PSEUDORABIES VIRUS ANTIBODY TEST KIT
LATEX AGGLUTINATION

For Veterinary Use Only

Manufactured by:

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INTRODUCTION

Pseudorabies virus infection, which causes Aujeszky's disease, occurs naturally in many species. In adult swine the disease symptoms usually are mild and the mortality is low; however, in other animals the disease is highly fatal. Swine are the primary hosts of pseudorabies virus and it is transmitted from the infected pigs to others in the herd. In pigs that have recovered from Aujeszky's disease the pseudorabies virus remains in a latent state where it resides quiescently in the trigeminal ganglion (1,2). Upon stimulation, the virus reactivates and produces an active infection. Although clinical illness may not be seen in swine after recrudescence, the virus may be shed by these animals (1,3,4). Therefore, any animal that has contracted a primary infection by pseudorabies virus must be considered a potential source for transmission of infectious virus.

Control of pseudorabies virus depends on elimination of the reservoir of infection. This requires the identification of any swine that have experienced an infection, since these animals should be considered potentially infectious due to recrudescence. Any previous or current infection with pseudorabies virus results in the presence of specific antibodies in the serum, and these are a good marker of infection. There are several methods by which antibodies against pseudorabies virus can be detected. The serum neutralization (SN) test and the ELISA are widely accepted; however, these are sophisticated assays requiring expensive equipment and highly-trained personnel. The latex agglutination test requires little equipment and is amenable to performance in the field. It provides rapid, sensitive, and accurate results.

PRINCIPLE

After infection with pseudorabies virus, the host develops an immune response against the antigens in the virion. This appears first as an IgM response and shortly thereafter an IgG response occurs. The IgM antibodies decline rapidly, whereas the IgG antibodies persist for the life of the host.

The latex agglutination test provides a simple, rapid method to detect IgM or IgG antibodies against pseudorabies virus in swine serum or plasma. The active reagent is a suspension of latex particles that has been sensitized with the antigens prepared from partially purified pseudorabies virus. When such latex is mixed, by rotation on a glass slide, with serum containing pseudorabies virus antibodies, the latex will agglutinate and form visible clumps; in the absence of antibody the latex remains smooth and evenly dispersed. The presence of antibody is indicative of previous infection or vaccination. These cannot be differentiated in the present configuration.

INTENDED USE

The latex agglutination test provides a specific and rapid assay for antibodies to pseudorabies virus. The test is configured to use swine serum or plasma and is to be used primarily as a qualitative assay to determine immune status.

MATERIALS PROVIDED

Materials provided in each kit to perform assays for pseudorabies virus antibodies include:

Reorder Number	**#9017**	**#9018**
Contains	(150 Determinations)	(750 Determinations)
PRV Latex Reagent	One (1) 2.1 mL bottle	Five (5) 2.1 mL bottles
PRV Sample Dilution Buffer	One (1) 30 mL bottle	Five (5) 30 mL bottles
PRV Positive Control Serum	One (1) 0.75 mL vial	One (1) 0.75 mL vial
PRV Negative Control Serum	One (1) 0.75 mL vial	One (1) 0.75 mL vial
Plastic Stirrers	150	750

MATERIALS REQUIRED BUT NOT PROVIDED

These items include: Pipettes or syringes capable of delivering 50 µl to prepare the 1:4 dilution of the specimen and to deliver 50 µl of the diluted specimen to the glass slide.

Test tubes to prepare the specimen dilution.

Reusable glass slide with 1.4 cm fused reaction wells (available separately).

Rotator with humidified cover, capable of rotating at 100 rpm. (Optional)

GENERAL PRECAUTIONS

Kit MUST be stored at 2 to 7ºC.

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Use good laboratory techniques and follow the procedure for accurate, reliable results.

Do not warm reagents to temperatures above 32ºC.

Clean the glass slide thoroughly after each use with soap and water. Discard slide when well surface is no longer glossy. Care must be taken to remove lint or dust before each use.

SPECIMEN INFORMATION

Fresh, refrigerated, or previously frozen serum or plasma can be used. Grossly contaminated sera should not be assayed.

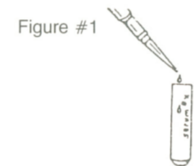
TEST PROCEDURE

Read entire circular before performing test.

1. Allow reagents to warm to room temperature (21 to 25ºC).

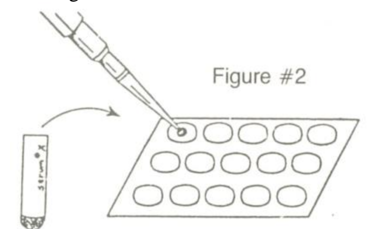
2. Label and identify one test tube for each serum sample, positive control, and negative control to be assayed. These tubes are for preparation of a 1:4 dilution of the serum sample, positive control, and negative control.

3. With a micropipettor, add 150 µl of Sample Dilution Buffer to each labeled test tube.



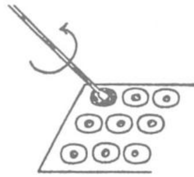
4. Vortex the serum samples, positive control, and negative control. Prepare a 1:4 dilution of each serum sample, positive control, and negative control. With a micropipettor, add 50 µl of each serum or control to a test tube containing 150 µl of sample dilution buffer. Mix by drawing up and down with the micropipettor six (6) times or by gently vortexing.

5. With the same pipettor or a clean one, if the sample is vortexed, transfer 50 µl of the diluted specimen to a 1.4 cm well on the glass slide.



6. When all of the specimens to be tested on one slide have been diluted and transferred to the slide, use a spreader to spread each 1:4 serum dilution to cover the entire well surface. Use a clean spreader for each sample. A positive and negative control sample should be placed on each slide.

