**INTERPRETATION**

**Calculation of the Ratio**

For each sample (S) and control, subtract the OD obtained in the well without Ag (2) from the OD obtained in the well containing Ag (1) to obtain the corrected OD. To obtain Ratio, divide each result by the one obtained for the positive control (P).

\[
\frac{(OD_{S1}(Ag) - OD_{S2}(without\ Ag))}{(OD_{P1}(Ag) - OD_{P2}(without\ Ag))} = RATIO
\]

**Validity Criteria**

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

- Negative control ratio must be less than 0.25.
- Positive control corrected OD must be greater than 0.8.

**Interpretation:**

- Sample ratio less than 0.4 is considered negative.
- Sample ratio greater or equal to 0.6 is considered positive.
- Sample ratio less than 0.6 but greater or equal to 0.4 is considered suspicious. It is recommended to collect a 2nd serum sample 2 weeks later and test it.

**BIBLIOGRAPHY**


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**Haemophilus parasuis Antibody Test Kit (ELISA) Swinecheck® HPS**

Insert

2012-03-01

This is an immunoenzymatic assay for the detection of antibodies against *Haemophilus parasuis* (HPS) in porcine serum regardless of the serotype.

HPS is a commensal organism of the upper respiratory tract of pigs. Fifteen different serotypes have been identified so far. Serotypes 1, 5, 10, 12, 13 and 14 are the most virulent. Under appropriate conditions virulent strains can cause a severe systemic disease known as Glässer’s disease. Clinical signs and lesions vary depending on the immune status of the animals and the virulence of the strain. Systemic infection of naive animals with virulent HPS strains is characterized by high fever, lethargy, swollen joints, coughing, dyspnoea, and central nervous system signs, such as trembling and paddling. Glässer’s disease may be controlled with antimicrobials and prevented by vaccination. Diagnosis of HPS infections is based on clinical signs, lesions at necropsy, and detection of the organism by isolation or PCR. Detection of HPS circulating antibodies may be useful to diagnose HPS infection in animals which have demonstrated suspect clinical signs or to evaluate levels of maternally derived antibodies prior to vaccination or antibody response after vaccination.

**PRINCIPLE OF THE TEST**

The diluted porcine serum samples and the controls are incubated in wells coated with HPS antigens (Ag) (odd columns), and in wells without Ag (even columns). The antibodies (Ab) specific to HPS present in positive serum samples bind to the Ag in the wells. After several washes to eliminate unbound substances, a conjugate (an Ab coupled to an enzyme) targeted at porcine Ab is added. After incubation, the excess of this conjugate is eliminated by a second wash and its attachment is revealed with a chromogenous substrate. Following this incubation, the enzyme, if present, reacts with the substrate and a green color develops. The reaction is then stopped and the optical densities are read. The intensity of the color allows the determination of the status of sample tested. A negative sample will show a weak reaction (pale green) whereas a strong positive will show a strong reaction (dark green). All shades of green between dark and pale represent various degrees of positivity.
### MATERIAL

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strips of 8 wells coated with HPS Ag</td>
<td>12</td>
</tr>
<tr>
<td>Strips of 8 wells without HPS Ag used as control (blue sticker)</td>
<td>12</td>
</tr>
<tr>
<td>Ready-to-use positive control</td>
<td>2 mL</td>
</tr>
<tr>
<td>Ready-to-use negative control</td>
<td>2 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>30 mL</td>
</tr>
<tr>
<td>Ready-to-use stop solution*</td>
<td>30 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.

The materials provided are sufficient for testing up to 92 samples in one test by using all the strips alternatively to form 2 microplates and distributing the appropriate controls on each microplate (see D. Test Procedure).

The quantity of reagents is sufficient for using the kit up to 10 times.

### 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., 50 mL of concentrated wash solution in 450 mL of purified water). Once prepared, the solution (1X) is stable for 1 week at 2-7°C.

#### B. Sample Preparation

Each sample and control needs to be tested in a well with Ag and in a well without Ag. Dilute porcine serum samples at 1/50 in 1X wash solution (see section A) (e.g., 6 µL sample in 294 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 to its use and always prepare a fresh solution.

#### D. Test Procedure

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

1. Put strips coated with HPS Ag on the odds columns of a frame, and strips without Ag (blue sticker) on the even columns. Put the rest of the strips in the plastic bags included for that purpose.
2. Make a schematic representation of the plate and the distribution of controls and samples.
3. Dispense 100 µL ready-to-use positive control into wells A1 and A2.
4. Dispense 100 µL ready-to-use negative control into wells B1 and B2.
5. Dispense 100 µL diluted samples (see section B) into wells C1/C2, D1/D2, …
6. Incubate at 23 ± 2°C for 30 minutes.
7. Wash each well with 4 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.
8. Dispense 100 µL diluted conjugate (see section C) into each well.
9. Incubate at 23 ± 2°C for 30 minutes.
10. Repeat step 7.
11. Dispense 100 µL ready-to-use substrate into each well.
12. Incubate, away from light, at 23 ± 2°C for 20 minutes.
13. Dispense 100 µL ready-to-use stop solution into each well.
14. 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.
15. Calculate the results.

### 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.
- The wash solution contains thimerosal (0.01%).
- Dispose of the wash solution, the substrate and the stop solution according to local regulations for chemicals.
- Keep all reagents at 2-7°C and bring to room temperature before use.