

---

## **Contagious Equine Metritis (CEM) Testing for Imported Horses at Approved Quarantine Facilities**

### **1. Purpose and Background**

This document outlines procedures for testing certain imported horses for CEM after arrival at approved testing facilities in the United States. Criteria for approval of CEM quarantine facilities are also provided.

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. The information it contains may be made 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. Valid through 02/13/2015

B. This document replaces Veterinary Services Memoranda No 558.3 and 558.4, which are cancelled.

### **3. Reason for Reissuance**

Not applicable.

### **4. Authority and References**

A. Authorities (*Code of Federal Regulations (CFR)*):  
7 CFR 371.4  
9 CFR 93.300  
9 CFR 93.301

B. Definitions:  
Horses: Horses, asses, mules, and zebras.

### **5. Audience**

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

**6. Guidance**

**A. General**

Section 93.301 in Title 9 of the *Code of Federal Regulations* (9 CFR) contains provisions for the importation of horses for permanent entry from regions that the Animal and Plant Health Inspection Service (APHIS) considers to be affected with CEM. After completion of Federal import quarantine, sexually intact horses over 731 days of age imported from CEM-affected regions must undergo a subsequent quarantine in participating States, where they are further tested for CEM at an approved facility supervised by State or Federal authorities prior to release for entry to the United States.

CEM is a venereal disease of horses caused by *Taylorella equigenitalis*, a Gram-negative coccobacillus. *T. equigenitalis* is a fastidious organism, and adherence to sampling protocols is necessary to reduce the risk of false negative results. Testing is done through a series of bacterial cultures, using samples obtained from various anatomical sites depending on gender, and by serological testing of mares using a complement fixation (CF) test for CEM antibodies. Cultures must be placed in Amies transport media with charcoal and shipped refrigerated (see section I for details). Samples must be received at the National Veterinary Services Laboratories (NVSL), or an APHIS approved CEM laboratory, and plated within 48 hours of collection.

An accredited veterinarian, a State veterinarian, or an APHIS veterinarian must perform the sample collection, test breeding, and treatments. The accredited veterinarian must be monitored by a State Animal Health Official (SAHO) or APHIS official. However, the SAHO or APHIS official may elect to monitor only the initial treatment when the series of treatments is performed by an experienced accredited veterinarian. An APHIS official or SAHO must be present during all test breeding.

To avoid false negative culture results, horses may not be treated with systemic antibiotics while undergoing *T. equigenitalis* testing. If a horse requires systemic antibiotic treatment, a minimum of 7 days must elapse between the last systemic treatment and an initial CEM culture. If a horse receives topical antibiotic treatment of the external genitalia, a minimum of 21 days must elapse between the last topical treatment and an initial CEM culture test.

**B. Standards for Approving CEM Testing Facilities**

The following are minimum facility requirements which States must use to approve premises to conduct quarantine and treatment of imported horses for CEM. Individual States may institute additional standards for approval and inspection of farms.

- 1) A proposed quarantine facility must be inspected and approved by State or Federal personnel prior to horses being placed in CEM quarantine. The facility must be inspected and re-approved at least annually.
- 2) The facility must be an enclosed building of sound permanent construction, capable of being effectively cleaned and disinfected.
- 3) The quarantine area shall be clearly identified by posted signs.
- 4) The facility must be large enough to accommodate all animals involved in the quarantine.
- 5) The facility must contain stalls where individual horses can be kept separate from each other by an empty stall, an empty area where horses cannot touch each other, or by a solid wall that is at least 8 feet (2.4 m) high. The facility must provide isolation from other horses and common traffic. Nose-to-nose contact may not occur.
- 6) The facility must be secure so that horses may not escape or be removed from the facility without authorization and to prevent other animals or unauthorized personnel from entering.
- 7) Drainage from the facility shall not flow into areas where other horses are being maintained on the premises.
- 8) During the quarantine period, all equipment used for feeding, watering, grooming, and cleaning must remain in the quarantine area and be limited only to the animals in quarantine.
- 9) After each culture or treatment, all gloves, sleeves, speculums, and other disposable material will be placed in plastic garbage bags and disposed of as regulated garbage through incineration, burial, or composting. These materials may alternatively be stored until the quarantine is released (and providing that all culture results are negative), when they may be disposed of as nonregulated garbage.
- 10) The facility shall be available to accredited attending veterinarians and State or APHIS personnel at any time necessary to conduct required testing or quarantine observation procedures.
- 11) Visitors and pets will not be permitted into the quarantine area unless authorized by State or APHIS personnel.
- 12) All horses under CEM quarantine must remain in the facility until released by a SAHO.

