

**EQUINE INFECTIOUS ANEMIA
VIRUS ANTIBODY TEST KIT**

USDA Product Code 5515.21

Assay Instructions for Catalog Number: 400-200

General Description

This equine infectious anemia virus (EIAV) immunodiffusion (ID) test detects precipitating antibodies in sera of Equidae to purified recombinant EIA virus core protein of 26,000 molecular weight (p26). Sample sera, if positive, will form a line that fuses with reference positive control lines or that deviate the reference positive control lines inward near the sample well without formation of a visible line. Negative sera will neither form a line that fuses with the reference positive control line nor cause deviation of the reference positive control lines.

Kit Contents

1 of bottle A EIAV p26 Antigen, 3.35 ml
1 of bottle R Reference Positive Control Serum, 10 ml
These reagents are sufficient to perform up to 200 tests.

Materials Required but not Included in the Test Kit

Hot plate, autoclave or microwave oven, balance, 250 ml flask, laboratory pipettes, refrigerator, standard gel cutter, vacuum pump or water-driven filter pump, micropipettor, disposable micropipettor tips, plastic container, high-intensity narrow-beam light source, 45°C waterbath (desirable, but not required), noble agar, NaOH, boric acid, distilled or deionized water, 100 mm diameter plastic petri plates, or 45 x 90 mm plastic tray.

Storage and Stability

Do not use components from other kits. Store all reagents at 2-7°C. **Do not freeze.** Reagents will remain stable until the expiration date when stored as instructed. **Do not use test kit past the expiration date printed on the box.** Use clean equipment to avoid contamination of reagents.

Preparation of Agar Gel

1. Prepare borate buffer by mixing 2 g NaOH and 9 g boric acid in 1 liter distilled water. The resulting pH should be approximately 8.6.
2. Prepare 1% solution of noble agar in borate buffer.
3. Boil solution until dissolved and then autoclave at 15 lbs pressure, 121°C for 7 minutes. Alternatively, microwave the agar/buffer solution at 30 second intervals for approximately 3 minutes or until the agar is completely dissolved.
4. Cool solution to 45°C and then transfer 15 ml of solution into 100 mm diameter petri dish or 11 ml into 45 x 90 mm trays. The agar should be 2.8 mm thick.
5. Allow the agar to cool in a relatively dust-free environment. Remove the lids during cooling to permit the escape of water vapor. For best results, we recommend that freshly poured plates be stored overnight at 2-7°C before use. The plates or trays of uncut agar may be stored inverted at 2-7°C in zip-lock bags for up to a week. Check stored agar before use to ensure that it is neither desiccated nor covered with moisture.

Cutting Wells in the Agar Gel

A seven-well pattern is used with one center well and six wells in a circle around it. The wells are 2.4 mm apart and 5.3 mm in diameter. The agar is cut after it has hardened sufficiently so that the cut edges of the wells do not break down when agar plugs are removed. The agar plug is suctioned from the wells using a metal or glass cannula drawn to a small opening (1-2 mm in diameter), connected to a vacuum line. Care should be taken to avoid separating the agar from the plate. If moisture is observed in the wells prior to introduction of reagents or samples, it should be removed by suction or allowed to evaporate. Plates should be used the same day they are cut.

Sample Requirements

The sample to be tested must be serum. Sample serum should be separated from the clot as soon as possible and stored at 2-7°C, or stored frozen, until testing. However, serum may be stored either on or off the clot at 2-7°C for up to 28 days until testing. Separated serum may be frozen indefinitely until testing. Hemolyzed serum may stain agar, but has not been demonstrated to affect test outcome. Nevertheless, testing of severely hemolyzed or contaminated samples is not recommended.

Filling Wells and Incubation of Agar Plates

Antigen (A) is placed in the center well with a micropipettor set at 50 µl. Fifty microliters of reference positive control serum (R) are placed in wells on each side of the sample(s) to be tested (Figure 1). This arrangement provides a positive control line on each side of the test serum, thus facilitating accurate determination of lines of identity. A total of three samples can be tested in each pattern, again using 50 µl for each well. The wells are filled level with the agar surface, leaving no meniscus. Serum or antigen must not run on top of the agar. Allow the plates to set a few minutes before moving to reduce the possibility of spillage. Plates are incubated at room temperature (23 ± 2°C) in a closed humid chamber. If room temperature is above 25°C or below 20°C, a 22°C incubator should be used.

Test Validation

For a test to be valid, the reference lines (between Positive Reference Serum wells and the center EIAV Antigen well) must be easily visualized. These lines should be clearly visible right to the edge of negative sample wells. If not, the assay cannot be accurately interpreted.

