



**United States  
Department of  
Agriculture**

**Animal and Plant  
Health Inspection  
Service**

**APHIS -91-55-064**

# **Equine Infectious Anemia: Uniform Methods and Rules**

January 10, 2007

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, sexual orientation, or marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 1400 Independence Avenue, SW, Washington, DC 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

Mention of companies or commercial products does not imply recommendation or endorsement by USDA over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

Issued January 1998  
Slightly revised March 2002  
Revised August 2006



## **Contents**

### **Introduction**

- I. Definitions and Abbreviations**
- II. Recommended Procedures (Minimum Requirements)**
  - A. Authority to Require Test**
  - B. Personnel Authorized to Submit Diagnostic Samples for EIA**
  - C. Approved Laboratories**
  - D. Laboratory and Testing Procedures**
    - 1. Official laboratory tests
    - 2. Supplemental laboratory test
    - 3. Official laboratory form
  - E. Testing Requirements**
    - 1. Categories of equines requiring testing
    - 2. Age of first testing
  - F. Procedures for Handling Infected or Exposed Equines**
    - 1. Quarantine
    - 2. Repeat testing and removal of reactors
    - 3. Epidemiologic investigation
    - 4. Quarantine release
    - 5. Identification of reactor equines
    - 6. Euthanasia and disposal
  - G. Procedures for Moving Restricted Equines**
    - 1. Restricted equines
    - 2. Permit
    - 3. Interstate movement
    - 4. Movement exceptions for exposed equines
  - H. Control Procedures**
    - 1. Annual EIA test
    - 2. Testing prior to exhibitions or competitive events
    - 3. Testing and isolation of additions to herds
    - 4. Testing of equines from high-risk areas at slaughter
    - 5. Individual equine identification
    - 6. Vector control
    - 7. Education

## **Introduction**

This publication—Equine Infectious Anemia: Uniform Methods and Rules (UM&R)—contains minimum standards for detecting, controlling, and preventing equine infectious anemia (EIA).

The provisions of this UM&R were approved by the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), with the recommendations of the United States Animal Health Association, the American Horse Council, and the American Association of Equine Practitioners. This UM&R may be amended in the future.

## **I. Definitions and Abbreviations**

### **Accredited veterinarian**

A veterinarian approved by the Deputy Administrator of USDA, APHIS, VS in accordance with provisions of Part 161, Title 9, *Code of Federal Regulations* (CFR). An accredited veterinarian is pre-approved to perform certain functions of Federal and cooperative State–Federal programs.

### **Agar gel immunodiffusion (AGID) test**

The primary official laboratory test for diagnosis of EIA in which precipitates are formed by interaction of EIA antigens and antibodies that diffuse through gel.

### **Approved laboratory**

A State, Federal, or private veterinary diagnostic laboratory for EIA testing that must be approved by USDA, APHIS, VS.

### **Approved laboratory tests**

Laboratory tests for diagnosis of EIA that are approved by and produced under license of USDA, APHIS, VS.

### **Certificate**

An official document issued by a VS representative, State representative, or accredited veterinarian at the point of origin of a shipment of equines. It includes all of the following:

1. The description, including age, breed, color, sex, and distinctive markings when present (such as brands, tattoos, scars, or blemishes) of each restricted equine to be moved, and any artificial identification;
2. The number of restricted equines covered by the document;
3. The purpose for which the equines are to be moved;
4. The points of origin and destination;
5. The consignor; and
6. The consignee.

### **Change of ownership**

Ownership of equines changing from one individual or entity to another either through selling, bartering, trading, or donating the equine to another individual.

### **Coggins test**

The common name for the agar gel immunodiffusion test for diagnosis of EIA.

**Enzyme-linked immunosorbent analysis (ELISA)**

A number of laboratory tests using ELISA formats are approved for the diagnosis of EIA and detect antibodies against one or more antigens of EIAV.

**Equine**

Any animal in the Family Equidae, including horses, asses, mules, ponies, and zebras.

**Equine infectious anemia (EIA)**

An infectious disease of equines caused by a lentivirus, equine infectious anemia virus (EIAV). The infection is characterized by three distinct forms: acute, chronic (both associated with clinical signs of disease), and inapparent.

