U.S. DEPARTMENT OF AGRICULTURE
Marketing and Regulatory Programs
Animal and Plant Health Inspection Service
Veterinary Services

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VS Guidance 13407.3

Testing of Equids During Import Quarantine

1. Purpose and Background

   This document establishes guidelines for testing quarantined equids to determine their import eligibility. These testing requirements apply to all equids, including horses, donkeys, mules, asses, and zebras. This guidance uses the terms "equid" and "horse" interchangeably.

   Veterinary Services (VS) personnel test horses presented for import to exclude four diseases from the United States: dourine, glanders, equine infectious anemia (EIA), and equine piroplasmosis (EP). Horses must test negative on official tests to these four diseases before VS will release them from quarantine and allow entry into the United States.

   This guidance document represents the Agency's position on this topic and is intended solely as guidance. It does not have the force and effect of law, does not create, or confer any rights for or on any person, and does not bind the U.S. Department of Agriculture (USDA) or the public. Language suggesting that this guidance is mandatory (e.g., "shall," "must," "required," or "requirement") should not be construed as binding unless the terms quote from a statutory or regulatory requirement. The information this document contains may be made available to the public. While this document provides guidance for users outside APHIS, APHIS employees may not deviate from the directions provided herein without appropriate justification and supervisory concurrence.

2. Document Status

   A. Review date: 8/15/2026.

   B. This document replaces VS Guidance 13407.2.

3. Reason for Reissuance

   VS is reissuing this guidance to update formatting, revise VS testing procedures for glanders, and add or amend several definitions to provide clarity to importers regarding animal classification and non-negative test results.

4. Authority and References

   A. Authorities (*Code of Federal Regulations* (CFR)):

   7 CFR 371.4
   9 CFR 93.304
   9 CFR 93.306
   9 CFR 93.308
   9 CFR 93.317
   9 CFR 93.324

B. References:

World Organisation for Animal Health (WOAH) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: Chapters 3. 6. 3 Dourine; 3. 6.11 Glanders and melioidosis; 3. 6. 6 Equine infectious anemia; and 3. 6. 8 Equine piroplasmosis.

VS Guidance 7000, Surveillance, Collection, and Submission of Suspected Exotic Ticks

VS Guidance 13424, Procedures for the Import of Equines into the United States and Approved Quarantine Facilities

C. Definitions:

1) Anticomplementary (AC): Non-specific consumption of complement; indeterminate reaction that occurs in the absence of antigen. The causes of anti-complementary results are unknown, but a fasting blood sample may improve the result. VS personnel should always submit clear serum without evidence of hemolysis for complement fixation tests.

2) Cohorts: Contact animals from the same shipment. Cohorts vary by disease. The specific definition of a cohort is described under each disease in Section 6. B.

3) Classification: The determination of an animal's regulatory health status as characterized by official diagnostic testing, including confirmatory or supplemental tests when necessary. Test results will classify an animal as negative, suspect, or positive for each disease. An animal must be classified negative to be considered eligible for release from quarantine.

4) Confirmatory test: Test method of high diagnostic specificity used to confirm results, usually positive results, derived from other test methods, and thereby determine the animal's classification. Horses determined to be negative on confirmatory testing will be classified negative for the target disease and may be eligible for release.

5) Non-negative test result: A test result reported as positive, inconclusive, or suspect. Test results establish the regulatory classification of an animal and are used to determine eligibility for release from quarantine.

6) Supplemental tests: Diagnostic tests beyond the primary official test. Supplemental tests are *only* performed on AC samples at the discretion of VS personnel.

5. Audience

   VS employees, other Federal and State agencies, and members of the public.

6. Guidance

   A. General

      1) All imported horses, regardless of age, are tested for diseases as specified in 9 CFR 93.308 using the following official tests:

         a. Complement fixation test (CFT) for EP and dourine.

         b. Agar gel immunodiffusion (AGID) test for EIA.

         c. Competitive enzyme-linked immunosorbent assay (cELISA) for EP.

         d. Double antigen enzyme-linked immunosorbent assay (daELISA) for glanders.

