United States Department of Agriculture
Marketing and Regulatory Programs
Animal and Plant Health Inspection Service
Veterinary Services

JACK SHERE

Digitally signed by JACK SHERE
Date: 2018.01.26 14:38:34 -05'00'

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.

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 for antibodies to these four diseases’ causative agents 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. It 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. VS may make this information available to the public. While this document provides guidance for users outside Veterinary Services (VS), VS employees may not deviate from the directions provided herein without appropriate justification and supervisory concurrence.

2. Document Status

A. Review date: January 5, 2021.

B. This guidance, which VS has updated to include tests currently in use, cancels and replaces VS Memorandum No. 591.58, dated Aug. 22, 2005.

3. Reason for Reissuance

Not applicable.

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 2.05.03 Dourine; 2.05.11 Glanders; 2.05.06 EIA; and 2.05.08 Equine piroplasmosis.
- VS Guidance 13405, "Importation of Horses from Mexico using Temporary APHIS-Approved and -Operated Quarantine Facilities at Land Border Ports."
- VS Memorandum 556.13, "Tick Surveillance, Collection, and Submission of Suspected Exotic Ticks."

C. Definitions:

1) Anti-complementary: Non-specific consumption of complement; indeterminate result. 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.

3) Non-negative: Positive, suspect, or anti-complementary test results.

4) Supplemental and additional tests: Assays beyond the primary official assay, conducted at VS discretion to provide additional information concerning an animal's serological status.

5) Suspect: An intermediate test result. A suspect test result may require submission of additional specimens and/or additional testing on the current specimen. VS will treat the suspect result as a positive result until it can make a clear determination. VS bases interpretations on the appropriate OIE manual chapter.

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 dourine and glanders.

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

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

2) Exceptions to the general 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.

   d. Canadian animal health officials will test horses from Canada in Canada with either the AGID test or the ELISA for EIA before entering 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 in the same shipment. 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 contact animals in the shipment.

   a. Contact animals vary for the disease:

      1. For dourine, only intact, mature animals (731 days or older) originating from the same premises are considered contact animals in the shipment.

      2. For glanders and EIA, VS considers all animals in the shipment contact animals.

      3. For EP, VS considers horses originating from the same premises and/or export isolation farm contact animals.

   b. VS can consider any additional epidemiological information presented to determine the status of the horses in the shipment.

5) Submitters (VS personnel at the port) collect blood samples, 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 appropriate section of the National Veterinary Services Laboratories (NVSL) (bacteriology, virology, etc.); the receiving NVSL lab will check that the sample seal is intact. If a horse routinely yields anti-complementary results, the owner may so notify the laboratory. The laboratory can then expedite supplementary testing.

6) If a horse tests positive on the initial blood collection, handlers will isolate it from the other horses in the shipment. The sample collector and all handlers will 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; 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).

8) As noted above, the laboratory may perform supplemental tests when the results of the official tests are non-negative. VS has no supplemental test for EIA. Supplemental tests include:

   a. Indirect fluorescent antibody (IFA) or other supplemental tests for dourine (on anti-complementary samples only).

   b. cELISA for glanders (on anti-complementary samples only).

   c. Western blot for glanders.

   d. IFA tests for EP (on anti-complementary samples only).

9) VS personnel may use other VS-approved assays as supplemental tests.

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) NVSL does not release any test results directly to importers, agents, or brokers. The quarantine station VMO releases NVSL test results to importers, agents, or brokers.

12) The quarantine station VMO advises the National Import-Export Services (NIES), Equine Imports veterinary staff officer of non-negative quarantine test results.

B. Specific Information and Procedures For Each Disease

1) Dourine

a. General information

Dourine is a venereal disease of horses transmitted by sexual contact and caused by the protozoan *Trypanosoma equiperdum*. The organism is 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.

b. Testing

1. The CFT is the official test for dourine. Positive CFT results are equal to or greater than a 4+ reaction at a 1:5 dilution. Suspect test results are 1+, 2+, and 3+ reactions at a 1:5 dilution.

2. VS personnel should immediately redraw blood samples from any horses testing positive or suspect on CFT and submit the samples to NVSL. NVSL will perform official tests on the redrawn sample.

3. When CFT results are anti-complementary, NVSL will immediately perform supplemental tests. If supplemental tests are positive or suspect, the port VMO should redraw blood samples immediately and submit to NVSL. NVSL will perform official and supplemental tests on the redrawn sample. See Figure 1.

c. Contact animals

1. Only intact, mature animals (731 days or older) originating from the same premises are considered contact animals in the shipment.

2. VS personnel will hold contact animals for 15 days after the initial blood collection then will rebleed and retest the animals. VS personnel may release contact animals if the retest results are negative.

