



VS Guidance 13407.2

Testing of Equidae During Import Quarantine

1. Purpose and Background

This document establishes guidelines for testing quarantined equidae to determine their import eligibility. These testing requirements apply to all equidae, including horses, donkeys, mules, asses, and zebras. This guidance uses the terms “equidae” 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 piroplasmiasis (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 VS, VS employees may not deviate from the directions provided herein without appropriate justification and supervisory concurrence.

2. Document Status

- A. Review date: 12/31/2023.
- B. This document replaces Veterinary Services Guidance 13407.1.

3. Reason for Reissuance

VS is reissuing this guidance to reflect changes in procedure and structure.



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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 \(OIE\) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: Chapters 3. 5. 3 Dourine; 3. 5.11 Glanders and melioidosis; 3. 5. 6 Equine infectious anemia; and 3. 5. 8 Equine piroplasmiasis.](#)
- [VS Guidance 7000.1, Tick Surveillance, Collection, and Submission of Suspected Exotic Ticks](#)
- VS Guidance 13424.1, 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 result 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) Confirmatory test: Test methods of high diagnostic specificity used to confirm results, usually positive results, derived from other test methods. Horses found negative on confirmatory testing will be considered negative and may be released.
- 3) Non-negative: Positive or suspect test results. VS bases interpretations on the appropriate OIE manual chapter. (OIE may describe suspect as “suspicious” or “doubtful.”)
- 4) Supplemental tests: Assays beyond the primary official assay. Supplemental tests are *only* performed for AC animals at the discretion of VS personnel.



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- 5) Retesting: Horses permitted to retest must be held fourteen (14) days after the initial blood collection and rebled for the disease of concern. The date of initial blood collection serves as day zero (0).

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, dourine, and glanders.
 - b. Agar gel immunodiffusion (AGID) test for equine infectious anemia (EIA).
 - c. Competitive enzyme-linked immunosorbent assay (cELISA) for EP.
- 2) Exceptions to the general testing requirements for imported horses:
 - a. Horses from Iceland are exempt from testing for EP, dourine, glanders, and EIA.
 - b. Horses from Australia and New Zealand are exempt from testing for dourine and glanders.
 - c. Horses 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 and/or isolation farm 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.
 - a. Cohorts vary for the disease:



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1. For dourine, only intact, mature animals (seven hundred and thirty-one (731) days or older) originating from the same premises are part of the cohort.¹
 2. For glanders and EIA, all animals in the shipment are cohorts.
 3. For EP, VS considers horses originating from the same premises and/or export isolation farm as cohorts.
- b. VS can consider any additional epidemiological information presented in addition to import testing to determine the import status of all horses in the shipment.
- 5) Submitters (VS personnel at the port) collect blood samples during the horse's first twenty-four (24) hours of arrival in quarantine, centrifuge them, and divide serum into two tubes from each animal in the shipment. VS packages tubes in a sealed container for shipment. VS personnel may ship samples by courier. Submit samples to the VS National Veterinary Services Laboratories (NVSL). The receiving NVSL laboratory checks that the sample seal is intact. If a horse routinely yields anti-complementary results, the responsible individual may notify the laboratory.
 - 6) If a horse tests non-negative on the initial blood collection, handlers isolate it from the other horses in the shipment. The sample collector and all handlers use appropriate personal protective equipment based on the disease of concern and the presence or absence of clinical signs (personnel should refer to their local/import center's biosafety guidance).
 - 7) The veterinary medical officer (VMO)/receiving officer at the port examines the animal and draws the samples; he or she 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 refused entry must be either exported out of the United States within ten (10) days of refusal notification or humanely euthanized.
 - 9) The laboratory performs supplemental tests when the results of the official CFT are anticomplementary. Supplemental tests include:
 - a. Indirect fluorescent antibody (IFA) or other supplemental tests for dourine and EP.