      2) Exceptions to the general testing requirements for imported horses:

         a. Horses originating and arriving directly from Iceland are exempt from testing for EP, dourine, glanders, and EIA.

         b. Horses originating and arriving directly from Australia and New Zealand are exempt from testing for dourine and glanders.

         c. Horses originating and arriving directly from Canada are exempt from testing for dourine, glanders, and EP. Canadian animal health officials test horses with either the AGID test or the ELISA for EIA before the horses enter the United States.

      3) Government officials from the exporting country must certify in writing the identity of the originating premises for all horses. This information must appear on each animal's endorsed export certificate or on additional certification the officials provide to VS.

      4) Unless otherwise specified, VS considers all horses transported in the same conveyance arriving at the U.S. port of entry one shipment for quarantine, testing, and release. Any non-negative test results for an individual animal may affect the release of the cohorts in the shipment. VS may consider any additional epidemiological information presented in addition to import testing to determine the import status of all horses in the shipment.

      5) During the horse's first twenty-four (24) hours in quarantine, submitters (VS personnel at the port) collect blood samples, process, package, and ship blood samples to the VS National Veterinary Services Laboratories (NVSL) in Ames, IA in accordance with port Standard Operating Procedures.

6) If a horse tests non-negative on the initial blood collection, handlers isolate it from the other horses in the shipment and may use additional personal protective equipment based on the disease of concern and the presence or absence of clinical signs, in accordance with port Standard Operating Procedures.

7) The veterinary medical officer (VMO)/receiving officer at the port examines the animal and draws the samples; they can, based on test results or physical examination, extend the quarantine period or refuse entry as prescribed in 9 CFR 93.308(a)(4). The importer of record must pay for any extended quarantine period and such extension is subject to the facility's ability to keep horses for extended periods.

8) Horses must be classified as negative for dourine, glanders, EP, and EIA, based on testing conducted by NVSL, to be considered eligible for entry into the United States. Horses refused entry must be either exported out of the United States within ten (10) days of refusal notification or humanely euthanized.

9) When the results of the official dourine and EP CFT are anticomplementary, the laboratory performs supplemental tests, such as an indirect fluorescent antibody (IFA) test. If a horse has yielded anticomplementary results on previous testing, the responsible individual may notify the laboratory for awareness. VS personnel may use other VS-approved assays as supplemental tests. VS has no supplemental test for EIA or glanders.

10) VS personnel must perform confirmatory testing using the Western blot on all samples that test positive on the EP cELISA for *Babesia caballi*.

11) VS personnel must perform confirmatory testing using the Western blot on all samples that test positive on the daELISA for glanders.

12) The quarantine station VMO releases NVSL test results to importers, agents, or brokers. NVSL does not release any test results directly to importers, agents, or brokers, except when given specific permission by the VMO at the quarantine station on a case-by-case basis.

13) NVSL advises the port VMO, Strategy and Policy Live Animal Import and Export Directors, and the Equine Import veterinary staff officer of non-negative quarantine test results.

B. Specific Information and Collection Procedures for Each Disease

1) Dourine

a. General information

Dourine is a venereal disease of horses transmitted primarily by sexual contact and caused by the protozoan *Trypanosoma equiperdum*. The organism may be present in the genital secretions of both male and female horses. The incubation period, severity, and duration of disease vary. Clinical signs can include fever, edema of the genitals and mammary glands, cutaneous eruptions, incoordination, facial paralysis, anemia, and emaciation. While the disease is often fatal, subclinical cases and latent carrier states can occur. Infections with other trypanosomes (e.g., *T. evansi*) may cause cross-reactions with *T. equiperdum* serological assays.

b. Testing

1. The CFT is the official test for dourine. Positive CFT results are equal to or greater than a 2+ reaction at a 1:5 dilution. Suspect test results are 1+ reactions at a 1:5 dilution.

2. VS personnel should immediately redraw blood samples from any horses testing positive or suspect and follow procedures outlined in section 6.C.

3. When CFT results are anticomplementary, NVSL performs supplemental tests on the sample obtained from the initial blood collection. If supplemental tests are positive or suspect, the port VMO should redraw blood samples immediately, submit to NVSL, and follow procedures outlined in section 6.C. NVSL performs official and supplemental tests on the redrawn sample.