**C. Quarantine and Biosecurity for Imported Horses**

- 1) Horses must be separated at all times; see B5 for details. Horses may be ridden, exercised, or turned out during CEM quarantine provided horses undergoing CEM testing do not have physical contact with any other horses. Grooming and cleaning supplies and tack must not be shared.
- 2) Horses under quarantine must not be bred, collected, or subjected to genital examination or cleaning beyond what is required for CEM testing as detailed below.
- 3) *T. equigenitalis* may be transmitted by fomites, especially contaminated reproductive equipment and contaminated artificial insemination equipment. Separate buckets, brushes, sponges, tack, etc., should be used for each horse under quarantine. All reusable equipment must be cleaned and disinfected between quarantines.
- 4) If an imported mare foals or aborts prior to completing CEM testing, placental tissue, fetal membranes and amniotic fluid should be considered a potential source of CEM transmission. These materials, along with soiled bedding and any other potentially contaminated materials from foaling, should be properly disposed of by burial, incineration, or composting to minimize any risk of *T. equigenitalis* transmission. Otherwise-soiled bedding and manure from the quarantine do not pose a risk for infection and can be disposed of normally.
- 5) Any horse found to be positive for *T. equigenitalis* on any of the testing performed (serology or culture) must remain in quarantine for additional treatment and testing until the horse is determined to not be infected with *T. equigenitalis*.

**D. Procedures for CEM Testing of Imported Stallions**

- 1) Culture the imported stallion
  - a. Collect one set of culture specimens from each stallion.
  - b. Culture the shaft of penis and prepuce, urethral sinus, fossa glandis, and distal urethra using a separate swab for each of these four sites. Swab sites may be cleaned with cotton and plain water if necessary to remove excess organic debris. Do not scrub or use disinfectant or detergent cleaners prior to swabbing.
  - c. If a stallion is determined to be positive at any time during the process, suspend further testing and initiate treatment (see section E). For example, if a stallion's cultures are positive, begin treatment without test breeding mares.

- 2) Test breed the imported stallion
  - a. Cleaning of the external genitalia should not take place prior to or during testing, since cleaning may lead to false negative culture results.
  - b. Do not begin test breeding until negative culture results have been obtained.
  - c. Do not scrub or use disinfectant or detergent cleaners on the test mare or the stallion prior to breeding.
  - d. Test breeding consists of live cover of two qualified mares (see section F). The goal is to maximize contact time between the external genitalia of the stallion and the mare. At a minimum, each test mare must experience at least two complete penetrations from the stallion, one of which must result in ejaculation. Complete penetration means full insertion of the shaft of the penis into the vaginal vault of the mare.
- 3) Treat the imported stallion
  - a. Do not begin treating the stallion until negative results from pre-breeding cultures are returned and the stallion has successfully completed test breeding of two qualified test mares.
  - b. After both test breedings are completed, the stallion's prepuce, penis, fossa glandis, and urethral sinus must be thoroughly cleaned with no less than 2 percent chlorhexidine scrub while the stallion is in full erection once a day for 5 consecutive days. After each cleaning, coat the penis, prepuce, and sheath with an antibiotic ointment with activity against *T. equigenitalis*, such as silver sulfadiazine or 0.2 percent nitrofurazone, once a day for 5 consecutive days.
  - c. Systemic antibiotics may be used in conjunction with topical treatment at the discretion of the attending accredited or regulatory veterinarian.
- 4) Quarantine release of CEM-negative imported stallions

If the stallion is negative on the prebreeding culture set, if postbreeding diagnostic tests are negative on the two qualified test mares (CF test and all post-breeding cultures; see section F), and if the stallion has completed treatment, then the stallion may be released from quarantine.

