Porcine Circovirus Type 2 Antibody ELISA

AniGen PCV 2 Ab ELISA

1. Explanation of the Test

Porcine Circovirus Type 2, or PCV2, is a very small circular-arranged DNA virus that belongs to the Circoviridae family. PCV2-infection is widespread and essentially all pig herds are infected with PCV2 but relatively few have PCV2-associated disease (PCVAD), which includes severe systemic PCV2 infection or Postweaning Multisystemic Wasting Syndrome (PMWS), PCV2-associated pneumonia as a part of the Porcine Respiratory Disease Complex (PRDC), PCV2-associated enteritis, PCV2-associated reproductive failure, and Porcine Dermatitis and Nephropathy Syndrome (PDNS).

Postweaning multisystemic wasting syndrome (PMWS) was first described in 1991 in Canada. PMWS is a serious manifestation of PCV2 infection. PMWS is characterized by severe loss of weight (wasting) and generalized lymph node enlargement. Today we know that there are several PCV2 associated diseases (PCVAD) and severe systemic PCV2 infection remains as the most important manifestation. PCV2-associated Porcine Respiratory Disease Complex (PRDC) is also a very commonly diagnosed PCVAD in the U.S. Less commonly diagnosed diseases include PCV2-associated Enteritis, PCV2-associated Reproductive Failure, and Porcine Dermatitis and Nephropathy Syndrome (PDNS).

PCV2-infection is widespread and essentially all pig herds are infected with PCV2 but relatively few have PCVAD. In many cases, PCV2 infection requires a trigger such as coinfection with other pathogens (PRRSV, Mycoplasma hyopneumoniae), immune stimulation of the host, or other stressors to trigger PCV2 infection to progress to PCVAD. Host genetics may also markedly affect the outcome of PCV2 infection and there is increasing evidence of differences in virulence among PCV2 isolates.

2. Sample Recommendation

For routine serologic herd monitoring, it is suggested that at least 10 or more sera per herd be randomly collected at standard time intervals (i.e. every four weeks). Proper sample collection procedures, serum harvest and serum sample storage(4℃ for up to four days or -20℃ for longer periods) are needed to provide reliable test results. To achieve better specificity and to minimize possible false positive reactions, serum samples that are contaminated with bacteria or are very fatty should be excluded from testing.

3. Materials Provided (480 wells/Kit)

AniGen PCV 2 Ab ELISA contains following items to perform the assay.

1) Antigen coated micro-assay plate (1) : 96 wells/plate x 5 plates, configured in twelve 1x8 strips.
2) Negative Control (2) : 1 vial (0.5 mℓ) of SPF piglet serum preserved in phosphate buffer with protein stabilizer. Proclin 300 (0.05%) added as preservatives.
3) Positive Control (3) : 1 vial (0.5 mℓ) of antibodies to PCV-2 preserved in phosphate buffer with protein stabilizer . Proclin 300 (0.05%) added as preservatives.
4) Sample Diluent (4) : 1 bottle (250 mℓ) of phosphate buffer, sodium azide(0.01%) added as a preservative.
5) Washing solution (20X concentrated) (5) : 1 bottle(250 mℓ) of PBS-Tween 20. Preservative is Proclin 300 (0.05%) Note: Before use, take one vial content, and then fill-up to 5,000 mℓ with distilled water. In presence of undissolved crystals, re-suspend the solution by placing the vial at 37℃ for few minutes.
6) Enzyme Conjugate (6) : 1 bottle (80 mℓ) of goat anti-swine IgG HRP, BSA and stabilizers. Preservative is Proclin 300 (0.05%). Ready for use.
7) Substrate (7) : 1 bottle (60 mℓ) of tetramethyl-benzidine with citrate-phosphate buffer : STORE IN THE DARK.
8) Stop solution(8) : 1 bottle (80 mℓ) of 1N sulfuric acid. Ready for use.
9) Adhesive plate sealer.
10) Instructions for use.