4. Refer to Figure 1.

c. Cohorts

1. Intact animals over seven hundred and thirty-one (731) days, as well as any younger animals which have been bred, that were resident on the same premises to the horses testing positive during the sixty (60) days immediately prior to export are part of the cohort.

2. VS personnel will release castrated and immature equids if the animals had negative test results on the initial serum collection.

3. VS personnel hold cohorts for fourteen (14) days after the initial blood collection, then rebleed and retest all cohorts. VS personnel may release cohorts if all retest results are negative. Cohorts that test positive must follow the procedures outlined in section 6.C.

2) Glanders

a. General information

Glanders is a highly contagious bacterial disease caused by *Burkholderia mallei*. Infected animals may have nodules, abscesses, and ulcers in the respiratory tract and skin. The average incubation period is two (2) weeks but varies from two (2) days to several months, and the disease may persist as chronic infections in inapparent carriers. In addition to affecting horses, glanders poses a significant public health risk. Humans can contract glanders through direct contact with diseased animals or contaminated materials, with up to a ninety-five (95) percent mortality rate in untreated cases. Infections with other bacteria (e.g., *B. pseudomallei*) may cause cross-reactions with *B. mallei* serological assays.

b. Testing

1. The daELISA is the official test for glanders. Results on the daELISA are only positive or negative. There is no suspect result. Positive ELISA results are equal to or greater than seventy (70) S/P percent. S/P is an optical density (OD) measurement, here referring to the ratio of the sample OD (S) to the positive control OD (P).

2. If the results of the initial daELISA results are positive, then the Western blot will be used as a confirmatory test. Horses that are found negative using the Western blot confirmatory test will be classified as negative for glanders and may be released. The final interpretation, including Western blot confirmatory testing, will be included on the final NVSL report.

3. VS personnel should immediately redraw blood samples from any horses testing non-negative using the daELISA and Western blot and follow the procedures outlined in section 6.C.

4. Refer to Figure 1.

c. Cohorts

1. All animals in the shipment are cohorts.

2. VS personnel hold cohorts for fourteen (14) days after the initial blood collection, then rebleed and retest all cohorts. VS personnel may release cohorts if all retest results are negative. Cohorts that test positive must follow the procedures outlined in section 6.C.

3) Equine piroplasmosis (EP)

a. General information

1. EP, or babesiosis, is a tick-borne protozoan disease of horses caused by *Babesia caballi* or *Theileria (Babesia) equi*. Co-infections with both

protozoan species may occur. Infections may occur from tick bites or through contaminated needles or transfusions (iatrogenic infection). The protozoan develops within the red blood cells of the host and within various cells of competent tick vectors. Tick-borne piroplasmosis infections have a one- (1-) to three- (3-) week incubation period; iatrogenic piroplasmosis infections may have a prolonged incubation period.

2. Clinical signs are variable and often nonspecific, but may include pale, icteric, or hemorrhagic mucous membranes; fever; anemia; depression; weakness; edema; and hemoglobinuria. Infected animals may remain asymptomatic carriers of these protozoan parasites for several years or more.

b. Testing

1. The official tests for EP are the cELISA and CFT. The CFT is more likely to detect early infections but may be negative when testing chronic carriers. The cELISA targets chronic asymptomatic carriers but may be negative during early stages of infection.

2. Results on the cELISA are only positive or negative. There is no suspect result. Samples are considered positive on cELISA if they exhibit a forty (40) percent inhibition or higher result for either organism that causes EP.

3. Results on the CFT are only positive or negative. There is no suspect result. Positive EP CFT results are equal to or greater than a 2+ reaction at a 1:5 serum dilution.

4. Horses must be negative on both cELISA and CFT to be considered negative for EP.

5. When CFT results are anticomplementary, NVSL performs supplemental tests on the sample obtained from the initial blood collection. If supplemental tests are non-negative, the port VMO should immediately redraw and follow procedures outlined in section 6.C.

6. Horses that test positive for *B. caballi* by cELISA, but which are found negative using the Western blot confirmatory test, will be classified as negative for *B. caballi* and may be eligible for release. The final interpretation, including Western blot confirmatory testing, will be included on the final NVSL report.