E. Procedures for Stallions Positive for *T. equigenitalis*

- 1) A stallion is positive for *T. equigenitalis* if positive results are returned on any of the assays performed: culturing of stallion, post breeding cultures of test mares, or post breeding CF testing of test mares. An infected stallion must remain in quarantine until the stallion is treated, retested, and determined to no longer be infected with *T. equigenitalis*.

- 2) If a stallion is determined to be positive for *T. equigenitalis*, then the stallion's prepuce, penis, fossa glandis, and urethral sinus must be thoroughly cleaned with 2 percent or stronger chlorhexidine scrub while the stallion is in full erection. After cleaning, coat the entire penis and prepuce with an antibiotic ointment with activity against *T. equigenitalis*, such as silver sulfadiazine or 0.2 percent nitrofurazone. This procedure must be repeated once a day for 5 consecutive days.
- 3) If the stallion is found to be positive based on test mare results and has already been scrubbed and treated immediately following breeding, then E1 above may be completed. However, repeat CEM culturing of the stallion may not be resumed until 21 days have elapsed since the final scrubbing and treatment.
- 4) The attending accredited veterinarian, stallion owner(s), and SAHO may also elect to treat the infected stallion with systemic antibiotics, but it is not required.
- 5) Following treatment, the stallion will then be retested by culture (using a separate swab for each site) the prepuce, urethral sinus, fossa glandis and the distal urethra, beginning no less than 21 days after the last day of topical antibiotic treatment, and no less than 7 days after systemic antibiotic treatment. If cultures are negative, the stallion should then be test bred to two mares, as per section D2, "Test Breed the Stallion."
- 6) Repeat the culture, test mare breeding, and treatment process for any positive stallions until negative on all pre-breeding and test mare breeding cultures.

**F. Procedures for Test Mares**

- 1) Qualify the test mares
  - a. Test mares must be permanently identified by hot branding, freeze branding, or tattooing with a capital letter "T", as applied by APHIS personnel, State authorities, or accredited veterinarians. Brands must be applied on the left shoulder or left neck area and be at least two inches high (lip tattoos must be applied to the inside surface of the upper lip and be at least 1 inch high and 0.75 inches wide).
  - b. Test mares must be tested and determined to be negative for CEM on culture prior to use.
    1. Culture the clitoral sinuses and clitoral fossa three separate times during a 12 day period, with at least 72 hours between sets of cultures. The third set of cultures must include a distal cervix or endometrium swab. Use one swab for the two sinuses and separate swabs for the clitoral fossa and the distal cervix or endometrium. Use dry clean-up or minimal water if necessary to remove excess organic debris. Do not scrub or use disinfectant or detergent prior to clitoral sinus or fossa swabs being taken.

If the distal cervix or endometrial swab is taken with the final set of swabs, it is acceptable to clean the external genitalia after swabbing the clitoral sinuses and clitoral fossa and before swabbing the distal cervix or endometrium.

2. Test mares must be negative for CEM by CF testing prior to use.

2) Post-breeding testing of qualified test mares

a. Counting the test breeding as day zero, culture the qualified test mares three separate times during a 12 day period beginning on day 3 post-breeding, with at least 72 hours between culture sets.

1. Culture the clitoral sinuses, clitoral fossa, and with the third set of swabs, the distal cervix or endometrium of each test mare. Use one swab for the two sinuses and separate swabs for the clitoral fossa and the cervix or endometrium.

2. Use dry clean-up or minimal water if necessary to remove excess organic debris. Do not scrub or use disinfectant or detergent prior to clitoral sinus or fossa swabs being taken.

3. After collecting the third set of swabs from the clitoral sinuses and fossa, the external genitalia can be scrubbed before swabbing the distal cervix or endometrium.

b. Between day 21 and day 28 post-breeding of the test mare, submit a serum sample to NVSL for CF testing.

3) Quarantine release of test mares

a. Test mares must remain in quarantine until negative results are known for all tests for both test mares.

b. If a test mare is positive on a CF test or culture, then that test mare shall not be used as a test mare ever again.

c. If a test mare is positive on CF test or culture as a result of post-breeding testing, then both test mares associated with the test breeding shall be treated in accordance with section H, "Procedures for Imported Mares Positive for *T. equigenitalis*" and re-cultured in accordance with section G, "Procedures for Imported Mares"

d. Negative post breeding cultures and CF tests on a test mare can serve as the qualifying tests for the test mare to be used again, provided the mare remains in the CEM testing facility.