3. VS personnel will release castrated and immature equidae if they had negative test results on the initial serum collection.

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 2 weeks, but varies from 2 days to several months. 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 95 percent mortality rate in untreated cases. Infections with other bacteria (e.g. *B. pseudomallei*) may cause cross-reactions with *B. mallei* serologic 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.

2. VS personnel should immediately redraw blood samples from any horses testing positive or suspect on CFT and submit to NVSL. NVSL will perform official and supplemental tests on both the original and redrawn samples.

3. When CFT results are anti-complementary, NVSL will immediately perform supplemental tests. If supplemental tests are positive or suspect, the port VMO should immediately redraw blood samples and submit them to NVSL. NVSL will perform official and supplemental tests on the redrawn sample. See Figure 2.

c. Contact animals

1. VS considers all animals in the shipment contact animals.

2. VS personnel will hold contact animals for 15 days after the initial blood collection then will rebleed and retest the animals. VS personnel may release contact animals if the retest results are negative.

3) 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 1 to 3 weeks, but can be as long as 3 months.

b. Testing

1. The official test for EIA is the AGID (Coggins) test.

2. The port VMO should immediately redraw and resubmit blood samples to NVSL for any horses testing positive on the initial AGID test. VS should immediately resample horses that test negative on redrawn samples and the samples submitted to NVSL for testing. VS will only release horses that are negative to two sequential subsequent tests after an initial positive test. See Figure 3.

c. Contact animals

1. VS considers all animals in the shipment contact animals.

2. VS refuses entry to contact animals without retesting. Owners may present contact animals for entry retesting after 45 days of isolation from EIA-positive horses after VS reviews further epidemiological information.

4) 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 1- to 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 cELISA is more likely to detect chronic asymptomatic carriers; the CFT is more likely to detect early infections.

2. Results on the cELISA are only positive or negative. There is no suspect range. The final report will include NVSL's interpretation.

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. VS will classify animals negative on both tests as EP negative and will allow the animals into the United States if all other required testing is negative.

5. When CFT results are anti-complementary, NVSL will immediately perform supplemental tests. If supplemental tests are positive or suspect, the port VMO should immediately redraw and resubmit blood samples to NVSL. NVSL will perform official and supplemental tests on the redrawn sample. See Figure 4.

6. VS will immediately re-sample animals with positive results on either test at the import facility for testing at NVSL if the owner continues to pursue importation. NVSL will perform 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.

7. Other than horses referenced in B) 4) b.5., VS should not release horses testing positive or suspect for EP on any rebleed samples by official or supplemental tests until VS completes additional review and consultation to adequately determine the horse's EP status. See Figure 4.

c. Contact animals

1. VS considers horses originating from the same premises and/or export isolation farm contact animals 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 syringes by personnel accompanying the horses on the aircraft) when determining contact animals.

3. VS personnel will hold contact animals for 15 days after the initial blood collection then rebleed and retest the animals using the cELISA and CFT. VS personnel may release contact animals if the retest results are negative.

d. Tick-infested horses

1. VS will treat all horses with an approved ectoparasite spray.

2. Horses found to have attached ticks when examined may have further action taken according to VS Guidance 13405.1.

3. VS personnel must submit the ticks collected to NVSL as outlined in VS Memorandum 556.13.

7. Inquiries

Please send all inquiries to:

National Import-Export Services, Live Animal Imports
301-851-3300, select option 2
Email: VS.Live.Animal.Import.Export@aphis.usda.gov.