**Equine infectious anemia laboratory test form**

The official Federal Government form (VS Form 10–11), or other approved form, required when submitting blood samples to an approved laboratory for EIA testing.

**Exposed animals**

Animals in the Family Equidae that have been exposed to EIA through direct/indirect association with equines having tested positive on approved diagnostic tests.

**Herd**

1. All animals of the Family Equidae, under common ownership or supervision that are grouped on one or more parts of any single premises (lot, farm, or ranch); or
2. All animals of the Family Equidae under common ownership or supervision on two or more premises that are geographically separated but in which the equines have been interchanged or had contact with equines from different premises. It will be assumed that contact between animals of the Family Equidae on the different premises has occurred unless the owner establishes otherwise and the results of the epidemiologic investigation are consistent with the lack of contact between premises; or
3. All animals of the Family Equidae on common premises, such as community pastures or grazing association units, but owned by different persons. Other groups of equines owned by the persons involved that are located on other premises are considered to be part of a herd unless epidemiologic investigation establishes that equines from an affected herd have not had the opportunity for direct or indirect contact with equines from that specific premises.

**Premises of origin**

A farm or other premises where the equines were born or where they have been kept for 30 days or more before the date of shipping. For the purposes of this UM&R, premises of origin has the same meaning as place of origin and farm of origin.

**High-risk area**

A geographic region in which EIA is known to be endemic and in which environmental conditions are conducive to the maintenance and spread of the infection.

**Identification**

Permanent notation of equines that are determined to be EIA reactors by application of a visible mark (e.g., hot iron, chemical brand, freezemarking, or lip tattoo), using the National Uniform Tag code number assigned by USDA to the State in which the reactor was tested, followed by the letter "A."

**Official seal**

A serially numbered metal or plastic strip, consisting of a self-locking device on one end and a slot on the other end that forms a loop when the ends are engaged. An official seal is tamperproof and cannot be reused if opened. It is applied to the doors of a transport vehicle by a representative of the APHIS Area Veterinarian-in-Charge or the State animal health official. A serially numbered, self-locking button that cannot be reused may be substituted for the metal or plastic strip type of seal.

**Official test**

Any test for the laboratory diagnosis of EIA that utilizes a diagnostic product that is: (1) produced under license from the Secretary of Agriculture, and found to be efficacious for that diagnosis, under the Virus-Serum-Toxin Act of March 4, 1913, and subsequent amendments (21 U.S.C. 151 *et seq.*); and (2) conducted in a laboratory approved by the Administrator of APHIS.

**Permit**

An official document (VS Form 1-27 or comparable State form) that is issued by a State or Federal representative or by an accredited veterinarian. The permit is required to accompany all EIA reactors and those EIA-exposed equines that are being moved under official seal during their movement to a specified destination.

**Quarantine**

The act of placing exposed or affected animals into isolation from other animals to prevent the transmission of a disease.

**Quarantined area**

A confined area under the direct supervision and control of a State or Federal animal health official who shall establish procedures for the accounting of all animals entering or leaving the area. All equines under EIA quarantine are considered to be exposed to EIA.

**Reactor**

Any equine that has been subjected to an official laboratory test whose result is positive for EIA and confirmed by the AGID or other approved reference laboratory tests if results are not concordant.

**Reference laboratory**

The national and international (World Organization for Animal Health [OIE]) reference laboratory for EIA serology is the National Veterinary Services Laboratories (NVSL) in Ames, Iowa. The University of Kentucky EIA Reference Laboratory is also nationally recognized as a reference laboratory for EIA research and may provide consultation. Reference laboratories must report results of all EIA tests to the appropriate State and Federal animal health officials.

**State**

Any State of the United States and the District of Columbia, Puerto Rico, the U.S. Virgin Islands, and Guam.

**State animal health official**

The chief State official responsible for disease control and eradication programs affecting livestock and poultry.



## **II. Recommended Procedures**

### **A. Authority to Require Test**

State laws and/or regulations shall provide authority to conduct an official laboratory test to diagnose EIA in any equine or herd at such times as may be deemed necessary by the cooperating State officials. These officials reserve the right to supervise any test conducted by an accredited veterinarian.

