EQUINE INFECTIOUS ANEMIA VIRUS ANTIBODY TEST

FUSION PROTEIN ENZYME-LINKED IMMUNOSORBENT ASSAY

(FP-ELISA II)

Manufactured by:
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SUMMARY AND EXPLANATION

The FP-ELISA II kit utilizes a synthetic antigen from the virus envelope and a core p26 antigen fused into one protein in an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of antibodies to Equine Infectious Anemia Virus (EIAV) in equine serum. The fusion protein (FP) is coated onto microwells and conjugated to horseradish peroxidase. In field trials, the results of testing by the EIAV antibody test show a high correlation with the agar gel immunodiffusion test of Coggins (1). Because there is no cure and no approved vaccine for EIAV, disease control measures are limited to the identification and isolation of infected animals (2, 3). The FP-ELISA II antibody test provides a rapid and reliable method for the detection of EIAV antibodies.

PRINCIPLES OF THE TEST

In the FP-ELISA II test, the wells of the microplate are coated with the fusion protein which contains amino acid sequences identical to a section of an EIAV envelope protein and a recombinant core antigen. The result is multiple epitopes of EIAV are available to bind antibody present in the serum specimen of an EIAV infected equid.
Serum samples are added to wells and incubated at room temperature for 10 minutes, then wells are washed with distilled or deionized water. Next, the fusion protein conjugated to horseradish peroxidase is added and incubated at room temperature for 5 minutes, then wells are again washed with distilled or deionized water. A single component TMB chromogen (tetramethylbenzidine) is added to the wells and incubated for 5 minutes at room temperature. Finally, a stop solution is added to end the reaction.
If antibodies are present in the test specimen, color develops in the well. If antibodies are absent, no color or only minimal color develops. Positive and negative control sera are run with each group of serum specimens to provide representative color development.

REAGENTS: All materials are provided ready-to use.

Materials Provided

1. Microplate wells, coated with fusion protein of EIAV envelope and core antigen
2. Conjugate of EIAV fusion protein and horseradish peroxidase in phosphate buffered saline with stabilizing proteins and Microcide III (green label)
3. Single Component TMB (Tetramethylbenzidine) Chromogen (black label)
4. Positive Control Serum with Microcide III (red label)
5. Negative Control Serum with Microcide III (blue label)
6. Stop Solution (contains 5% phosphoric acid) (pink label)

Materials Required But Not Provided

1. Wash bottle
2. Micropipettor capable of delivering 50 microliters
3. Disposable tips
4. Distilled or de-ionized water

Optional Materials Not Provided

1. Microplate wash system
2. Multichannel pipettor (50 microliter) and disposable tips
3. Microplate reader

PRECAUTIONS

1. Do not mix reagents or microplate wells of one kit with those from another serial or lot number. Do not use after the expiration date shown on the package label.
2. For veterinary use only and for sale only to USDA/APHIS approved laboratories.
3. Vigorous and thorough removal of conjugate from the wells is more critical for assay performance and avoiding false positive reactions than the momentary contact diluted fluid may have with adjacent wells during washing.
4. The Conjugate has a green dye to facilitate noticing which wells it has been added to, and how thoroughly it has been removed during the wash procedure.
5. Warm reagents to room temperature (15° to 25°C) prior to each use.

STORAGE AND STABILITY

All materials provided in the test kit should be stored at 2° to 7°C. Unused wells of the microplate should be stored, and re-sealed properly, with the desiccant in the bag provided.

SPECIMEN COLLECTION AND PREPARATION

Equine serum is recommended for use with the FP-ELISA II test kit. Whole blood may be collected by venipuncture and the serum fraction separated by centrifugation for 10 minutes at approximately 2500 rpm. Serum separators may be used. Anticoagulants are not recommended.

Specimens may be stored at refrigerator temperature (2° to 7°C) for five days. If longer storage is needed, specimens should be frozen at -20°C. Repeated freezing and thawing should be avoided. Frozen samples should thaw at room temperature and should be mixed by gentle inversion before testing begins.

