

USDA APHIS VETERINARY SERVICES

FY 2010 CONTAGIOUS EQUINE METRITIS NATIONAL TESTING PROJECT

TESTING PROTOCOL FOR ACTIVE BREEDING STALLIONS

January 2010

I. General Information

The purpose of this protocol is to provide guidelines for testing active breeding stallions as part of the FY 2010 Contagious Equine Metritis (CEM) National Testing Project. CEM is a venereal disease of horses caused by *Taylorella equigenitalis*.

Beginning in February 2010, swab samples will be collected from 3,000 active breeding stallions from around the United States and tested for *T. equigenitalis*. Active breeding stallions are considered to be those that had semen collected, or were bred to a mare by live cover, in 2008 or 2009.

Specific sampling targets have been set for each State in order to produce the overall national sampling total of 3,000 breeding stallions. The number of stallions to be sampled from each State is based on APHIS National Animal Health Monitoring System estimates of breeding stallion populations in each State and on the number of equine practitioners indicating a reproduction focus to their practice in each State as compiled in the directory of the American Association of Equine Practitioners (see Table 1 for State sampling targets). State of sampling is considered to be the State in which the stallion is standing at the time of sampling, regardless of the usual State of residence of the stallion or its owner.

Enrollment of stallions in the national testing will be voluntary in all cases; for information on the potential benefits of enrollment and how to volunteer, see section VI, Enrollment. Sample collection will be performed by an accredited equine veterinarian. Stallion owners are required to pay the accredited equine practitioner for sample collection costs.

No quarantine restrictions will need to be imposed at the time of sample collection or while test results are pending. If a stallion is found positive, the positive stallion must be placed under State quarantine or hold order until he is treated, retested, and determined to no longer be infected with *T. equigenitalis*. APHIS will pay all costs associated with the necessary treatment and retesting procedures of stallions that test positive in this national testing project.

No Federal or State regulatory oversight of sample collection is required. However, any State animal health authority that desires to have oversight can make it a requirement. Extra costs incurred from that oversight will be at State expense.

Sampling will consist of one set of swabs for culture. One set includes three swabs: one swab collected from the fossa glandis, one from the urethral sinus, and one from the distal urethra. No post-testing antibiotic treatment is required (unless the stallion is found positive).

Diagnostic testing will be performed only at APHIS-approved CEM laboratories or at the National Veterinary Services Laboratories (NVSL). All suspect positive cultures must be sent to the NVSL for confirmation.

APHIS will pay the laboratory diagnostic testing costs for one set of swabs, including shipment of samples to an approved CEM laboratory or to the NVSL. Collection, shipping, and testing of any additional swab sets are at the stallion owner's discretion and expense.

T. equigenitalis is a fastidious gram-negative coccobacillus. Adherence to sampling and submission protocols is necessary to reduce the risk of false negative results. Cultures must be placed in Amies transport media with charcoal and shipped refrigerated (e.g., with ice packs). Samples must be received and plated at an APHIS-approved CEM laboratory or at the NVSL within 48 hours of collection.

Stallions should not be treated with antibiotics while undergoing *T. equigenitalis* testing. If a stallion requires systemic antibiotic treatment, then a minimum of 7 days must elapse between the last systemic treatment and any sample collection. If a stallion requires topical treatment of the penis or prepuce with an antiseptic, a disinfectant, or an antibiotic, then a minimum of 21 days must elapse between the last topical treatment and the collection of a sample for *T. equigenitalis* culture.

No cleaning of the external genitalia should take place prior to testing except as described in Section III of this document, since cleaning of the external genitalia prior to or during culturing can lead to false negative results.

II. Eligibility of Active Breeding Stallions for Testing

Active breeding stallions are considered to be those that had semen collected, or were bred to a mare by live cover, in 2008 or 2009. However, some active breeding stallions are not eligible for enrollment in the national testing project. Stallions that are **not** eligible for testing include:

- Stallions known to have been exposed to a positive horse in the current CEM outbreak.
- Stallions tested for *T. equigenitalis* in the previous 6 months.
- Stallions that received any systemic antibiotic treatment in the previous 7 days, or any topical treatment of the penis or prepuce with an antiseptic, a disinfectant, or an antibiotic in the previous 21 days. (These stallions could be eligible for testing, given they meet other eligibility requirements, if they are sampled after an appropriate waiting period following treatment (i.e., 7 to 21 days).)

In addition, the maximum number of stallions to be sampled at any one premises or facility will be limited to 50 percent of the total number of active breeding stallions present, but not more than 20 stallions in total. Sampling of more than 20 stallions at a given facility or premises may be approved by APHIS in some situations.

