Instruction Manual

VDPro® PPV HI Reagent

Cat. No. 2061

MEDIAN Diagnostics

Antigen : Store at -70 ℃

1. INTRODUCTION

Porcine Parvovirus (PPV) hemagglutination inhibition reagent is for the detection of antibodies in pleural and ascites fluids of aborted fetus, serum of pregestation and postpartum of sow, and boar serum. We used genetic recombination PPV VP2 hemagglutination antigen.

2. CONTENTS

Reagents		100 tests
1)	PPV HI Antigen (Liquid)	1.0㎖ X 1
2)	PPV Positive control	1.0㎖ X 1
3)	PPV Negative control	1.0㎖ X 1
4)	RBC Washing Buffer	120㎖ X 1
5)	RBC Dilution Buffer	120㎖ X 1
6)	Dilution Buffer	120㎖ X 1
7)	25% Kaolin Solution	120㎖ X 1
8)	User Manual	1 copy

3. MATERIALS

1) Alsever's solution

Glucose	2.05g
Sodium citrate	0.80g
Sodium chloride	0.42g
D.W. to 100㎖	

❖ Please store at 4℃ after filtering (0.45㎛).

2) Guinea pig RBCs
3) U-bottom microplate

4. PREPARATION OF RBCs

1) Collect 5㎖ of guinea pig blood from guinea pig's heart by using 10㎖ syringe including 5㎖ of Alsever's solution.
2) Add guinea pig blood to tube and centrifuge at 845 xg for 10 min. Remove the supernatant by pipette.
3) Fill with RBC Washing Buffer which is 2 to 3 times of RB Cs volume and prepared packed RBCs by repeat 2) step three times.
4) Packed guinea pig RBCs should be diluted to 0.6% guinea pig RBCs before use in HA, HI test.

❖ **0.6% Guinea pig RBCs**
Packed RBCs 60㎕ + 10 ㎖ RBC Dilution Buffer

❖ Packed RBCs should be used in one or two days.

5. SAMPLE TREATMENT

1) The specimen should be inactivated in 56℃ water bath for 30 min.
2) Add 25% Kaolin Solution (600㎕) to inactivated specime n (200㎕) in the tube and mix well.
3) Incubate for 30min at room temperature (20-25℃) and mix by inverting the tube every 5 min during the incubation. (Removing non-specific reaction)
4) After centrifugation (8,000rpm, 5min), transfer the supernatant to a new tube then add 1/40 volume(20㎕) of packed guinea pig RBCs to each tube.
5) Incubate for 1hrs at room temperature and mix by inverting the tube every 5 min during the incubation.
6) Centrifuge for 5min at 8,000rpm. The supernatants is used for HI test. **(Initial dilution fold: 1/4)**

➢ **Caution : There is no need to pretreat the Positive and Negative control**

❖ In the case of more than 70 day-old aborted fetus (more than 16cm of body length), pleural effusion or ascites fluids are able to be used for test by the specimen after pretreatment.

❖ When the fetus are frozen and it is difficult to collect the body fluids, please keep the fetus in the plastic bags for overnight at 4℃ and then the exudation from the fetus can be used in the HI test after pretreatment.

6. ANTIGEN PREPARATION

1) PPV HI Antigen is supplied as frozen solution. Thaw and then keep on ice during use. Store at -70℃ below after using.

❖ After using antigens, keep it under -70℃.

❖ Frequent freezing and thawing are reduces antigen titer.

2) Dilution method of PPV HI Antigen (8HA unit; 8HAU)

PPV HI Antigen should be diluted to 8HA Unit in HI test.

➢ When the PPV HI Antigen titer is 2,048 HA Unit :
Add 0.05㎖ of Dilution Buffer to 12.8㎖ of Antigen.

7. TEST PROCEDURE

7.1 Hemagglutination Test (HA test)

1) Add 50㎕ of Dilution Buffer to all test well.
   (Use the RBC control group)
2) Add 50㎕ of the PPV HI Antigen to the first well.
   Two-fold serial dilution of the antigen.
   Last diluted solution (50㎕) is discarded.
3) Add 50㎕ of 0.6% guinea pig RBCs in each well.
4) Mix it well by shaking of the plate.
   Incubate for 1hr at room temperature (20-25℃),
   then read the result.
5) Please calculate the HA titer from the reciprocal of last dilution in which the hemagglutination is occurred.
6) Dilute the PPV HI Antigen to make 8 HAU and used for HI test.