ASSAY PROTOCOL

Preliminary Steps

1. Remove the FP-ELISA II test kit from the refrigerator about one hour before use to allow reagents and microplate wells to come to room temperature.
2. Use a single microplate well for each serum specimen, Positive Control Serum and Negative Control Serum. Return the remainder of microplate wells in the plate to the storage bag with the desiccant. Re-seal bag.
3. Identify and record a well location for each control and serum specimen. Alternatively, identification may be written directly on the side of the well.

Testing Procedure

1. **Add 50 microliters of control or serum specimen to the appropriate wells. Use a new pipette tip for each sample and control.**
2. **Incubate the uncovered wells at room temperature (15° to 25°C) for ten (10) minutes.**
3. **At the end of the incubation, invert wells and flick out contents into an appropriate container. Using a wash bottle filled with distilled or deionized water, rinse wells to overflowing with a *forceful stream* of water. Flick water from wells and repeat the wash step until a total of 5 washes has been completed.**
4. **Tap the inverted wells vigorously on absorbent material to remove any residual wash solution.**
5. **Add one (1) drop or 50 microliters of fusion protein HRP conjugate to each microplate well.**
6. **Incubate the uncovered wells at room temperature (15° to 25°C) for five (5) minutes.**
7. **At the end of the incubation, invert wells and flick out contents into an appropriate container. Using a wash bottle filled with distilled or deionized water, rinse wells to overflowing with a *forceful stream* of water. Flick water from wells and repeat the wash step until a total of 5 washes has been completed.**
8. Tap the inverted wells vigorously on absorbent material to remove any residual wash solution.
9. Add two (2) drops or 100 microliters of TMB Chromogen to each test microwell.
10. Incubate for five (5) minutes to allow for blue color development. If interpreting results visually, read immediately. The use of a white background facilitates the observation of color.
11. A stop solution is included in the test and recommended if there will be any delay in reading the results, or if a spectrophotometer will be used, (or if there is blue-green color blindness). Add two (2) drops or 100 microliters of Stop Solution to each well. The blue color will now become yellow. If a spectrophotometer is used, the microplate may be read at 450nm.

QUALITY CONTROL

The Positive Control Serum produces an intense color in the assay. When read spectrophotometrically with the spectrophotometer blanked on air, the Positive Control Serum should yield an O.D. of 0.3 to 1.5. If color development in the Positive Control Serum is weak or appears to be less than usual to the experienced user, the assay should be repeated. A lack of color development in the Positive Control Serum may be due to the use of expired or damaged reagents or wells. The Negative Control Serum produces either no color or only a faint tint. When read spectrophotometrically, the Negative Control Serum should yield an O.D. of 0.0 to 0.100. The presence of color could be due to cross contamination during the test procedure or inadequate washing of the wells.

RESULTS

For visual determination, any test well yielding color development greater than the Negative Control Serum should be considered positive for antibodies to EIAV. Wells which visually show color development equal to or less than the Negative Control Serum should be considered to contain serum specimens which are free of detectable antibody to EIAV.

For spectrophotometric determination, blank the spectrophotometer on air. Add 0.05 OD to the negative control value to establish the test cutoff value. Specimen wells with values above the cutoff should be considered positive for antibodies to EIAV. Specimen wells with values equal to or below the cutoff should be considered negative for antibodies to EIAV. Any positive ELISA evaluation should be confirmed using the agar gel immunodiffusion (AGID) test. Discrepant samples should be re-evaluated according to State regulations or be sent to the National Veterinary Services Laboratory in Ames, Iowa for confirmation before being reported as positive.

TECHNICAL SERVICES

If you have any questions regarding the use of this test, please call Centaur, Inc. in the USA at 800-236-6180 or outside the US (913) 390-6184.

REFERENCES

1. Pearson, J.E. and Coggins, L.: Protocol for the Immunodiffusion (Coggins) Test for Equine Infectious Anemia. American Association Veterinary Laboratory Diagnosticians. 22nd Annual Proceedings, 449-462 (1979).
2. Issel, C.J. and Coggins, L.: Equine Infectious Anemia: Current Knowledge. JAVMA 174 (7): 727-733 (1979).
3. Mohanty, S.B. and Dutta, S.K.: Equine Viruses in Veterinary Virology, Lea and Febiger (1981), pages 177-182.