### **B. Personnel Authorized to Submit Diagnostic Samples for EIA**

Diagnostic samples for EIA may be submitted only by a State or Federal animal health official or accredited veterinarian.

### **C. Approved Laboratories**

Tests for EIA are to be conducted in USDA-approved laboratories by individuals who have been properly trained in the laboratory procedures involved. These laboratories must use USDA-approved laboratory tests, follow official protocols, and perform annual proficiency tests using the EIA test they routinely employ. Laboratories and personnel will be subject to inspection as required by NVSL. Laboratories will require accurate and detailed identification of equines, owners, and submitting veterinarians and will report all EIA test results as required by State and Federal regulations. Individual States may have additional laboratory standards of their own, in addition to those prescribed by USDA.

### **D. Laboratory Testing**

1. Official laboratory tests—The following laboratory tests are approved by USDA for the diagnosis of EIA:
  - a. Agar gel immunodiffusion (AGID).  
Also known as the Coggins test, AGID is the most widely accepted procedure for the diagnosis of EIA. The test detects antibody against the viral p26 antigen (major core protein). It is the only procedure that has been statistically correlated with the presence of EIA virus in blood. False-positive AGID test results appear to be rare and are generally caused by technician errors and corrected by repeated tests. False-negative AGID test reports are generally related to faulty interpretation of the test reaction by the technician or to low levels of antibody in the test serum. Results are recorded as either positive or negative.
  - b. ELISA tests.  
A number of ELISA test formats are used in approved test kits.

Results of ELISA tests can be obtained within a few hours, compared to the 24 hours minimum required with the AGID test. Since ELISA test results can be read by spectrophotometer (giving rise to less human error in interpretation) and less antibody is required to produce the color change in ELISA tests than is needed to produce a visible line of precipitation in AGID tests, fewer false-negative results are seen with these tests than with the AGID test. However, a higher number of false-positive results are expected in ELISA tests than in AGID tests. ELISA test results are recorded as either positive or negative. Positive tests must be confirmed using the AGID test or other reference laboratory test before regulatory actions are taken.

2. **Supplemental laboratory test**—The Western blot test is not an official laboratory test but may be used to resolve equivocal results on official laboratory tests. Also called the immunoblot test, it may be used to reach consensus when other diagnostic tests have yielded contradictory results. The immunoblot test for EIA is approved to be conducted only at the NVSL and at the University of Kentucky.
3. **Official laboratory form**—All laboratory submissions must be accompanied by an EIA laboratory test form, as defined in this document. Forms without adequate descriptions of the equine and complete addresses, including zip codes, counties, and telephone numbers, will not be processed. The VS Form 10-11 “Equine Infectious Anemia Laboratory Test” provides sections for mandatory certification by an accredited veterinarian and optional certification by the horse’s owner or owner’s agent at the request of the veterinarian. In compliance with U.S.C. Section 1001, falsification of the form or knowingly using a falsified form is a criminal offense and may result in a fine or imprisonment.

## **E. Testing Requirements**

Intervals between tests should be based on risk, supported by State or regional regulations reflective of this risk. Until such risk-based programs are established, uniform interstate movement standards of 12-month testing intervals are recommended.

1. **Categories of equines requiring testing**—The following categories of equines must be tested for EIA:
  - a. **Equines being entered into exhibitions or competitive events:** All equines entered in exhibitions or competitive events must have been tested for EIA with a negative result within the time prescribed by local authorities and be documented on an official

EIA laboratory test form, as defined in this document. Event officials must review official test papers of all equines entered into an event to ensure that all participating equines are test-negative for EIA.

