Porcine Reproductive & Respiratory Syndrome Virus Antibody ELISA

Explanation of the Test

Porcine Reproductive and Respiratory Syndrome (PRRS) is considered the most economically important viral disease for the swine industry worldwide. The syndrome first began causing swine herd problems in the late 1980's and, prior to isolation of the causative agent, was often referred to as mystery swine disease.

The most commonly used serological tests for PRRS diagnosis are the indirect fluorescent antibody (IFA) test and enzyme linked immunosorbent assay (ELISA).

The BIONOTE PRRS Ab ELISA 4.0 contains microplates, which are pre-coated with recombinant PRRSV antigens on the well. For testing, ELISA plates are incubated with samples and controls(1:39 dilution with the Sample Diluent) for 30 minutes at room temperature(18~25 ℃). During first incubation, anti-PRRSV antibodies present in sample bind to the antigens coated on the well. Following this incubation, all unbounded materials are removed by washing step. After that, rabbit anti-pig IgG-HRPs are dispensed into the wells and incubated for 30 minutes at room temperature. The enzyme activity will be in proportion to the anti-PRRSV antibodies in sample and evidenced by incubating the solid-phase with a Substrate Solution. The reaction is stopped by adding a Stop Solution, and colorimetric reading is performed using a spectrophotometer at 450nm and reference wavelength at 620nm.

The highly specific selected recombinant PRRSV antigens are used as capture material in this test. These enable the BIONOTE PRRS Ab ELISA 4.0 to identify to anti-PRRSV antibodies in sample, with a high degree of accuracy.

Materials Provided

1) Antigen Coated Microplate (1) : 96 wells/plate, configured in 8x12 strips,
2) Negative Control (2) : SPF piglet serum preserved with Proclin 300(0.05%).
3) Positive Control (3) : Anti-serum to PRRSV preserved with Proclin 300(0.05%).
4) Sample Diluent (4) : Phosphate buffer preserved with Proclin 300(0.01%).
5) 20X Washing Solution (5) : PBS-Tween 20 preserved with Proclin 300 (0.05%).
6) Enzyme Conjugate (6) : Rabbit anti-pig IgG-HRP in BSA preserved with Proclin 300(0.05%). Ready to use.
7) TMB Substrate (7) : Tetramethyl-benzidine with peroxide : STORE IN THE DARK. Ready to use.
8) Stop Solution (8) : 1N sulfuric acid. Ready to use.
9) Adhesive Plate Sealer
10) Instructions for Use

Materials required, but not provided

1) Disposable microplate or test tube for dilution
2) Micropipette
3) ELISA washer
4) ELISA reader
5) Distilled/deionized water

Precautions

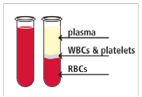
In order to obtain reproducible results, the following must be observed:

1) This kit is suited as a herd test for routine serologic herd monitoring, not an individual test.
2) S/P value means antibody titer. Basic test is needed for each herd because antibody titer is different depending on the types of vaccine and vaccine dose.
3) At least 5 or more samples per herd and age should be randomly collected at standard time intervals (i.e. every four weeks).
4) For *in vitro* diagnostic use only.
5) Use disposable gloves while handling potentially infectious materials and performing the assay. After assay, wash hands with sanitizers.
6) Use fresh sample. Hemolyzed or contaminated sample might cause false result.
7) Remove the blood corpuscle in samples before use. They may cause non-specific reaction.
8) TMB Substrate and Stop Solution can cause irritation or burns to the skin and eyes. In case of accident, rinse immediately with fresh cold water.
9) Dispose of containers and residues safely in accordance with national and local regulations.
10) Do not mix reagent of different lots.

Preparation of Sample

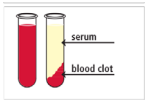
1) BIONOTE PRRS Ab ELISA 4.0 has been evaluated with swine samples only. Samples from other animals have not been evaluated.
2) Fresh serum or plasma samples should be used with this assay.
3) Mix samples thoroughly by gentile inversion. If necessary, any visible particulate matters in the samples should be removed by low-speed centrifugation.

[Preparation of plasma]



(1) Blood should be collected with a disposable syringe and transferred to a tube containing anticoagulant (Heparin, EDTA, or Citrate), and then separate plasma by centrifugation.
(2) Plasma should be stored at 2~8 °C for up to 3 days. For longer storage, freeze at below -20 °C.

[Preparation of serum]



(1) Blood should be collected with a disposable syringe and transferred to a serum collection tube (no anticoagulant).
(2) Collected blood should be left at room temperature for 30 minutes to coagulate, and then separate serum by centrifugation.
(3) Serum should be stored at 2~8 °C for up to 3 days. For longer storage, freeze at below -20 °C.

