

Equine Infectious Anemia Antibody Test Kit

LAB-EZ™/EIA

SALE AND USE RESTRICTED TO LABORATORIES APPROVED BY
STATE AND FEDERAL (U.S.D.A.) ANIMAL HEALTH OFFICIALS



For Veterinary Use Only
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I. INTRODUCTION:

The agar gel immunodiffusion (AGID) test for the diagnosis of Equine Infectious Anemia (EIA) was described by Coggins and Norcross, *Cornell Veterinarian*, April 1970. The test has proven to reliably diagnose infection by detecting specific antibody against EIAV in the serum of infected horses.

II. TEST PRINCIPLES:

The immunodiffusion test is based upon the concurrent movement of antigen and corresponding specific antibody toward each other in an agar gel, forming a visible precipitin line. Making use of this principle, the AGID test can reliably detect specific antibody that is formed after one to four weeks of infection with the EIA virus.

LAB-EZ™/EIA AGID uses a highly purified recombinant protein from the EIA virus which will form a specific line of identity with infected serum antibody. No precipitin lines will form if the serum is negative for EIAV.

III. CONTENTS OF EIA/AGID:

EIA Antigen (Bottle A)	1 vial
EIA Positive Control Serum (Bottle B)	3 vials
EIA Negative Control Serum (Bottle C)	1 vial

Package insert with instructions for conducting the test.

IV. PRECAUTIONS:

Store contents of kit at 2°-7°C (36°-45°F). Antigen and accompanying antiserum have been standardized and should be used together. Do not allow reagents to stand at room temperature for excessive periods of time while performing tests. Handle all reagents and equipment as if capable of transmitting EIA. Burn all containers and all unused contents. Autoclave all disposable test components and test specimens after use. The negative control is to be used in place of a test serum. DO NOT SUBSTITUTE THE NEGATIVE CONTROL SERUM FOR A POSITIVE CONTROL SERUM WELL.

V. SPECIMEN INFORMATION:

Use only horse serum for test specimens. Specimens may be stored at 2°-7°C for up to five days. If longer storage is desired, store at -20°C (-4°F). The presence of gross turbidity, hemolysis or bacterial growth may interfere with the performance and accuracy of the test.

VI. TEST PROCEDURE:

A. Preparation of Agar Gel

1. Borate Buffer is prepared by mixing:

2g Sodium Hydroxide (NaOH)
9g Boric Acid (H₃BO₃)
1 liter distilled water
The resulting pH should be adjusted to 8.6 ± 0.2.

2. A one percent solution of Noble agar is prepared in the borate buffer and dissolved by either of two methods:

- a. Boil the suspension to dissolve the agar and autoclave for seven minutes.
- b. Microwave agar solution for a total of 3 minutes at 30 second intervals or until agar dissolves.

3. Add 15 mls of agar to a 100mm diameter petri dish.
4. Plates are cooled for 1 hour at room temperature and then stored at 2°-7°C. If uncut, plates can be stored up to one week.

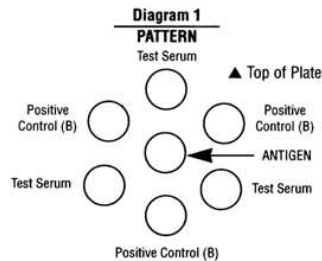
B. Cutting Wells in Agar

1. A seven-well pattern is used with one center well encircled by 6 wells. The wells are 2.4 mm apart and 5.3 mm in diameter. Cutting tools can be obtained from the National Veterinary Services Laboratory, P.O. Box 844, Ames, Iowa 50010
2. Wells are cut while the agar is cold and the same day as used. Remove the agar plugs and leave lids ajar for 30 minutes before adding reagents and serum samples. Any remaining moisture in the wells should be suctioned out or allowed to evaporate.

C. Filling Wells and Incubation of Agar Plates

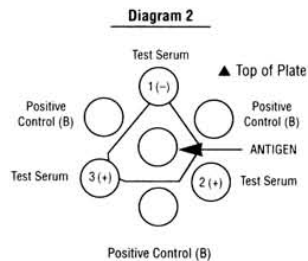
NOTE: THE NEGATIVE CONTROL (BOTTLE C) SHOULD BE RUN IN AT LEAST ONE WELL FOR EVERY GROUP OF PLATES. IT SHOULD BE PIPETTED INTO A TEST WELL IN PLACE OF A TEST SERUM.

1. Fill each alternate outside well (see diagram 1) with one of the three test sera (or the kit negative control) but without overflowing onto the agar surface. Use a separate disposable pipette or pipette tip for each sample.
2. Fill the center well with purified EIA Antigen (Bottle A) in the same manner.
3. Fill the three remaining outside wells with EIA Positive Control Serum (Bottle B) in the same manner.
4. Incubate plates for 24-48 hours at room temperature in a moist chamber.



VII. INTERPRETATION OF RESULTS (SEE DIAGRAM 2):

1. Negative – Reagent serum control lines continue into the test sample well without bending or with a slight bend back towards the reagent serum well.
2. Positive – Control lines join with and form a continuous line with the line between the test serum and antigen.
3. Weak positive – Control lines bend slightly towards the antigen well and away from the positive control serum well but do not form a complete line between antigen and test serum.
4. Very strong positive – Control lines turn towards the antigen well before they reach the well containing the test serum, and may continue on as a broad or hazy line which will be close to the antigen well. A more distinct line will usually form if the sample is diluted 1:2, 1:4 and 1:8 and retested.
5. Weak immunodiffusion reactions may be due to the following:
 - a. Foals nursed by infected mares may produce positive results. The foal should be retested at 6 months of age to determine whether it is negative. If a mare is negative her positive foal should be considered infected.



VIII. CONTROLS

- a. Weak positives have been observed during the incubation period of EIA. If a second sample is obtained 2 to 3 weeks later, the reaction should be stronger.
- b. Inapparent carriers that have no clinical signs of EIA for long periods of time may have weak reactions in the AGID. In these cases, retesting rarely results in a change in the strength of the reaction.
6. Any questionable sample should be sent to the National Veterinary Services Laboratory (NVSL) for verification.

Positive Control Serum (Bottle B) –

If the positive reagent serum included in the kit does not react by forming a precipitin line with the EIA antigen (Bottle A), do not use the kit. Call Synbiotics' Technical Services at 800-228-4305

Negative Control Serum (Bottle C) –

The negative control can be used as a comparison when testing weak reacting samples. If the negative control produces any precipitin line with the EIA antigen reagent, do not use the kit. Call Synbiotics' Technical Services at 800-228-4305.