7. Refer to Figure 1.

c. Cohorts

1. Horses resident on the same premises within the sixty (60) days immediately prior to export are cohorts to the horses testing positive on the cELISA or CFT.

2. VS considers additional epidemiological information (such as the presence of ticks or the use of blood-contaminated medical equipment by personnel accompanying the horses on the aircraft) when determining cohorts.

3. VS personnel hold cohorts for fourteen (14) days after the initial blood collection, then rebleed and retest all cohorts using the cELISA and CFT for *B. caballi* and/or *T. equi* as appropriate. VS personnel may release cohorts if all retest results are negative. Cohorts that test positive must follow the procedures outlined in section 6.C.

d. Tick-infested horses

1. VS thoroughly inspects and treats all horses with an approved ectoparasite spray as outlined in VS Guidance 13424.

2. Horses found to have attached ticks when examined will be held for fourteen (14) days in quarantine and re-tested for piroplasmosis or may be refused entry.

3. VS personnel must submit the ticks collected to NVSL as outlined VS Guidance 7000.

4) Equine infectious anemia (EIA)

a. General information

EIA is an acute or chronic viral disease of horses characterized by intermittent fever, depression, weakness, weight loss, edema, and anemia. EIA is transmitted through blood from an infected animal by contaminated needles, bloodsucking flies, or other blood contact. The incubation period usually ranges from one (1) to three (3) weeks but can be as long as three (3) months.

b. Testing

1. The official test for EIA is the AGID (Coggins) test. Results of the AGID are only positive or negative. There is no suspect result. If the EIA AGID test is positive on initial screening, NVSL performs the test again in duplicate on the sample obtained from the first blood collection.

2. The port VMO should immediately redraw and resubmit blood samples to NVSL for any horses testing positive on the initial AGID test. If immediate rebleed samples are positive, the horse will be refused entry. VS should

immediately resample horses that test negative on redrawn samples and submit the samples to NVSL for testing. VS will only release horses that are negative to two sequential subsequent tests after an initial positive test.

3. Refer to Figure 2.

c. Cohorts

1. All animals in the shipment are cohorts.

2. VS refuses entry to cohorts without retesting and they are returned to the country of origin; they cannot stay in import quarantine. The importer of record may present cohorts for entry retesting after forty-five (45) days of isolation from EIA-positive horses after VS reviews further epidemiological information.

C. Procedures for Horses with Non-Negative Results on Initial Sample Testing for Glanders, Dourine, and EP

1) VS personnel should immediately redraw blood samples from any horses testing non-negative for dourine, glanders, or EP and submit the samples to NVSL. The importer of record can withdraw importation at any time. In this case, testing of the cohorts may proceed as described for each disease (Sections 6.B.1 – 6.B.3).
   a. If negative test results are received on the redrawn sample, the horse will be allowed entry into the United States.

   b. If non-negative test results are received, the importer of record, on behalf of the owner, may choose to withdraw importation or continue to pursue importation as described below.

2) Horses testing non-negative on the immediate rebleed may be held for fourteen (14) days from the initial blood collection, which serves as day zero (0). On day fourteen (14), horses must be retested for the disease of concern.

   a. If negative test results are received on the day fourteen (14) test sample, the horse is eligible for entry into the United States.

   b. If non-negative test results are received, and the importer of record continues to pursue importation, the horse may be held for an additional fourteen (14) days and retested.

3) Horses testing non-negative on day fourteen (14) may be held for an additional fourteen- (14-) day period and must be retested for the disease of concern.

   a. If negative test results are received on the day twenty-eight (28) test sample, the horse will be allowed entry into the United States.

b. If non-negative test results are received, the horse will be refused entry with no option for further testing.

