Instruction Manual

VDPro® JEV HI Reagent

CAT.NO. RM-JEV-31

MEDIAN Diagnostics

Antigen : Store at -70℃

1. INTRODUCTION

Swine Japanese Encephalitis Virus (JEV) 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. JEV HI reagent used hemagglutination antigen which is made from JEV infected mouse brain tissue.

2. CONTENTS

Reagents		100 tests
1)	JEV HI Antigen (Liquid)	1.0㎖ X 1
2)	JEV Positive control	1.0㎖ X 1
3)	JEV 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)	14% 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
Distilled Water to	100㎖

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

2) Goose blood (male goose)

3) U-bottom microplate

4. GOOSE BLOOD CELL PREPARATION

Preparation of standard RBCs

a. Collect 5㎖ of goose blood by using 10㎖ syringe including 5㎖ of Alsever's solution.

b. Add the goose blood to tube and centrifuge at 845 xg for 10 min. Remove the supernatant by pipette.

c. Fill with RBC Washing Buffer which is 2 to 3 times of RBCs volume and prepared packed RBCs by repeat b) step three times.

d. Add 13.3㎖ of RBC Washing Buffer to 1㎖ of prepared packed RBCs for producing of standard RBCs.

e. The standard RBCs should be diluted to 1:24 by RBC Dilution Buffer before use in HA, HI test.

❖ The standard RBCs should be used to adjust the concentration depending on the result.

❖ The standard RBCs is possible to use for 2 weeks at 4℃.

5. ANTIGEN PREPARATION

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

❖ Frequent freezing and thawing are reduces antigen titer.

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

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

➢ When the JEV HI Antigen titer is 512 HA Unit :

Add 6.3㎖ of Dilution Buffer to 0.1㎖ of Antigen.

6. SAMPLE TREATMENT

1) The specimen should be inactivated in 56℃ water bath for 30 min.

2) Add the 900㎕ of 14% Kaolin Solution to 100㎕ of inactivated specimen in the tube and mix well.

3) Incubate for 30 min at room temperature (20-25℃) and mix by inverting the tube every 5 min during the incubation.

4) After centrifugation (8,000 rpm, 5min), transfer the supernatant to a new tube then add 1/50 volume(20㎕) of packed goose 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 8,000 rpm. The supernatants is used for HI test.

➢ 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.

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 JEV HI Antigen to the first well.
   Two-fold serial dilution of the antigen.
   Last diluted solution (50㎕) is discarded.
3) Add 50㎕ of RBCs diluted to 1:24 to each well.
4) Mix it well by shaking of the plate.
   Incubate for 30min at 37℃, 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 JEV 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 second well to twelfth well.
2) Add 50㎕ 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 JEV HI Antigen diluted to 8HAU to wells (from first to eleventh well).
6) Incubate for 1hrs at 37℃ after plate sealing.
7) Add 50㎕ of the RBCs diluted to 1:24 to each well.
8) Incubate for 30min at 37℃, then read the result.

8. INTERPRETATION

1) It should be interpreted that the HI titer of Positive control is more than 40 fold and the HI titer of Negative control is less than 10 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 1/10, the HI titer should be calculated from 10 fold.
4) Interpretation of the results
   An HI titer of 10 fold or higher is considered positive. It was suspected to natural infection in the field in the case of more than 640 fold in sow.

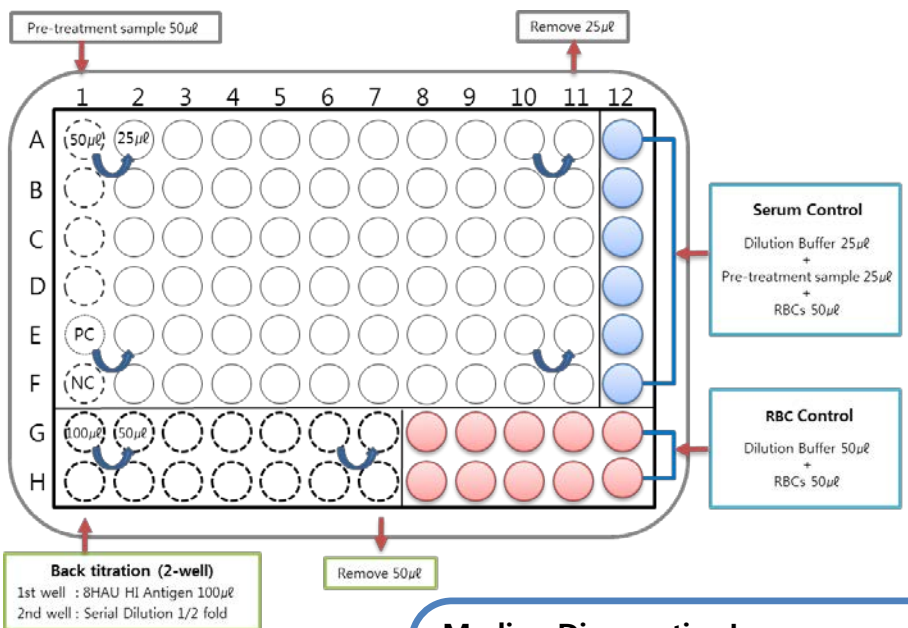
❖ **Notes**

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**



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