Figure 1 Testing of Imported Equines for Dourine

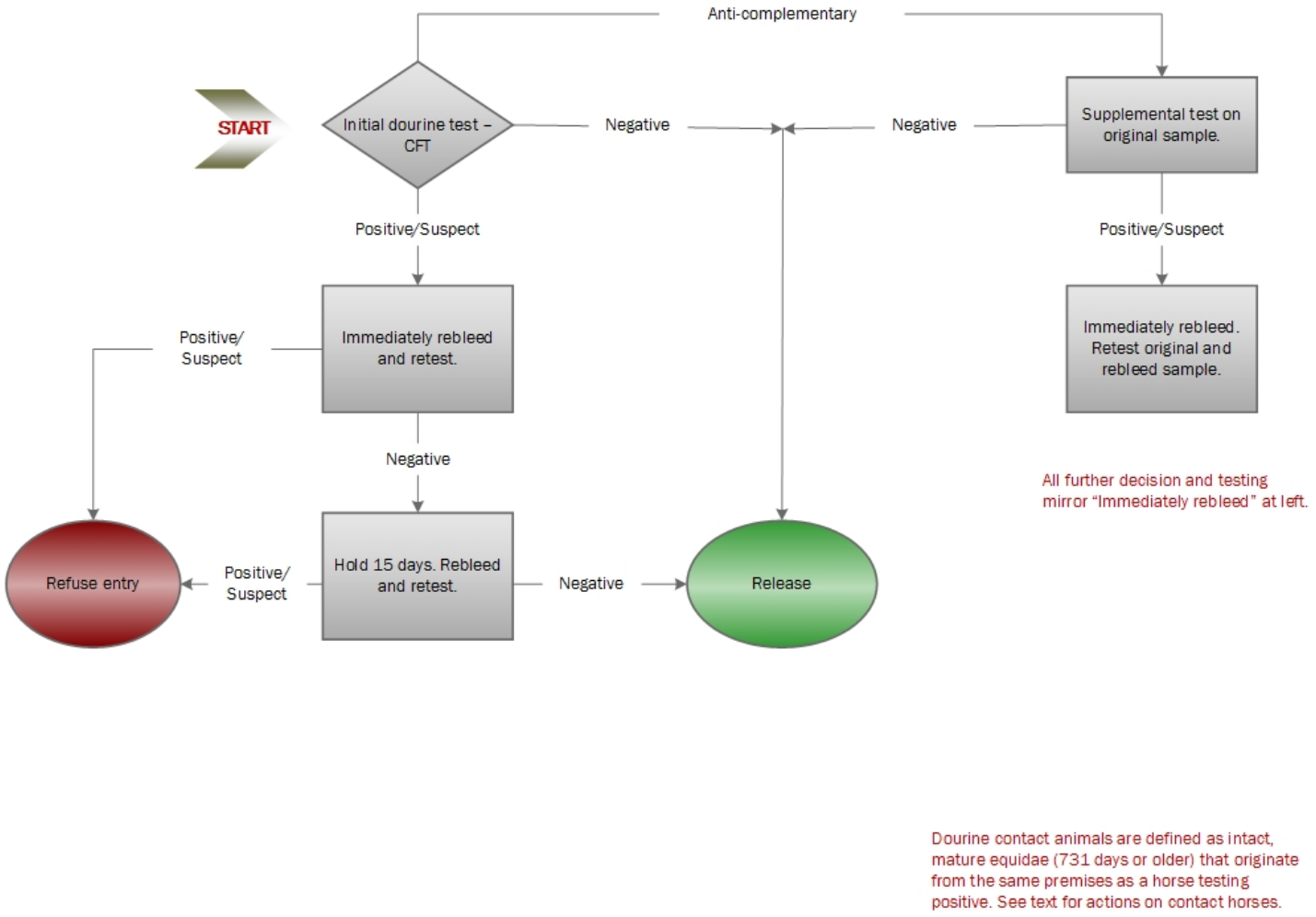

Figure 2 Testing of Imported Equines for Glanders

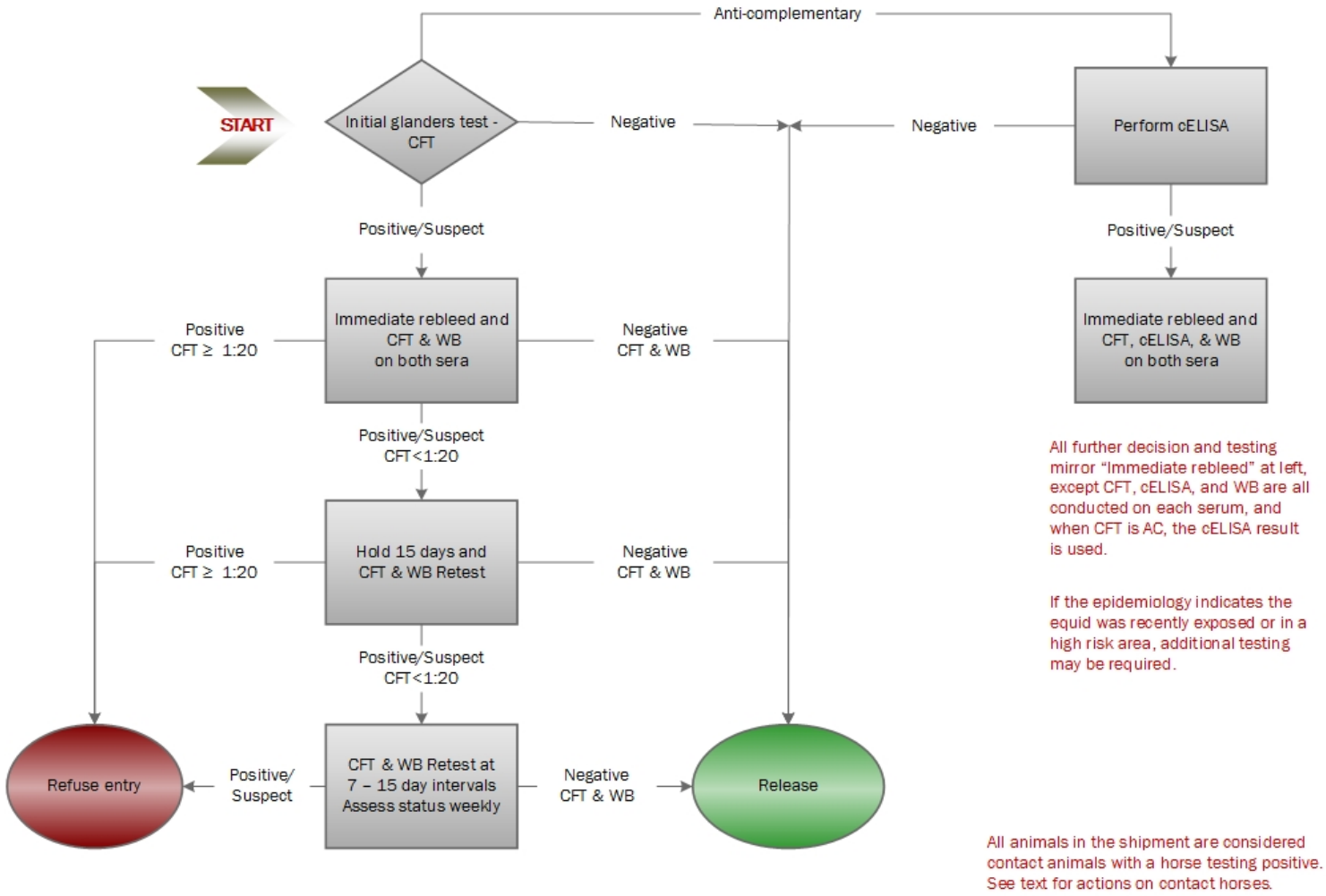