¹ Contact animals within the same shipment, imported from the same originating country.



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- b. ELISA for glanders.
 - c. VS has no supplemental test for EIA.
- 10) VS personnel may use other VS-approved assays as supplemental tests at their discretion.
- 11) VS personnel must perform confirmatory testing using the Western blot on all samples that test positive on the EP cELISA for *Babesia caballi*, except in cases where the sample is also positive on another EP test, in which case the horse would be refused entry.
- 12) The quarantine station VMO releases NVSL test results to importers, agents, or brokers. NVSL does not release any test results directly to imports, 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.



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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 anti-complementary, 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.
- c. Cohorts
1. Only intact, mature animals (seven hundred and thirty-one (731) days or older) originating from the same premises are part of the cohort.
 2. VS personnel will release castrated and immature equidae 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.
- 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 CFT is the official test for glanders. Positive CFT results are equal to or greater than 4+ at a 1:5 dilution. Suspect test results are 1+, 2+, and 3+ reactions at a 1:5 dilution.



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2. VS personnel should immediately redraw blood samples from any horses testing positive or suspect and follow procedures outlined in section 6.C. NVSL performs the official test on the initial and redrawn samples.
 3. When CFT results are anti-complementary, NVSL performs supplemental tests on the sample obtained from the initial blood collection. If supplemental tests are positive or suspect, the port VMO should immediately redraw blood samples and submit them to NVSL. NVSL performs official and supplemental tests on the redrawn sample.
- c. Cohorts
1. All animals in the shipment are cohorts.
 2. VS personnel hold cohorts for 14 days after the initial blood collection, then rebleed and retest all cohorts. VS personnel may release cohorts if all retest results are negative.
- 3) Equine piroplasmiasis (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 piroplasmiasis infections have a 1- to 3- week incubation period; iatrogenic piroplasmiasis 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.



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2. Results on the cELISA are only positive or negative. Samples are considered positive on cELISA if they exhibit a forty (40) percent inhibition or higher result for either organism that causes EP. There is no suspect range.
 3. Positive EP CFT results are equal to or greater than a 2+ reaction at a 1:5 serum dilution. There is no suspect result.
 4. Horses must be negative on both cELISA and CFT to be considered negative for EP.
 5. When CFT results are anti-complementary, 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. NVSL performs official and any needed supplemental tests on both the original and redrawn samples. Horses that test positive for *B. caballi* by cELISA, but which are found negative using the Western blot confirmatory test, will be considered as negative for *B. caballi* and may be released. The final interpretation including Western blot confirmatory testing will be included on the final NVSL report.
- c. Cohorts
1. Horses originating from the same premises and/or export isolation farm 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.
- d. Tick-infested horses
1. VS thoroughly inspects and treats all horses with an approved ectoparasite spray as outlined in VS Guidance 13424.1.
 2. Horses found to have attached ticks when examined may be refused entry.
 3. VS personnel must submit the ticks collected to NVSL as outlined in [VS Guidance 7000.1](#)



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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. 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. 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. NVSL performs official tests on the redrawn samples alongside the initial samples. The importer of record can withdraw importation at any time.
 - a. If negative test results are received on the redrawn sample, the horse will be allowed entry into the United States.



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- 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 where the horse may be held for fourteen (14) days and retested.
 - 2) Horses testing non-negative on the immediate rebleed may be held for fourteen (14) days from the initial blood collection. 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 will be allowed 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.