- b. Equines being moved interstate: All equines being moved interstate must have been tested for EIA with a negative result within 12 months prior to movement and must be accompanied by a permit describing the equine, and signed by an accredited veterinarian.
  - c. Equines changing ownership: All equines sold, traded, or donated within a State must have been tested for EIA with a negative result no more than 12 months prior to change in ownership and, preferably, no more than 60 to 90 days. It is recommended that all equines originating in high-risk areas be tested for EIA. In areas of highest risk, one negative test may not be sufficient to ensure that the equine is not infected with the EIAV. In such cases, it is recommended that the new owner retest 60 days after obtaining ownership and make the sale contingent on a negative retest result. All change of ownership transactions must be accompanied by a certificate describing the equine, and signed by an accredited veterinarian.
  - d. Equines entering horse auctions or sales markets: All auction or sale markets, regardless of size, should be licensed by the State and are required by the State to keep records to expedite traceback capabilities. A negative EIA test is required for all equines prior to sale. If an EIA test is not possible prior to each sale, then the equines must be held in quarantine within the State until the test results are known. Markets should employ an accredited veterinarian to attend sales, interpret the validity of test papers presented, and draw blood for testing equines that have no current negative EIA test result.
2. Age of first testing—Equines of any age can be tested for EIA because equines at all ages are susceptible to infection and, if their immune systems are competent, can respond to infection by producing antibodies to EIAV. To determine if a foal is infected with EIAV, several strategies must be used, based primarily on the status of the foal's contacts. If the status of all contacts is not known, it is recommended that the foal be tested for the first time at weaning (less than six months) and again after a suitable quarantine period (more than 60 days) to protect against possible exposure to EIA from untested contacts. If its dam is test-positive, the foal will acquire passive antibody to EIA in the colostrum and may test

positive for more than six months. In these cases, the foal must be quarantined for at least 60 days and have a negative test at the end of the quarantine period before being commingled with other equines. If a foal less than six months of age is accompanying a test-negative dam, the testing requirements can be waived on practical grounds.

#### **F. Procedures for Handling Infected Equines**

1. **Quarantine**—When an equine has a positive result on an official test for EIA, the animal must be placed under quarantine within 24 hours after positive test results are known in order to permit confirmation testing and to prevent further exposure of other equines. The equine must remain in quarantine until final infection status and disposition are made.

All exposed equines, either individual or within a herd, within 200 yards of the location where a reactor equine is or was maintained must also be placed under quarantine.

The quarantine area must provide no less than 200 yards' separation from all other equines. The quarantine area, and the quarantined equines therein, must be monitored periodically by regulatory personnel to ensure that provisions of the quarantine are not being violated.

2. **Repeat testing and removal of reactors**—When a reactor is detected in a herd and removed, testing for EIA must be repeated until all remaining equines on the premises test negative. All subsequent reactors must be removed from the herd within 24 hours after positive test results are known. The remaining animals in the herd must be retested at 30- to 60-day intervals, or more frequently, until no new cases are found. Once the remaining equines in the herd have negative test results for a minimum of 60 days, the quarantine may be lifted.
3. **Epidemiologic investigation**—It is recommended that epidemiologic information be collected for all animals whose EIA test results are positive. Information should be entered on the VS Form 10–12, “Equine Infectious Anemia Supplemental Investigation.” Factors to be investigated should include (a) potential source(s) of infection, (b) the equine’s movement history, (c) the EIA test history for equines on that premises, (d) the history of the equine’s contact with needles or surgical instruments, and (e) the location of the equine prior to testing. The form should also include a sketch of the EIA reactor’s location relative to major highways and primary and secondary roads. This information will allow for computerized geographic information systems to analyze spatial and biological factors with respect to the presence and maintenance of EIAV

in areas where the disease may be localized, and provide an epidemiologic assessment of risk factors associated with new cases.

4. Quarantine release—Equines under quarantine due to exposure to a reactor equine may be released from quarantine when tests on the entire herd have been negative at least 60 days after the reactor equines have been removed. Herd testing during the quarantine period may be conducted to minimize the spread of the disease within the herd and to reduce the threat of exposure to equines adjacent to the quarantined herd.
5. Identification of reactor equines—Equines that are determined to be reactors must be permanently identified using the National Uniform Tag code number assigned by USDA to the State in which the reactor was tested, followed by the letter “A.” Markings must be permanently applied to the reactor by an APHIS representative, State representative, or accredited veterinarian using a hot iron, chemical brand, freezemarking, or lip tattoo. If hot iron, chemical branding, or freezemarking is used, the markings shall be not less than two inches high and shall be applied to the left shoulder or left side of the neck of the reactor. If a lip tattoo is used, each character of the tattoo shall be not less than one inch high and three-fourths of an inch wide and shall be applied to the inside surface of the upper lip of the reactor.