Figure 3 Testing of Imported Equines for Equine Infectious Anemia

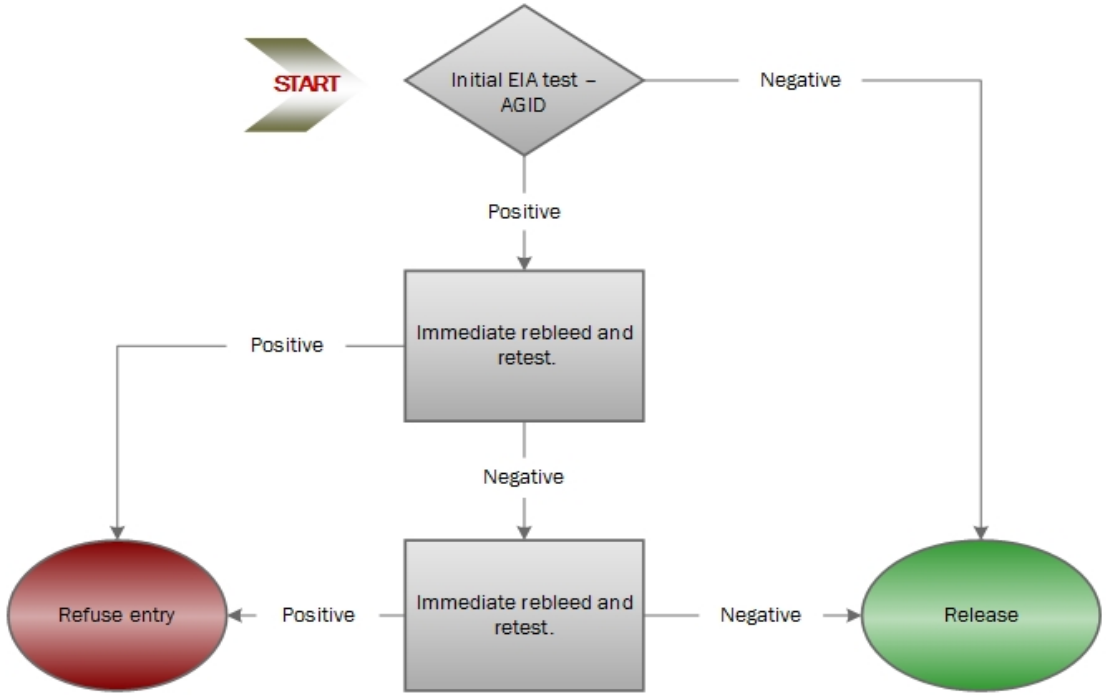

Figure 4. Testing of Imported Equines for Equine Piroplasmosis