- Descriptive statistics value
- One way ANOVA
- Linear regression

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**Internal trial** Linearity

4 replicates of each dilution of the OIE reference standard run on 5 plates on 5 separate occasions

![Graph showing linearity](image)

Results are expressed as the mean of the natural logarithm ODs compared to natural logarithm of theoretical FAVN titers (ln Titer), IU/ml, expected upon serial dilutions of the OIE reference standard.
Internal trial  Repeatability

4 replicates of weak positive OIE reference standard dilution run on 5 plates on 5 separate occasions

<table>
<thead>
<tr>
<th></th>
<th>Plate 1</th>
<th>Plate 2</th>
<th>Plate 3</th>
<th>Plate 4</th>
<th>Plate 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>7.6</td>
<td>10.3</td>
<td>3.8</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Run 2</td>
<td>6.7</td>
<td>4.1</td>
<td>7.7</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>Run 3</td>
<td>3.8</td>
<td>2.1</td>
<td>7.1</td>
<td>4.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Run 4</td>
<td>5.5</td>
<td>3.5</td>
<td>9.7</td>
<td>5.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Run 5</td>
<td>5.2</td>
<td>3.4</td>
<td>5.4</td>
<td>6.3</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Preliminary estimation of repeatability expressed by the coefficient of variation (%) calculated on ODs obtained for the 0.667 IU/ml OIE titer

All Coefficient of variation <10%

Internal trial  Sp & Se

FAVN test results cut off: 0.5 IU/mL

<table>
<thead>
<tr>
<th>Naive animals</th>
<th>Vaccinated animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>241</td>
</tr>
<tr>
<td>Positive</td>
<td>7*</td>
</tr>
</tbody>
</table>

ELISA test results cut off: 0.6 EU/mL

<table>
<thead>
<tr>
<th>ELISA /Status</th>
<th>ELISA /FAVN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se = 231 / 303 = 76,2 %</td>
<td>Se = 229 / 265 = 86,4 %</td>
</tr>
<tr>
<td>Sp = 246 / 253 = 97,2 %</td>
<td>Sp = 282 / 291 = 96,9 %</td>
</tr>
</tbody>
</table>

Frequency tabulation on n = 556 pets, (253 naïve or non vaccinated animals and 303 vaccinated pets) tested by using FAVN reference method (in two reference laboratories)
**Field trial Analytical Sensitivity**

<table>
<thead>
<tr>
<th>FAVN Expected OIE titers</th>
<th>Ref Lab A (in EU/mL)</th>
<th>Ref Lab B (in EU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>STD</td>
</tr>
<tr>
<td>3.33</td>
<td>4.22</td>
<td>1.4</td>
</tr>
<tr>
<td>2.22</td>
<td>3.01</td>
<td>0.8</td>
</tr>
<tr>
<td>0.667</td>
<td>1.29</td>
<td>0.35</td>
</tr>
<tr>
<td>0.5</td>
<td>1.15</td>
<td>0.4</td>
</tr>
<tr>
<td>0.13</td>
<td>0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>0.0667</td>
<td>0.14</td>
<td>0.015</td>
</tr>
<tr>
<td>0.022</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*ELISA mean titer and standard deviation, studied on different OIE reference standard titers*

**Field trial Accuracy**

Correlation between OIE FAVN expected titres and ELISA observed titres

- **Ref Lab A**
- **Ref Lab B**
- **Theoric R²=1**
Field trial Correlation

<table>
<thead>
<tr>
<th></th>
<th>ELISA test results cut off: 0.6 EU/mL</th>
<th>FAVN test results cut off: 0.5 IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>22 (2.2%)</td>
<td>67 (6.7%)</td>
</tr>
<tr>
<td>Positive</td>
<td>11 (1.1%)</td>
<td>900 (90%)</td>
</tr>
</tbody>
</table>

Frequency tabulation on Ref Lab A pet population, n = 1000. Samples were tested at the Ref Lab A

<table>
<thead>
<tr>
<th></th>
<th>ELISA test results cut off: 0.6 EU/mL</th>
<th>FAVN test results cut off: 0.5 IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>19 (4.32%)</td>
<td>91 (20.68%)</td>
</tr>
<tr>
<td>Positive</td>
<td>1 (0.23%)</td>
<td>329 (74.77%)</td>
</tr>
</tbody>
</table>

Frequency tabulation on n = 440 Ref Lab B pets. Samples were tested in Ref Lab B

Conclusion

ELISA for neutralizing Ab titration test
Analytical sensitivity and linearity of result quantitative test
Use of dilutions of the OIE standard reference serum obtention of titer
Very good repeatability accuracy
Efficient and useful alternative technique for screening