Interpreting the Results

An intense narrow beam of light adjustable for varying intensities and positions should be used to provide illumination for reading. The reaction should be observed against a black background. A magnifying lens is helpful in some cases. Viewers made for observing stained immunodiffusion reactions are not suitable for reading this test. A minimum of 24 hours are required for a complete immunodiffusion reaction to take place. If the reaction is complete after 24 hours incubation, the result can be reported. The reaction is complete when the positive control lines go into the well containing a negative sample or when a distinct specific line of identity is formed between the sample and the positive control sera. Some weak positive samples require 48 hours before the reaction is complete. These should be retested before the results are reported. The type of reaction will vary with the concentration of antibody in the sample being tested. The reference positive control serum line is the basis for reading the test. If it is not a distinct line, then the test is invalid and must be repeated.

The following types of reactions are observed:

1. **Negative:** The reference positive control lines continue into the test sample well without bending or with a slight bend away from the antigen well (A) and toward the positive control serum (Figure 2, S1, negative sample).
2. **Positive:** Control lines join with and form a continuous line with the line between the test serum (S2) and antigen (A) (Figure 2).
3. **Weak Positive:** The reference positive control lines bend slightly toward the antigen well (A) and away from the reference positive control serum wells (R) but do not form a complete line between antigen (A) and test serum (S3) (Figure 2). These reactions require careful observation and can be easily overlooked. All weak positive samples should be retested before reporting the results.
Weak immunodiffusion reactions have been observed in three types of cases:
 - a) Foals nursing infected mares have weak to fairly strong reactions which may persist for up to 5 months of age due to colostrum antibodies. If the mare and foal are both positive, the foal should be retested at 6 months of age to determine if it becomes negative. If a mare is negative, her positive foal should be considered infected.
 - b) Weak positives have been observed during the incubation period of EIA. If a second sample is obtained two to three weeks later, the reaction should be stronger.
 - c) Inapparent carriers that have no clinical signs of EIA for long periods of time may have weak reactions in the test. In these cases, retesting rarely results in a change in the strength of the reaction.
4. **Very Strong Positive:** The reference positive control lines turn toward the antigen well before they reach the well containing the test serum (S1) and there is a broad, hazy line between the test serum (S1) and antigen (A) (Figure 3). This line is situated very near the antigen well (A), especially if the plate is observed at 24 hours.
5. **Non-Specific Lines:** These lines are observed between the antigen and test serum well. However, the reference positive control lines will pass through the non-specific line and continue on into the test serum well of negative sera (not shown). The non-specific line does not form a continuous line with the reference positive control lines. The reference positive control lines will form more acute angles with a non-specific line than with an EIAV-specific line of identity. The non-specific lines are formed by sample-antibody reactions with antigens other than EIAV p26. A sample serum may produce a specific EIAV line as well as a non-specific line (Figure 3, S2). Care must be taken to be certain a specific reaction is not obscured by a non-EIAV line. Retesting such samples and observing the reactions at frequent intervals may facilitate making determinations if the samples are positive or negative.
6. **Haze Around Well:** Occasionally a haze, due to lipids or other material in the serum, will form around the test serum well that may obscure the reference positive control lines near the sample well (Figure 3, S3). If the test is read at 24 and 48 hours, sometimes the results can be determined before the haze obscures the reaction. However, in some cases a determination cannot be made and another sample should be requested.

All positives should be confirmed by retesting before the results are reported. Also, samples which produce questionable reactions should be retested in duplicate before reporting results.

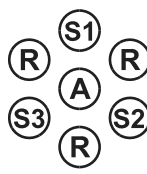


Figure 1.

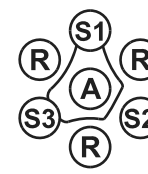


Figure 2.

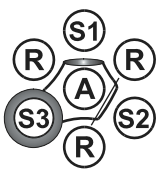


Figure 3.

EIAV-infected horses are generally accepted to be virus carriers for life. Infected horses may not exhibit clinical signs for months or years but still have EIA infectious virus in the blood. Therefore, any adult horse that is positive on the ID test should be considered to be infected.

Notes to US customers:

Sale and use in the U.S. restricted to laboratories approved by State and Federal (USDA) animal health officials. The National Veterinary Services Laboratories will periodically supply coded check test samples to evaluate the competency of the USDA-approved laboratories. Any questionable sample should be sent to the National Veterinary Services Laboratories for verification.

Precautions

Kit components should be handled and disposed of as potentially hazardous. Do not eat, drink, or smoke where serum samples and kit reagents are handled. Do not pipette by mouth. Some reagents may be harmful if ingested. If ingested, seek medical attention. Do not use expired or contaminated reagents, or reagents from other kits or serials. Do not mix reagents from different serials of this same product.

Component A, EIAV p26 Antigen, contains sodium azide as a preservative.
Component R, Reference Positive Control Serum, contains sodium azide as a preservative.