INTERPRETATION

Calculation of the S/P Ratio

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

$$\frac{OD_S - M\ OD_N}{M\ OD_P - M\ OD_N} = S/P\ RATIO$$

Validity Criteria

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

- Negative control's MOD must be less than 0.40
- Positive control's corrected OD must be greater than 0.70

Interpretation

- Sample S/P ratio less than 0.4 is considered negative.
- Sample S/P ratio greater or equal to 0.4 is considered positive.

Porcine Epidemic Diarrhea Virus Antibody Test Kit, ELISA
Swinecheck® PED indirect – 2 plates
Insert

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Swinecheck® PED indirect is an immunoenzymatic assay for the detection of antibodies to porcine epidemic diarrhea virus (PEDV) in porcine serum samples.

Porcine epidemic diarrhea (PED) is a swine disease characterized by acute diarrhea and vomiting. The disease is very contagious and affects pigs of all ages. The mortality may reach 100% in young piglets but is negligible in mature pigs.

PED is caused by a coronavirus distinct from the other porcine coronaviruses more especially the transmissible gastroenteritis virus (TGEV). PED was first reported in Europe in the late '60 and is now prevalent in China, the Asian South East and more recently in the USA.

PED diagnosis relies on the demonstration of PEDV nucleic acids or antigens in various specimens (e.g. intestines, feces or oral fluids) or of PEDV antibodies in serum. Several serological tests have been developed to detect PEDV antibodies (e.g. IFA, ELISA). Enzyme-Linked Immuno Assays (ELISA) are easy to perform and to be automatized.

PRINCIPLE OF THE TEST

Controls and diluted porcine serum samples are incubated in wells coated with PEDV antigens (Ag). The antibodies (Ab) specific to PEDV present in positive samples bind to the Ag in the wells. After several washes to eliminate unbound substances, biotinylated polyclonal Ab targeted at porcine immunoglobulins G (IgG) (conjugate A) are added. After incubation, the unbound material is eliminated by several washes and streptavidin conjugated to horseradish peroxidase (HRPO) (conjugate B) is added. After incubation, the excess of conjugate is eliminated by several washes and a chromogenous substrate (TMB) is added. During the incubation of the substrate, HRPO, if present, reacts with the substrate and a blue color develops. The reaction is then stopped (the color changes from blue to yellow) and the optical densities (OD) 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 yellow) whereas a strong positive will show a strong reaction (dark yellow). All shades of yellow between dark and pale represent various degrees of positivity.

MATERIAL

Components	Quantity
• 6 strips of 16 wells coated with PEDV antigen	2
• Ready-to-use positive control*	2.5 mL
• Ready-to-use negative control*	2.5 mL
• Concentrated conjugate A	100 - 300 µL**
• Concentrated conjugate B	500 - 1000 µL**
• Ready-to-use dilution buffer*	3 x 100 mL
• Concentrated wash solution (10X)*	2 x 100 mL
• Ready-to-use substrate	25 mL
• Ready-to-use stop solution	25 mL

*** Clumps, precipitates or crystals may form in the dilution buffer, the controls or the wash solution when stored at 2-7ºC. This does not affect the assay performance. However, warm these solutions in a water bath at 37°C for 30 minutes and mix them vigorously prior to use.**

** Precise volume depends on the lot of conjugate and the recommended working dilution (see Section C).

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
- Containers for dilution of other solutions
- ELISA microplate washer (optional)
- ELISA 96-well microplate reader equipped with 450 nm filter.

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 stop solution contains a strong acid and must be manipulated with caution to avoid contact with skin or mucous membranes.
- Dispose of 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 (unused conjugates should be stored back at 2-7°C as soon as possible).
- If using partial plates, remove only the wells required for the samples to be tested. Then put the remaining wells, along with the supplied desiccant, in a sealed bag and store them at 2-7°C.

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., 10 mL 10X concentrated wash solution in 90 mL purified water). Once diluted, the solution (1X) is stable for 1 week at 2-7°C.

B. Sample Preparation

Dilute porcine serum samples in dilution buffer at 1/200 (e.g. 4 µL sample in 796 µL of dilution buffer). 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 conjugates A and B in the dilution buffer according to the dilution indicated on the Final Control Sheet. Dilute conjugates a few minutes prior to their use and always prepare fresh solutions. Return the concentrated conjugates at 2-7°C immediately after dilution.

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 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 60 minutes.
4. Wash each well 4 times with 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.
5. Dispense 100 µL of diluted conjugate A (see section C) into each well.
6. Cover the wells and incubate at 23 ± 2°C for 60 minutes.
7. Repeat step 4.
8. Dispense 100 µL of diluted conjugate B (see section C) into each well.
9. Cover the wells and incubate at 23 ± 2°C for 60 minutes.
10. Repeat step 4.
11. Dispense 100 µL of ready-to-use substrate into each well.
12. Cover the wells and incubate, away from light, at 23 ± 2°C for 10 minutes.
13. Dispense 100 µL of ready-to-use stop solution into each well.
14. Measure optical densities (OD) at 450 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
15. Calculate the results.