**INTERPRETATION**

Quality control and interpretation of results

For each sample (S) and control, calculate the mean (M) of ODs obtained. To obtain **Ratio**, divide each sample's mean OD by the positive control's (P) mean OD.

\[
\frac{M_{OD_S}}{M_{OD_P}} = \text{RATIO}
\]

The following criteria must be met in order to validate the analysis:

- Negative control ratio must be less than 0.15.
- Mean of positive control ODs must be greater than 0.6.

**Interpretation:**

- Sample ratio less than 0.35 is considered negative.
- Sample ratio greater or equal to 0.5 is considered positive.
- Sample ratio less than 0.5 but greater or equal to 0.35 is considered suspicious.

**Test evaluation**

<table>
<thead>
<tr>
<th>This test</th>
<th>ELISA Reference Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>55</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Suspicious</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
</tr>
</tbody>
</table>

- When considering suspicious results as positive, the relative sensitivity of this test is 91.9% (95% CI = 82.8 – 96.7%) and the relative specificity is 94% (95% CI = 86.9 – 97.5%).
- When considering suspicious results as negative, the relative sensitivity of this test is 98.2% (95% CI = 89.2 – 99.9%) and the relative specificity is 97.4% (95% CI = 87.7 – 97.4%).

**BIBLIOGRAPHY**

MATERIAL

Materials required but not provided:
- Purified water
- Adjustable single- and multi-channel micropipettes
- Single-use micropipette tips
- ELISA microplate washer
- Test tubes for sample dilution
- ELISA 96-well microplate reader equipped with 405 nm filter
- Containers for dilution of other solutions

Reagents provided with the kit:

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 strips of 8 wells coated with APP 4, 7 antigens</td>
<td>2</td>
</tr>
<tr>
<td>Ready-to-use positive control</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Ready-to-use negative control</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Concentrated conjugate</td>
<td>50 μL</td>
</tr>
<tr>
<td>Concentrated wash solution (10X)*</td>
<td>2 X 100 mL</td>
</tr>
<tr>
<td>Ready-to-use substrate</td>
<td>24 mL</td>
</tr>
<tr>
<td>Ready-to-use stop solution*</td>
<td>24 mL</td>
</tr>
</tbody>
</table>

* Crystals may form when stop solution and wash solution are kept at 2-7°C. This will not affect the efficiency of the products. In order to use these solutions, simply bring them to room temperature and the crystals will dissolve.

PRECAUTIONS

- For in vitro veterinary use only.
- The materials used in this kit must be considered as infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents from different serial numbers.
- The sensitivity and specificity of this test are guaranteed only if the procedures are strictly observed.
- Do not expose the substrate to either light or oxidizing agent. Always keep the substrate in a plastic container. This solution might cause skin or eye irritation.
- Keep all reagents at 2-7°C and bring to room temperature before use.

EXECUTION

A. Preparation of wash solution
After homogenizing the concentrated wash solution (no evidence of crystals), dilute at 1/10 with purified water (e.g., 100 mL 10X concentrated wash solution in 900 mL purified water for each plate). Once diluted, the solution (1X) is stable for 1 week at 2-7°C.

B. Sample preparation
It is strongly recommended to test the samples and controls as duplicate. Dilute porcine serum samples in 1X wash solution (see section A) at 1/200 (e.g., 4 μL sample in 796 μL 1X wash solution). Make sure you use a new tip for each sample. Also make sure each dilution is properly mixed before being distributed into the wells.

C. Conjugate preparation
Dilute the conjugate with 1X wash solution (see section A) according to the dilution indicated on the Quality Control Certificate. Dilute conjugate a few minutes prior its use and always prepare a fresh solution.

D. Test procedures
Bring all reagents to room temperature and mix well manually before use.

1. Make a schematic representation of the plate and the distribution of controls and samples.
2. Dispense 100 μL ready-to-use positive control into wells A1 and A2.
3. Dispense 100 μL ready-to-use negative control into wells B1 and B2.
4. Dispense 100 μL diluted samples (see section B) into wells C1/C2, D1/D2, …
5. Incubate at 23 ± 2°C for 30 minutes.
6. Wash each well with 5 times 300 μL 1X wash solution (see section A). Throw away all liquid contained in the plate after each wash. After the last wash, dry the plate by tapping it on absorbent paper.
7. Dispense 100 μL diluted conjugate (see section C) into each well.
8. Incubate at 23 ± 2°C for 30 minutes.
10. Dispense 100 μL ready-to-use substrate into each well.
11. Incubate, away from light, at 23 ± 2°C for 20 minutes.
12. Dispense 100 μL ready-to-use stop solution into each well.
13. Measure optical densities (OD) at 405 nm. If the microplate reader is equipped with a reference filter, set it at 490 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
14. Calculate the results.