7.2 Hemagglutination Inhibition test (HI test)

1) Add 25㎕ of Dilution Buffer to the plate from first well to twelfth well.
2) Add 25㎕ of the pretreated specimen to the first well. Two-fold serially dilute it by 25㎕ to eleventh well.
3) Add 25㎕ of the pretreated specimen to twelfth well.
4) Use the Positive and Negative Control in the same method above.
5) Add 25㎕ of the PPV HI Antigen diluted to 8HAU to wells (from first to eleventh well).
6) Incubate for 1hrs at room temperature (20-25℃) or 37℃ after plate sealing.
7) Add 50㎕ of 0.6% guinea pig RBCs in each well.
8) Incubate for 1hrs at room temperature (20-25℃), then read the result.

8. INTERPRETATION

1) It should be interpreted that the HI titer of Positive control is more than 64 fold and the HI titer of Negative control is less than 8 fold.
2) There should be no non-specific agglutination on the Serum and RBC control.
3) Please calculate the serum HI titer in which the reciprocal of the serum dilution in the last well with complete hemagglutination inhibition. As the initial dilution is 1/8, the HI titer should be calculated from 8 fold.
4) Interpretation of the results
   An HI titer of 8 fold or higher is considered positive. It was suspected to natural infection in the field in the case of more than 512 fold in sow.

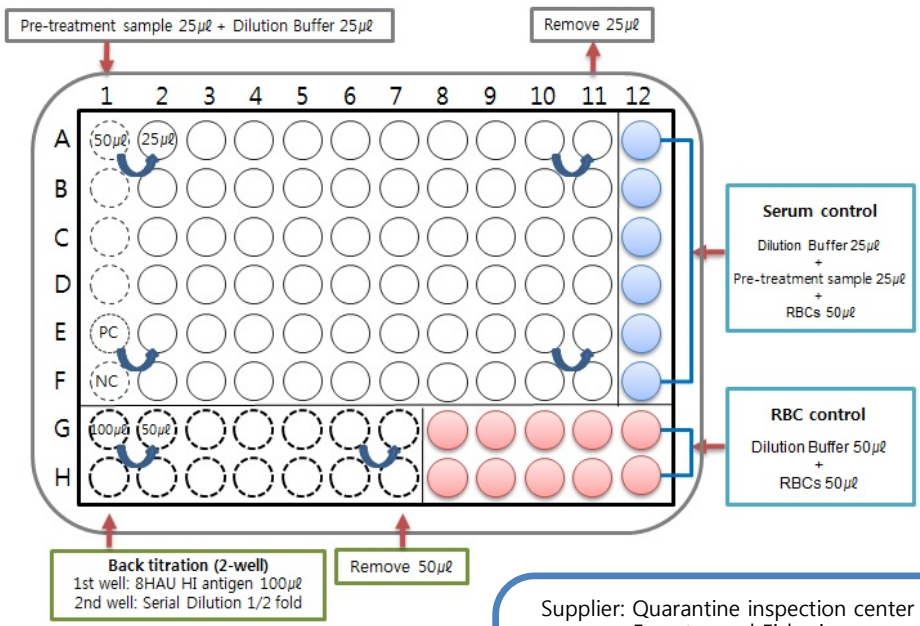
❖ **Note**

When the aborted fetus is tested to infectious reproductive disturbance, the antigen detection or causative agent isolation should be carried out because the fetus before 70 day-old has none of antibody production. In the case of the pregnancy in the sow lasts for about 70 days, it is possible to test using the fetal serum, ascites fluids, etc.

PRECUTION

1) The test should be used to the Positive and Negative control.
2) The controls are already diluted and it is not necessary to pretreatment.
3) Please perform the back titration of 8HAU diluted antigen.
4) Please confirm the non-specific agglutinin by using the Serum control.

* HI test Diagram



Supplier: Quarantine inspection center of Agriculture, Forestry and Fisheries
Manufacturer: Median Diagnostics Co.
Technological Support: Median Diagnostics Co.
878, Sunhwan-daero, Dongnae-myeon, Chuncheon-si, Gangwon-do, 24399, Republic of Korea
Tel 033 244 0100, fax 033 244 4634
median@mediandx.com