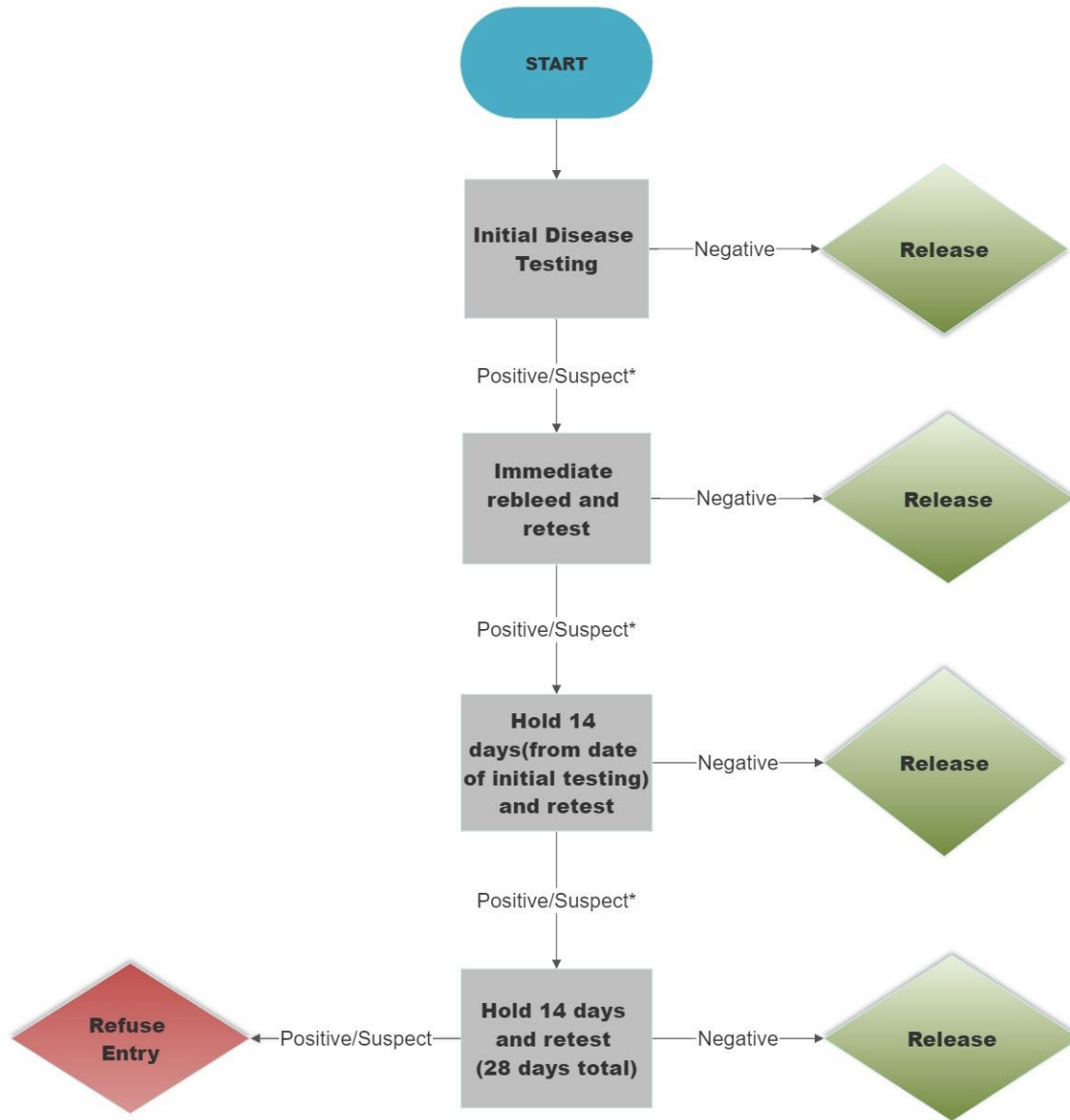
7. Inquiries

Please send all inquiries to:

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

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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 or to hold for 14-day retest, if the facility can accommodate the request. Withdrawn importation will result in refused entry. Horses remaining non-negative after 28 days will be refused entry.
- * If importer of record request to hold for 14-day intervals, the date of initial blood collection (or day 14 rebleed) serves as day zero.

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Figure 2: Testing of Imported Equines for Equine Infectious Anemia