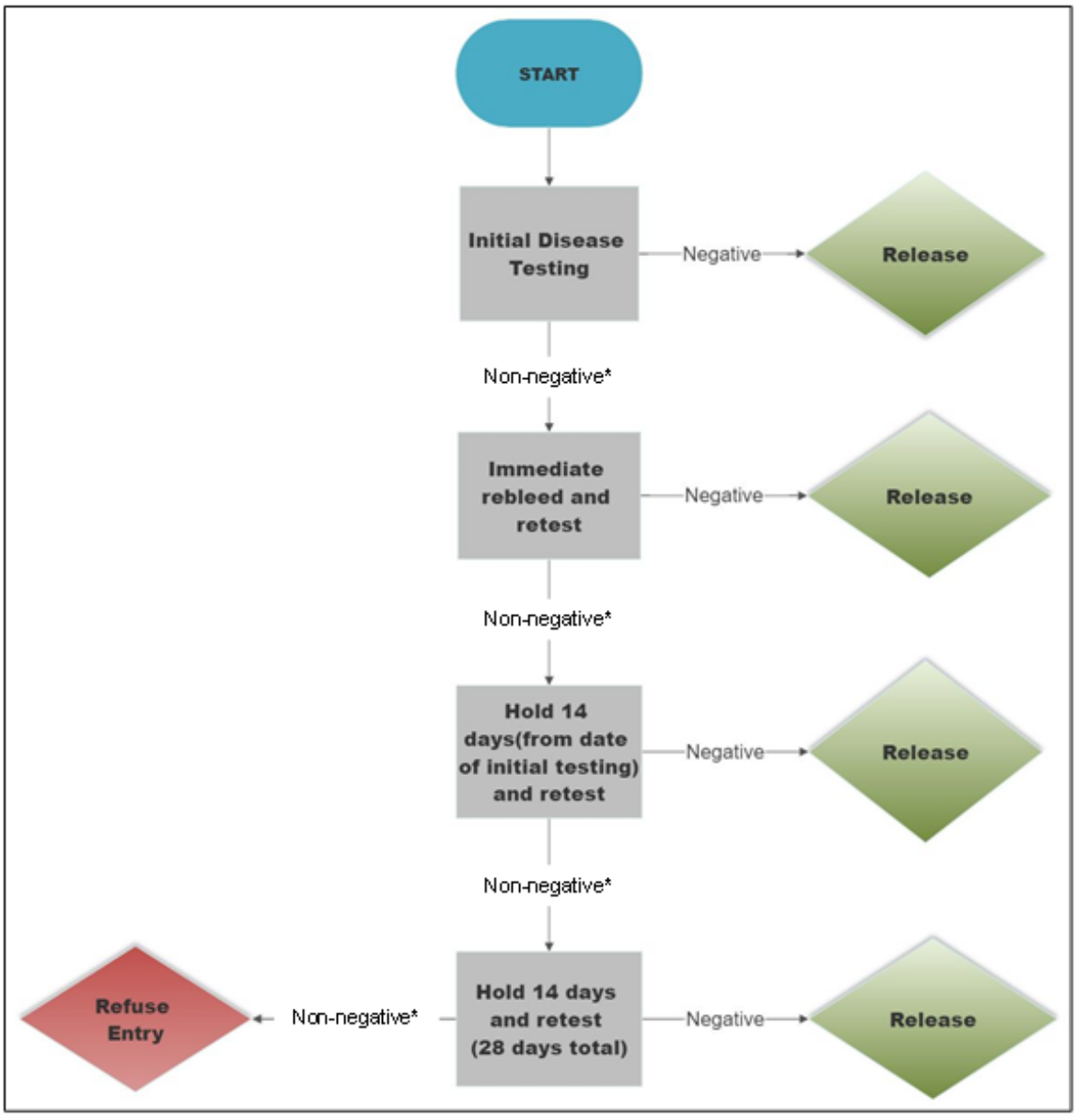
4) Refer to Figure 1.

7. Inquiries

Please send all inquiries to:

Strategy and Policy, Live Animal Imports
301-851-3300, select option 2
Email: LAIE@usda.gov.

Figure 1: Testing of Imported Equines for Glanders, Dourine and Piroplasmosis



* If non-negative after immediate rebleed, importer of record has discretion to withdraw importation with resulting refused entry or to hold for 14-day retest, provided that the facility can accommodate the request.
* If importer of record requests to hold for 14-day intervals, the date of initial blood collection (or day 14 rebleed) serves as day zero. Horses remaining non-negative after 28 days will be refused entry.
* VS will refuse the entire shipment if a glanders cohort horse tests positive on the 14-day rebleed.

Figure 2: Testing of Imported Equines for Equine Infectious Anemia