Preparation of Reagent

[Precautions]

1) Allow all reagents to come to room temperature(18~25 °C) for 30 minutes before use.
2) Unused microplate wells must be sealed with silica gel using the enclosed sealing bag and stored at 2~8 ℃.

[Preparation of reagents]

1) 20X Washing Solution (5) : The 20X Washing Solution must be diluted 1 to 19 using distilled/deionized water before use.
   For example, mix 50 ㎖ of 20X Washing Solution with 950 ㎖ of distilled/deionized water.

[Storage and stability of reagents]

Material / reagent	State	Storage	Stability
Working washing Solution	Once prepared	Room temp. (18 ~ 25 ℃)	1 week

Test Procedure

[Simple procedure]

1) Prepare Antigen Coated Microplate and all reagents.
2) **Dilute samples and controls** with Sample Diluent. (1:39 dilution)
3) **Add 100 µl of diluted samples and controls** to wells.
4) Incubate plate for **30 minutes at room temperature** (18~25 °C).
5) **Wash** plate 5 times using the diluted washing solution.
6) **Add 100 µl of Enzyme Conjugate** to wells.
7) Incubate plate for **30 minutes at room temperature** (18~25 °C).
8) **Wash** plate 5 times using the diluted washing solution.
9) **Add 100 µl of TMB Substrate** and incubate for 15 minutes at room temperature in the dark.
10) **Add 100 µl of Stop Solution**.
11) Measure the optical density (OD) at 450 nm with reference wavelength at 620 nm.

Interpretation of the Result

[Test validation]

① If the mean OD_{sample} is less than the mean OD of negative control (OD_{NCx}), the S/P ratio can be interpreted as 0.
② The mean OD of positive control (OD_{PCx}) minus the mean OD of negative control (OD_{NCx}) must be more than 0.200 (at 450 nm with reference wavelength at 620 nm).
③ The OD_{NCx} must be less than 0.200 (at 450 nm with reference wavelength at 620 nm).
④ If these specifications are not met, the test has to be repeated.

[Results calculation]

① Calculation of OD_{NCx}: $\frac{OD_{NC1} + OD_{NC2}}{2}$
② Calculation of OD_{PCx}: $\frac{OD_{PC1} + OD_{PC2}}{2}$
③ Criteria: The criteria is based on following formula.

$$\text{S/P ratio} = \frac{(OD_{sample} - OD_{NCx})}{(OD_{PCx} - OD_{NCx})}$$

[Result interpretation]

① Positive : If the S/P ratio is equal or more than 0.4, the sample is regarded as positive for PRRSV antibodies.
② Negative : If the S/P ratio is less than 0.4, the sample is regarded as negative for PRRSV antibodies.

(For example)

- OD_{NCx}: 0.112, OD_{PCx}: 0.514,
- OD_{sample}: 0.324

$$\text{S/P ratio} = \frac{(0.324 - 0.112)}{(0.514 - 0.112)} = \frac{0.212}{0.402} = 0.53$$

→ This sample is classified as positive for PRRSV antibodies.

* OD_{NCx}: mean OD of negative control
* OD_{PCx}: mean OD of positive control

Limitations and Interferences

1) Failure to add sample in the procedure could lead to a false negative result. Repeat testing should be considered where there is clinical suspicion of infection.
2) Other clinically available tests are required if questionable results are obtained. As other diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test. It is recommended the diagnosis decision is made by a veterinarian after all clinical and laboratory findings have been evaluated.

Storage and Stability

1) All reagents should be stored at 2~8 ℃ (35.6~46.4 °F).
2) Shelf life is 18 months. This test kit is stable through the expiration date printed on the package and on the label of each material / reagent in an unopened state.

Trouble Shooting

If the test does not perform satisfactorily, check if the test procedures were carried out correctly.

1) No color after 30 minutes incubation
   - Enzyme Conjugate contaminated.
   - Enzyme Conjugate was not dispensed into sample well.
   - Stop Solution was added instead of TMB Substrate.
2) Color develops too slowly
   - After washing plate, the plate became dried out.
   - The TMB Substrate was not allowed to come to room temperature before use.
3) Color develops too quickly
   - Poor washing.
   - Enzyme Conjugate contaminated.
4) All wells are colored
   - Poor washing.
   - TMB Substrate contaminated.
5) Patchy or poor color
   - Poor pipetting or washing.
   - Poor mixing of reagents.
   - Dirty glassware.