Figure #3



7. Mix the latex reagent by vortexing for a few seconds or by inverting the vial several times and remove the cap. Hold the latex reagent bottle in an inverted vertical position perpendicular to the slide and drop one free-falling drop of sensitized latex on each slide well containing a diluted serum sample. IMPORTANT! You must hold the latex reagent bottle in a vertical position to avoid excessive drop size.

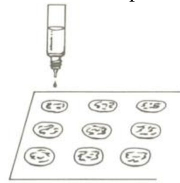


Figure #4

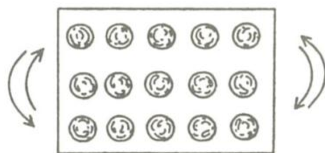
8.
   a. Place the slide under a moistened humidified cover on a rotator and rotate for eight (8) minutes at 80 to 120 rpm in a circumscribed circle of approximately two (2) centimeters in diameter.

   or

   b. Mix the latex and specimen under a moistened humidified cover by rocking the glass slide by hand for five (5) minutes. Rock and tilt the slide slowly (approximately 20 to 25 to-and-fro motions per minute) for the entire five (5) minute period.

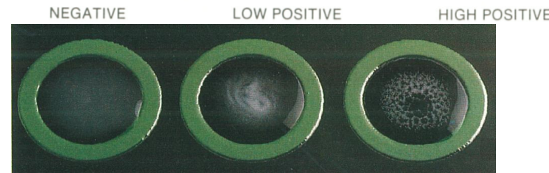
9. The slide should be read in the wet state immediately. Examine under incandescent light or sunlight for the presence of visible aggregates of latex that are similar to those observed with the positive control serum. Gently rotating the slide by hand while reading facilitates the visualization of weak positive reactions.

Figure #5



10. Agglutination of the latex is indicative of the presence of antibody and should be reported as positive. The negative serum produces no agglutination and the latex remains as a smooth dispersed solution. For the test to be valid, the positive control sample must be positive and the negative control sample must be negative.

Figure #6



INTERPRETATION: The presence of antibodies is an indication of previous infection with pseudorabies virus or vaccination with a pseudorabies vaccine.

RETURN REAGENTS TO REFRIGERATOR IMMEDIATELY AFTER USE.

PROTOCOL FOR EVALUATING SERA POSITIVE BY LATEX AGGLUTINATION

Occasional false positive results may be obtained with the Latex Agglutination test. Most of these have low latex agglutination titers. Therefore, it is recommended that all samples testing POSITIVE at the initial 1:4 screening dilution be tested according to the following protocol.

> HEAT INACTIVATE an aliquot of the original serum, an aliquot of the positive control, and an aliquot of the negative control by placing the samples in a water bath set at 56 (±0.5) ºC for 30-33 minutes. DO NOT HEAT INACTIVATE the 1:4 dilution. Test the inactivated samples at a 1:4 serum dilution as specified in the test procedures.

Serum samples converting to NEGATIVE at the 1:4 dilution after HEAT INACTIVATION should be considered NEGATIVE.

Serum samples remaining POSITIVE at the 1:4 dilution after HEAT INACTIVATION should be considered POSITIVE.

The Latex Agglutination Test has been shown to detect Pseudorabies virus antibodies as early as six (6) days after infection, 4-6 days earlier than the ELISA or SN test. Because of the increased sensitivity (5) in the detection of early antibody, a second bleeding (7-10 days later) and retest of a suspected positive animal is suggested.

CLINICAL EVALUATIONS

Four clinical studies were conducted. to determine the sensitivity and specificity of the latex agglutination test compared with the SN test in diagnostic laboratories and in the field by area veterinarians (6). Serum samples were from: (i) Pseudorabies-free herds, (ii) Pseudorabies-infected herds, (iii) swine experimentally infected with Pseudorabies, (iv) swine inoculated with an attenuated strain of Pseudorabies virus, and (v) sera routinely sent to the diagnostic laboratories. Swine sera were tested at a 1:4 dilution. The overall performance of the latex agglutination test is presented in Table 1.

Table 1

Results of Studies Comparing
The Latex Agglutination Test with the SN Test

	Serum Neutralization	
Latex Agglutination	Positive	Negative
Positive	531	35[a]
Negative	0	3104

[a] Eleven of these samples were tested by Western Blot and confirmed to be positive.

Sensitivity relative to SN = 531/ 531 = 100%
Specificity relative to SN = 3104/3139 = 99%

TECHNICAL ASSISTANCE

For technical assistance, contact Calbiotech Veterinary Diagnostics at (619) 660-6162.

REFERENCES

1. Beran, G.W.; Davies, E.B.; Arambule, P.V.; et al. (1980). Persistence of pseudorabies virus in infected swine. J. Am. Vet. Med. Assoc. 176: 998-1000.
2. Gutekunst, D.E.; Pirtle, E.C.; Miller, L.D.; et al. (1980). Isolation of pseudorabies virus from trigeminal ganglia of a latently infected sow. Am. J. Vet. Res. 411: 1315-1316.
3. Sabo, A. and Grunert, Z. (1971). Persistence of virulent PRV in herds of vaccinated and non-vaccinated pigs. Acta Virol. 15: 87-94.
4. Gutekunst, D. E. and Pirtle, E.C. (1979). Humoral and cellular immune responses in swine after vaccination with inactivated pseudorabies virus. Am. J. Vet. Res. 40: 1343-1346.
5. Schoenbaum, M.A.; Beran, G.W.; Murphy, D.P.; (1990). A study comparing the immunologic responses of swine to pseudorabies viral antigens based on the ELISA, serum virus neutralization, and latex agglutination tests. J. Vet. Diagn. Invest. 2:29-34 (1990).
6. Data available upon request.

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