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 of intensive swine farms in Europe and North America. The disease may also be referred to as Swine Infertility and Respiratory Syndrome (SIRS) by some veterinarians and swine industry professionals. 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 a microplate, which is pre-coated with recombinant PRRSV antigen on the well. For testing, ELISA plates are incubated with samples and controls(1:39 dilution with the sample diluents) for 30 minutes at room temperature(18~25℃). During first incubation, anti-PRRSV antibodies present in sample bind to the antigen coated on the well. Following this incubation, all unbounded materials are removed by washing step. After that, rabbit anti-pig IgG-HRP is 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 stopping 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 micro-assay plate (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 Diluents (4) : Phosphate buffer preserved with Proclin 300(0.01%).
5) Washing solution (20X concentrated) (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) Substrate (7) : Tetramethyl-benzidine with peroxide : STORE IN THE DARK. Ready to use.
8) Stopping 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) Micro pipette
3) ELISA Washer
4) ELISA Reader
5) Distilled/deionized water

Precautions

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

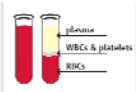
1) This kit is proper to herd test for routine serologic herd monitoring, not individual test.
2) S/P value means antibody titer. Basic test is needed for each herd because antibody titer is different depending on types of vaccine and vaccine dose.
3) At least 5 or more samples per herd and age should 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) Substrate and stopping 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 Pig 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]

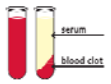
(1) Blood should be collected with a disposable syringe and transferred to a tube containing anticoagulant (Heparin, EDTA, or Citrate), and then separate plasma by centrifugation.



(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 30minutes before use.
2) Unused microplate wells must be sealed with silica gel in enclosed sealing bag and stored at 2~8℃.

[Preparation of Reagents]

1) Washing solution (20X concentrated)(5) : The concentrated washing solution must be diluted 1 to 19 using distilled/deionized water before use.

   For example, mix 50㎖ of washing solution (20x concentrated) with 950㎖ of distilled/deionized water.

[Storage and stability of Reagents]

Material / reagent	State	Storage	Stability
Working wash solution	Once prepared	Room temp(18 ~ 25℃)	1 week

Test procedure

[Simple procedure]

1) Prepare antigen coated micro assay plate and all reagents.
2) **Dilute samples and controls** with sample diluents. (1:39 dilution)
3) **Add 100µl of diluted samples and controls** to wells.
4) Incubate plate for **30minutes at room temperature** (18~25°C).
5) **Wash** plate 5 times using the diluted washing solution.
6) **Add 100µl of enzyme conjugate** to wells.
7) Incubate plate for **30minutes at room temperature** (18~25°C).
8) **Wash** plate 5 times using the diluted washing solution.
9) **Add 100µl of substrate** and incubate for 15minutes at room temperature in the dark.
10) **Add 100µl of stopping solution**.
11) Measure the optical density (OD) at 450nm with reference wavelength at 620nm.

Interpretation of the Result

[Test validation]

① If the mean OD_{450} of a sample is less than the mean OD_{450} of negative control(OD_{450}NCx), the S/P ratio can be interpreted as 0.

② The mean OD_{450} of positive control (OD_{450}PCx) minus the mean OD_{450} of negative control (OD_{450}NCx) must be more than 0.200 (at 450nm with reference wavelength at 620nm).

③ The OD_{450}NCx must be less than 0.200 (at 450nm with reference wavelength at 620nm).

④ If these specifications are not met, the test has to be repeated.

[Results calculation]

① Calculation of OD_{450}NCx: $\frac{NC\ 1 + NC2}{2}$

② Calculation of OD_{450}PCx: $\frac{PC\ 1 + PC2}{2}$

③ Criteria: The criteria is based on following formula.

$$S/P\ ratio = \frac{(OD_{450}\ sample - OD_{450}\ NCx)}{(OD_{450}\ PCx - OD_{450}\ NCx)}$$

[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_{450}NCx: 0.112, OD_{450}PCx: 0.514,
- OD sample: 0.324

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

→ This sample is classified as positive for PRRSV antibodies.