**G. Procedures for Imported Mares**

- 1) Collect a blood sample and submit to NVSL for CF testing.
- 2) Culture the mare.
  - a. Culture the clitoral sinuses and clitoral fossa three separate times during a 12 day period, with at least 72 hours between culture sets.
  - b. For nonpregnant mares, include a swab of the distal cervix or endometrium with the third set. Use one swab for the two sinuses and separate swabs for the clitoral fossa and the cervix or endometrium. Pregnant mares do not need to have the distal cervix or endometrium cultured.
  - c. Use dry clean-up or minimal water if necessary to remove excess organic debris. Do not scrub or use disinfectant or detergent prior to clitoral sinus or fossa swabs being taken. After collecting the third set of swabs from the clitoral sinuses and fossa, the external genitalia can be scrubbed before swabbing the distal cervix or endometrium.
  - d. Do not begin treatment until culture results are returned. If a laboratory reports that a culture cannot be used—for example, if there is overgrowth on the plate or a sample does not arrive at the lab within 48 hours—then the mare will need to be recultured. If treatment has already begun, then 21 days must elapse from the end of treatment until the mare can be recultured.
- 3) Treat the mare for 5 consecutive days. On day 1 of treatment:
  - a. Infuse the clitoral sinuses with a small volume of a ceruminolytic agent (e.g., 5.0-10.0 ml of Cerumene [squalene 2.5 percent]) using either a curved tip 12-cc syringe or a syringe attached to a disposable 1-1/3" plastic teat infusion cannula, or bathe the clitoral area with the ceruminolytic agent and infuse the sinuses using digital manipulation. The amount of ceruminolytic agent required will be determined by the difficulty encountered in softening any smegma or other debris sufficiently so that it can be removed as described below.
  - b. Manually express the smegma ("bean") from the central sinus of the clitoris by grasping the clitoris between the thumb and forefinger and gently squeezing, while simultaneously pulling down and out. This method should displace the frenulum sufficiently to allow access to the central sinus. The bean, if present, will be dislodged and removed.
  - c. Flush the sinuses with a small volume (e.g., 5.0-10.0 ml) of 2 percent or greater chlorhexidine scrub to remove all residual smegma and ceruminolytic agent.



- d. Following flushing, clean and scrub the clitoral sinuses and clitoral fossa area using 2 percent or greater chlorhexidine scrub. Rinse the areas that were scrubbed.
  - e. After cleaning and rinsing, infuse the sinuses with an antibiotic ointment effective against *T. equigenitalis*, such as silver sulfadiazine or 0.2 percent nitrofurazone. Infusion can be accomplished by digital manipulation, working the ointment into the sinuses by hand.
  - f. After infusing the sinuses, coat the entire clitoral area, sinuses, and fossa with the antibiotic ointment.
- 4) On days 2 through 5 of treatment:
- a. Clean and scrub the clitoral sinuses and clitoral fossa area using not less than 2 percent chlorhexidine scrub. Rinse the areas that were scrubbed.
  - b. After cleaning and rinsing, infuse the sinuses with an antibiotic ointment designated as effective against *T. equigenitalis*, such as silver sulfadiazine or 0.2 percent nitrofurazone. Infusion can be accomplished by digital manipulation, working the ointment into the sinuses by hand.
  - c. After infusing the sinuses, coat the entire clitoral area, sinuses, and fossa with the antibiotic ointment.
- 5) Systemic and/or intrauterine antibiotics are not required, but may be used in conjunction with topical treatment at the discretion of the attending accredited veterinarian in consultation with regulatory officials.
- 6) If all cultures are negative, the mare may be released from quarantine and no further treatment is required. If any culture results are positive, the mare must be treated and re-cultured according to section H before release.
- H. Procedure for Imported Mares Positive for *T. equigenitalis*
- 1) A mare is considered positive when the CF test or culture results are positive.
  - 2) Treat the mare as described in sections G3 and G4.
  - 3) No sooner than 21 days after the last topical or intrauterine treatment and 7 days after the last systemic treatment (if used), repeat culture sets on the mare.
  - 4) If all culture sets are negative, repeat the treatment steps described in sections G3 and G4. The mare may be released following this treatment.