Packaging Unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit	960 Tests/Kit
Antigen Coated Microplate	1 ea	5 ea	10 ea
Negative Control	0.2 ml/vial x 1	1.0 ml/vial x 1	2.0 ml/vial x 1
Positive Control	0.2 ml/vial x 1	1.0 ml/vial x 1	2.0 ml/vial x 1
Sample Diluent	50 ml/bottle x 1	250 ml/bottle x 1	250 ml/bottle x 2
20X Washing Solution	50 ml/bottle x 1	250 ml/bottle x 1	250 ml/bottle x 2
Enzyme Conjugate	15 ml/bottle x 1	80 ml/bottle x 1	200 ml/bottle x 1
TMB Substrate	12 ml/bottle x 1	60 ml/bottle x 1	120 ml/bottle x 1
Stop Solution	15 ml/bottle x 1	80 ml/bottle x 1	200 ml/bottle x 1
Adhesive Plate Sealer	2 ea	10 ea	20 ea

Precision

Within-run and between-run precisions have been determined by testing 10 replicates of 2 samples : negative control and positive control. The C.V (%) of negative and positive control values are within 10 %.

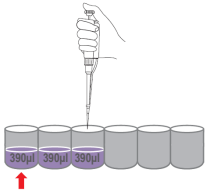
Detailed Procedure by Step

Allow all reagents and samples to come to room temperature before use and prepare the strip well for use. Store the unused strip well in the pouch (Provided).

[Sample dilution procedure (1:39 dilution)]

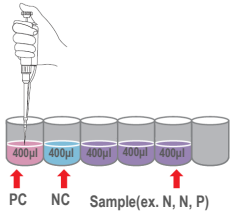
1) Prepare the microplate or test tube for dilution (Not provided).

2) Add 390 µl of Sample Diluent into each well/tube.



1 well of dilution microplate

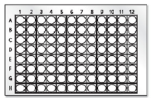
3) Add 10 µl of Negative Control(NC), 10 µl of Positive Control(PC) and 10 µl of sample into each 2) well/tube containing Sample Diluent.



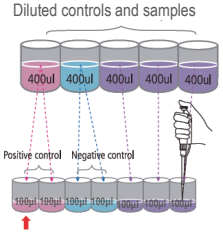
(N: Negative sample, P: Positive sample)

[Test procedure]

1) Use the Antigen Coated Microplate (Provided).

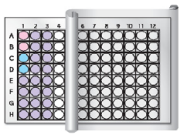


2) Add 100µl of diluted positive control to two (2) wells, 100 µl of negative to two (2) wells and each sample into each of appropriate wells, respectively. Run each of the controls in duplicate.



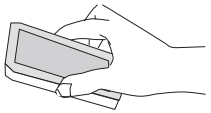
1 well of Antigen Coated Microplate

3) Cover the plate with the Adhesive Plate Sealer and incubate for 30±1 minute at room temperature (18~25 °C).

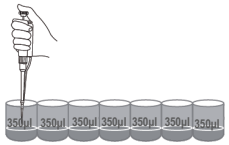


4) Rinse the plate 5 times using ELISA washer or micropipette as following.

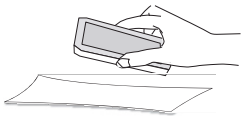
① Remove controls and samples. Tap hard to remove all remains of fluid.



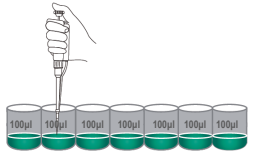
② Add 350 µl of diluted washing solution and remove it.



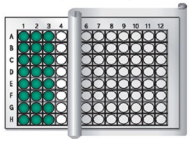
③ Remove diluted washing solution by tapping thoroughly on absorbent paper towel.



5) Add 100 µl of Enzyme Conjugate into each well.

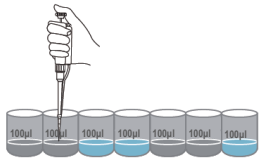


6) Cover the plate with the Adhesive Plate Sealer and incubate for 30±1 minute at room temperature (18~25 °C). [Repeat step #3]

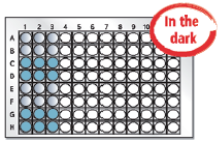


7) Wash plate 5 times using ELISA washer or micropipette. [Repeat step #4]

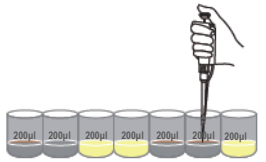
8) Add 100 µl of TMB Substrate solution into each well.



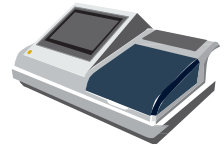
9) Incubate for 15 minutes at room temperature (18~25 °C) in the dark.



10) Add the 100 µl of Stop Solution into each well.



11) Read the absorbance values of the wells at 450 nm in a bichromatic spectrophotometer (with reference wavelength at 620 nm) right after from the end of assay.



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