4. Precautions

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

1) For in vitro diagnostic use only.
2) Do not mix reagent of different lots.
3) Use thoroughly cleaned glassware, free from contamination of metal ion or oxidating substances.
4) Use disposable gloves while handling potentially infectious material and performing the assay.
5) Substrate and stopping solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident, rinse thoroughly with running water.

5. Specimen Collection and Storage

1) This ELISA was evaluated at swine. Other species are not evaluated.
2) Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Any visible particulate matters in the sample should be removed by centrifugation at 3,000 rpm for at least 20 minutes.
3) If specimens are not immediately tested they should be refrigerated at 2~8℃. For storage periods greater than three days, freeze the specimen at 20℃ or below(serum, plasma). They should be brought to room temperature prior to use.
4) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

6. Reagent preparation

1) Allow all reagents to come to room temperature before use.
2) **Washing solution (20X concentrated)**: The concentrated washing solution (5) must be diluted 1 to 20 with distilled/deionized water before use.

7. Procedure of the Test

1) Prepare the strip wells (1) for negative control (2) 2 wells, positive control (3) 2 wells and each of the samples to be tested.
2) Add 390±2ul of sample diluent (4) to dilution microplate or disposable test tube. (Not provided).
3) Add 10±1 μℓ of each of the negative control (2) and positive control (3) to 2 wells, and 10 μℓ of each of the samples to each wells and mix well (Sera are diluted at 40).
4) Add 100±2 μℓ of diluted samples to each well of antigen micro-assay plate (1).**For confirmation purpose it is recommended to run the control sera in duplicates.**
5) Cover the test plate and incubate at 18~25℃ for 30±2 minutes.
6) Wash the wells at 5 times with 350±5 μℓ of diluted washing solution. Aspirate all liquid from the wells.
7) Add 100±2 μℓ of conjugate to each well.
8) Cover the test plate and incubate at 18~25℃ for 30±2 minutes.
9) Wash the wells at 5 times with 350±5 μℓ of diluted washing solution. Aspirate all liquid from the wells.
10) Add 100±2 μℓ of substrate solution to each well.
11) Incubate at 18~25℃ for 15±1 minutes **in the dark**.
12) Add 100±2 μℓ of stop solution (9) to each well.
13) Read the absorbance of the wells with a bichromatic spectrophotometer at 450nm with reference wavelength at 620nm. **Reading must be completed within 20±2 minutes from the end of assay**.

8. Interpretation of the Test

1) Test validation

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

② The absorbance of the positive control mean (PCx) should be above 0.250. If these specifications are not met, the test is to be repeated. The negative control mean (NCx) must be less than or equal to 0.200

2) Results calculation

①Criteria : The criteria is based on following formula.

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

②Positive : If the S/P ratio is greater than or equal to 0.4 , the sample is regarded as positive for PCV-2 antibodies.

③Negative : If the S/P ratio is less than 0.4 , the sample is regarded as negative for PCV-2 antibodies.

For example)

- Mean OD negative : 0.112
- Mean OD negative : 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 PCV-2 antibodies.

9. Limitations and Interferences

1) The test procedure, precautions and interpretation of results sections for this test kit must be followed closely when testing.

2) Samples

① Samples containing sodium azide do not affect the test result.

② Pasteurized samples (no less than 10 hours at 60℃) may lead to diminished reactivity and therefore should not be used.

③ Heat-inactivated samples (30 minutes at 56℃) do not impair the test.

④ Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.

⑤ Hemolytic samples should be centrifuged before use to avoid interference by cellular constituents.

⑥ Lipaemic and icteric samples do not impair the test results.

3) Failure to add specimen in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection.

10. Storage

Store at 2~8℃. This test kit is stable through the expiration date printed in the package and in the label of each material / reagent as unopened state.

11. Stability

1) Do not use after the stated expiry date.

2) Stability of once prepared reagents

Material / reagent	State	Storage	Stability
Working Washing Solution	Once opened	Room Temp.	1 week

12. Packaging Unit

96 Tests/kit, 480 Tests/kit

13. Precision

Within-run and between-run precisions have been determined by the testing 10 replicates of three specimens: a negative serum, a low positive serum and a strong positive serum. The C.V (%) of negative, low positive, and strong positive values were within 10% of the time.

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