* OD_{450}NCx: mean OD_{450} of negative control
* OD_{450}PCx: mean OD_{450} of positive control

Limitations and Interferences

1) Failure to add specimen in the procedure could result a falsely negative. 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 the clinician 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 the test procedure instructions were carried out correctly.

1) No color after 30 minutes incubation
   - Enzyme conjugate contaminated.
   - Enzyme conjugate was not dispensed into sample well.
   - Stopping solution was added instead of substrate.
2) Color develops too slowly
   - After washing plate, the plate became dried out.
   - The 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.
   - 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 plate (8wells x 12 strips)	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 diluents	50 ml/bottle x 1	250 ml/bottle x 1	250 ml/bottle x 2
Washing solution (20X concentrated)	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
Stopping 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 specimens : negative control and positive control. The C.V (%) of negative and positive control values are within 10%.

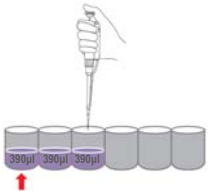
Detailed procedure by step

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)]

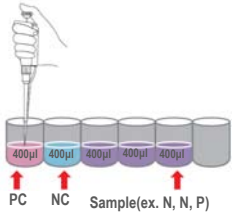
1) Prepare the micro plate or test tube for dilution (Not provided).

2) Add 390µl of sample diluents into each well/tube.



1 well of dilution microplate

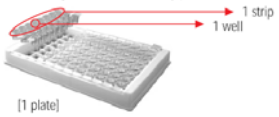
3) Add 10µl of negative control(NC), 10µl of positive control(PC) and 10µl of sample into each 2) well/tube containing sample diluents.

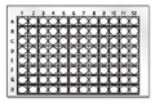


(N: Negative sample, P: Positive sample)

[Test procedure]

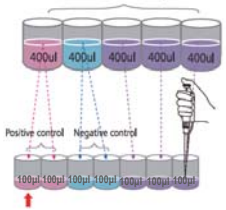
1) Use the antigen coated micro-assay plate (Provided).





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

Diluted controls and samples

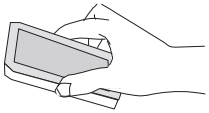


1 well of coated micro-assay plate

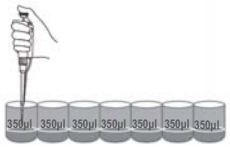
3) Cover the plate with the adhesive plate sealer and incubate for 30±1 minute at room temperature (18~25°C).

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

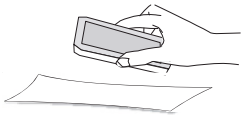
① Remove controls and samples. Tap hard to remove all remains of fluid.



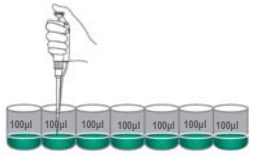
② Add 350µl of diluted washing solution and remove it.



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



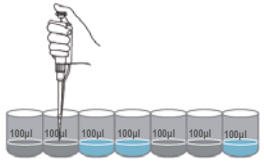
5) Add 100µl of Enzyme conjugate into each well.



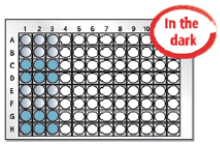
6) Cover the plate with the adhesive plate sealer and incubate for 30±1 minute at room temperature (18~25°C). [Repeat step #3]

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

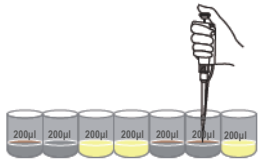
8) Add 100µl of Substrate solution into each well.



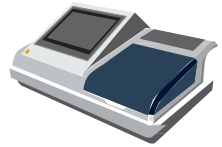
9) Incubate for 15 minutes at room Temperature (18~25°C) in the dark.



10) Add the 100µl of stopping solution into each well.



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



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