**I. Sample Collection and Submission**

- 1) All initial samples are to be sent to a National Animal Health Laboratory Network (NAHLN) laboratory approved to conduct CEM testing or to NVSL. All suspect samples must be confirmed at NVSL. A list of laboratories approved by APHIS to process CEM cultures can be found at [http://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/downloads/ApprovedLabs\\_CEM.pdf](http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/ApprovedLabs_CEM.pdf)
- 2) Swabs must be placed in Amies transport medium with charcoal (each swab in a single tube) and refrigerated.
- 3) Approved CEM laboratories will send a swab of suspect colonies to NVSL in Amies media with charcoal. If suspect colonies are noted on multiple anatomical locations from the same set of swabs, send a suspect colony from each location in individual Amies media. The original swabs do not need to be sent to NVSL.
- 4) The swabs that come in the Amies tubes are adequate for collection of stallion samples and clitoral fossa samples from mares, but are too large for clitoral sinuses of mares. Cut the large swab off, swab the sinuses with a small tipped swab, put the swab(s) in the Amies, and replace the cap.
- 5) Specimens must be submitted with the name, address, and phone/fax number of the submitting veterinarian, location of horse, complete animal identification, anatomical location sampled, and date and time of collection.
- 6) Swabs must be shipped with ice packs by an overnight service provider as identified by the VS Area or Regional Office (or State CEM Coordinator, as applicable) to the APHIS approved CEM diagnostic laboratory. Specimens shipped to NVSL should be addressed to: National Veterinary Services Laboratories, Diagnostic Bacteriology Laboratory (DBL), 1800 Dayton Ave., Ames, IA 50010.
- 7) All samples must reach the lab and be plated within 48 hours after collection. If the arrival of swabs is delayed beyond 48 hours after submission, the cultures must be repeated.
- 8) Incubation for *T. equigenitalis* takes a minimum of 7 days.

**J. Communications**

- 1) Each State participating in CEM testing of imported horses will identify a State CEM coordinator. The coordinator may be a State or APHIS employee. A National CEM Coordinator will be designated by VS. Updates and other communications concerning CEM import testing will be relayed by the National CEM coordinator, through the VS Regional Import-Export Coordinators (RIECs), to State CEM coordinators.

- 2) Identification and treatment records at CEM quarantine facilities are to be retained by the Federal or State official monitoring the testing and treatment. Copies are to be sent to the State CEM coordinator (unless the State coordinator is a VS employee; in that case, a copy should be sent to the appropriate SAHO) and APHIS Area Office by mail, fax, or electronically. The State CEM coordinator and APHIS Area Office should retain records for a minimum of 7 years.
- 3) If a horse tests positive for CEM while in quarantine, the State CEM coordinator should notify the appropriate VS RIEC. The RIEC will inform the National CEM Coordinator.

**K. Recordkeeping and CEM Database**

- 1) A CEM Quarantine Tracking Spreadsheet will be used to capture testing and other information pertinent to susceptible stallions and mares imported from CEM-affected countries while those horses are in CEM quarantine facilities. The spreadsheet will also be used to record test mare information. The most recent version of the Quarantine Tracking Spreadsheet will be maintained on the APHIS SharePoint site at the following link: <http://animalhealth/equine/CEM/default.aspx>. Individuals without access to the APHIS SharePoint site should obtain a copy of the spreadsheet from the National or Regional CEM coordinators.
- 2) The RIECs will provide the spreadsheet to the State CEM Coordinators. The State CEM coordinators should enter information for imported stallions, imported mares, and test mares into the spreadsheet per the instructions.
- 3) The State CEM coordinator should forward the spreadsheet on a quarterly basis to the National CEM Coordinator at NCIE (copy the RIEC) for entry into a national CEM database.
- 4) Requests for information regarding the national CEM database should be made to the National CEM Coordinator.

**7. Inquiries**

For inquiries, contact the National Center for Import and Export at (301) 851-3300, menu option 2.



John R. Clifford  
Deputy Administrator