INTERPRETATION

Calculation of the Ratio

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

$$\frac{OD_S}{M\ OD_P} = \text{RATIO}$$

Validity Criteria

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 0.80.

Interpretation

- Sample ratio less than 0.30 is considered negative.
- Sample ratio greater or equal to 0.40 is considered positive.
- Sample ratio less than 0.40 but greater or equal to 0.30 is considered suspicious.

BIBLIOGRAPHY

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2. Sassu EL, Bossé JT, Tobias TJ, et al. Update on *Actinobacillus pleuropneumoniae*-knowledge, gaps and challenges. Transbound Emerg Dis. 2018;65 Suppl 1:72-90.
3. Trottier, Y.L. 1991. Évaluation des paramètres impliqués dans l'ELISA et leur application au sérodiagnostic de la pleuropneumonie porcine causée par *Actinobacillus pleuropneumoniae* sérotype 5. Masters Memoir. University of Montréal, Montréal. Canada. 252 p.

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Actinobacillus pleuropneumoniae 13 Antibody Test Kit (ELISA)
Swinecheck® APP13
Insert

v.1 2019-05-27

This is an immunoenzymatic assay for the detection of antibodies against *Actinobacillus pleuropneumoniae* (APP) serotype 13 in porcine serum.

Porcine pleuropneumonia caused by APP is an important porcine disease that can result in considerable economic losses. A remarkable feature of APP is that its virulence greatly varies depending on the isolates. This results in clinical situations varying from subclinical infections to acute disease resulting in severe respiratory distress with high lethality. Interestingly the virulence of isolates correlates quite well with the serovars in a given geographical location (18 serotypes have been identified so far).

Due to virulence variability the control of APP mainly focuses on the most virulent serotypes. Serological testing is the most efficient tool to monitor APP infections on a herd basis. Serogroup/serotype specific assays are used to allow discriminating serotype/serogroup antibodies. The most sensitive and specific assays are indirect ELISA using highly purified long chain lipopolysaccharides (LC-LPS) as antigen.

Swinecheck APP13 ELISA is using highly purified LC-LPS from APP13 as antigen. As APP13 LC-LPS is very similar to APP7 LC-LPS, cross-reactions between these serotypes do occur in LC-LPS ELISA. However, reactions are stronger with the homologous antigens. The use of APP13 LC-LPS ELISA thus allows maximizing the sensitivity of detecting animals infected with this serotype.

PRINCIPLE OF THE TEST

Controls and diluted porcine serum samples are incubated in wells coated with APP13 antigens (Ag). The antibodies (Ab) specific to APP13 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 the 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.

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MATERIAL

Components	Quantity
• 12 strips of 8 wells coated with APP 13 antigen	2
• Ready-to-use positive control	2.5 mL
• Ready-to-use negative control	2.5 mL
• Concentrated conjugate	50 µL
• Concentrated wash solution (10X)*	2 X 100 mL
• Ready-to-use substrate	24 mL
• Ready-to-use stop solution*	24 mL

* Crystals may form when stop solution and wash solution are kept at 2-8ºC. 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 184 samples.

Materials Required but not Provided:

- Purified water
- Adjustable single- and multi-channel micropipettes
- Single-use micropipette tips
- Test tubes for sample dilution
- ELISA 96-well microplate reader equipped with 405 nm filter
- Containers for dilution of other solutions

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.
- Dispose of the substrate and the stop solution according to local regulations for chemicals.
- Keep all reagents at 2-8°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 2-8°C.

B. Sample Preparation

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). It is important to use a new tip for each sample. 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 Final Control Sheet. Dilute conjugate a few minutes prior to its use and always prepare a fresh solution.

D. Washings

THE WASHING STEPS ARE CRITICAL TO GET SUITABLE RESULTS. Perform washings manually using a wash bottle or a multi-channel pipette. Gentle washings are required. Empty the contents of the wells into the sink. Blot the wells dry on a clean absorbent paper. Flood the wells with about 300 µL of 1X wash solution (see section A). Avoid cross-contamination of the wells. Make sure each well is filled and no air bubbles are trapped. Throw away all liquid contained in the plate. Repeat the process as indicated in the test procedure. After the last wash, dry the plate by tapping it on absorbent paper.

E. 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 of ready-to-use positive control, ready-to-use negative control or diluted samples (see section B) into appropriate wells (it is recommended to run control sera in duplicates).
3. Cover the wells and incubate at 23 ± 2°C for 30 minutes.
4. Wash each well 5 times with 300 µL 1X wash solution (see sections A and D).
5. Dispense 100 µL of diluted conjugate (see section C) into each well.
6. Cover the wells and incubate at 23 ± 2°C for 30 minutes.
7. Repeat step 4.
8. Dispense 100 µL of ready-to-use substrate into each well.
9. Cover the wells and incubate, away from light, at 23 ± 2°C for 20 minutes.
10. Dispense 100 µL of ready-to-use stop solution into each well.
11. 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.
12. Calculate the results.

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