Official identification is not necessary if the reactor is moved directly to slaughter under a permit and is in a conveyance sealed with an official seal. If, however, the interstate movement to the destination slaughtering establishment cannot be completed without a stop for resting, feeding, and watering a reactor, the equine must be officially identified and may be moved interstate through no more than one approved stockyard for sale for immediate slaughter. In this case, the reactor must be accompanied by a permit during the interstate movement and moved within five days of its arrival at the approved stockyard directly to slaughter.

6. Euthanasia and disposal—Once an equine has been classified as a reactor, it must be separated and removed from the herd. This can be accomplished by euthanasia, slaughter, or quarantine at the premises of origin. If slaughter is chosen, the equine must be moved either to a federally or State-inspected slaughtering establishment per the *Code of Federal Regulations*, Part 75.4. Permits (VS Form 1–27) attesting to said slaughter must be signed and returned to the animal health authorities in the State of origin in a timely manner by the inspecting authority at the slaughtering facility.

## **G. Procedures for Moving Restricted Equines**

1. Restricted equines—All reactor and exposed equines must be quarantined and their movement restricted by State animal health officials within 24 hours after positive test results are known.
2. Permit—Restricted equines may move interstate only if accompanied by an official permit. The permit is an official document (VS Form 1–27 or a State form that contains the same information, but not a “permit for entry”) issued by an APHIS or State representative or accredited veterinarian. This document lists the owner’s name and address, points of origin and destination, number of equines covered, purpose of the movement, and one of the following: the individual equine registered breed association registration tattoo, individual equine registered breed association registration number, or similar individual identification, including name, age, sex, breed, color, and markings.
3. Interstate movement—Reactors may only move interstate under permit to the following locations:
  - a. Federally inspected slaughter facility
  - b. Federally approved diagnostic or research facility
  - c. Premises of origin

The individual issuing the permit must consult with the State animal health official in the State of destination for approval and must determine that the reactor to be moved interstate will be maintained in isolation sufficient to prevent the transmission of EIA to other equines. The reactor will remain quarantined under State authority at the above locations until natural death, slaughter, or until disposed of by euthanasia.

4. Movement exceptions for exposed equines—Individual exposed equines may be allowed to move from a quarantined area for specific purposes if they have a negative test at the time of movement. These equines must be retested at 15-day intervals until their EIA tests are negative at least 60 days after the last exposure to EIA.

## **H. Control Procedures**

1. EIA testing frequency—It is recommended that all equines be tested for EIA as part of a routine health program at an interval consistent with the risk of acquiring EIA in the local area and, if possible, the region. Until such risk-based programs are established, uniform interstate movement standards of 12-month testing intervals are recommended.

2. All equines offered for entry into exhibitions or competitive events must present proof to event officials of a negative EIA test as documented on an EIA laboratory test form, as defined in this document.
3. Testing and isolation of additions to herd—All introductions of equines into a herd must have a negative EIA test conducted prior to entry or must be tested while in isolation on the farm.
4. Testing of equines from high-risk areas at slaughter—All untested equines consigned to slaughter channels from areas with a high incidence of EIA or areas where the disease is endemic must have blood drawn for EIA testing at the most efficient time for obtaining identification and history on the farm of origin for each equine. This may be at sale yards, gathering points, or at the premises of licensed slaughter equine dealers.
5. Individual equine identification—Individual equine identification is critical to the control and surveillance of EIA and can be facilitated by using a uniform electronic identification standard. For those States not yet utilizing this technology, a complete and accurate written and graphic description of all markings is necessary for proper identification.
6. Vector control—Vector control practices should be followed to reduce exposure in herds. These practices shall include direct insect control, such as periodic application of repellants and insecticides to equines and facilities occupied by equines, and environmental insect control such as proper manure management and moisture management to discourage insect populations.
7. Biosecurity—Control of personnel access to equines, cleaning and disinfection of equipment between uses on multiple equines, and proper disposal of “single use” material such as needles and syringes should be encouraged.
8. Education—A successful EIA control program should include an educational program directed toward equine owners in all facets of the industry.