Of the active breeding stallions otherwise eligible for testing, those of highest priority for enrollment are:

- Stallions collected or bred at a facility where at least one other stallion was collected or bred on the same day in 2008 or in 2009;
- Stallions collected or bred on two or more days in 2008 or in 2009;
- Stallions collected or bred in both 2008 and 2009;
- Stallions potentially collected or bred outside the United States at any time.

After using the priority categories to target stallions for enrollment, some attention should be given to their breed. Participating stallions in any given State should not all be the same breed. Rather, the breeds of the stallions enrolled should reflect the breeds found in the State's overall breeding stallion population.

III. Collecting Samples from Active Breeding Stallions

Sample collection will be performed only by a USDA-accredited equine veterinarian. In addition to accreditation, State animal health officials may require sample collection training or experience for equine practitioners collecting samples in their jurisdiction.

Accredited equine veterinarians must have prior approval from the APHIS-VS Area Veterinarian in Charge (AVIC), or from the State Animal Health Official, of the State in which sampling will be performed. The stallion owner is responsible for the cost of sample collection.

APHIS will pay for the shipping and laboratory diagnostic testing of one set of swabs per eligible breeding stallion. One set includes three swabs: fossa glandis, urethral sinus, and distal urethra. Collection, shipping, and testing of any additional swab sets are at the stallion owner's discretion and expense.

Samples must only be collected on a Monday, Tuesday, Wednesday, or Thursday, in order to allow samples to reach the diagnostic laboratory on a weekday via overnight delivery. Also, samples should not be collected on any day immediately prior to a holiday. Samples must be plated within 48 hours of collection and laboratories will not authorize overtime work (e.g., weekends, holidays) for this national testing project.

Culture the urethral sinus, the fossa glandis, and the distal urethra, using a separate swab for each of the three sites. Swab sites may be cleaned with cotton and plain water to remove excess organic debris, but no residual water should be present at the time of swabbing. Do not scrub or use antiseptics or disinfectants prior to swabbing. If antiseptics or disinfectants have been used, stallions must wait 21 days to have samples collected for *T. equigenitalis* culture.

The accredited equine veterinarian collecting the samples must fax a copy of the VS Form 10-4 for each stallion sampled to the AVIC for the State in which the samples were collected.

IV. Stallions Found Positive for *T. equigenitalis*

A stallion is positive for *T. equigenitalis* if positive results are returned from the NVSL on any of the testing performed. A positive stallion must be placed under a State quarantine or hold order until the stallion is treated, retested, and determined to no longer be infected with *T. equigenitalis*.

Stored (frozen) semen collected from a stallion found positive for *T. equigenitalis* should be quarantined, or otherwise held without use, pending an epidemiological determination of the year of infection of the donor horse. Semen collected during or after the year of initial *T. equigenitalis* exposure may pose a risk for exposure to recipient horses.

Stallions confirmed as positive at the NVSL will be re-sampled and cultured at the NVSL, at APHIS expense, to reconfirm the status of the stallion to be treated. Procedures for treating a positive stallion are detailed in Section IV of the “*USDA APHIS Veterinary Services FY 2009 CEM Incident Testing Protocol for Investigations.*”

APHIS will pay all costs associated with the necessary treatment and retesting procedures of stallions that test positive in this national testing project. However, cost sharing and procedures for associated tracing and testing/treatment of horses exposed to a positive stallion will be the same as in the current outbreak response (see “*FY 2009 CEM Incident Manual*”). APHIS and State animal health authorities share the cost of tracing activities. APHIS pays for laboratory diagnostics and shipping of samples, but not for sample collection, test mares, or treatment for exposed horses. Owners of the exposed horses are responsible for the cost of sample collection, test mares, or treatment. If any exposed horses are found positive, those horse owners are responsible the cost of treatment and retesting of that horse until it is determined to be negative.

V. Sample Collection and Submission

1. Animal health officials and accredited veterinarians must refer to the “Laboratory Submission and Reporting Protocol for Active Breeding Stallions” for specific sample collection and submission guidance.
2. General guidance:
 - a. Active breeding stallion samples are to be sent to an approved CEM laboratory or to the NVSL. All suspect positive samples must be confirmed at the NVSL.
 - b. Swabs must be placed in Amies transport medium with charcoal (each swab in a single tube) and refrigerated. (Amies media is available at no charge through VS Area Offices.)
 - c. The swabs that come in the Amies tubes are adequate for collection of stallion samples.

- d. VS Form 10-4 must be used for all submissions, whether to an approved CEM laboratory or to the NVSL. A separate 10-4 should be used for each stallion sampled. Required forms and specimens should be identified with the name, address, and phone/fax number of submitting veterinarian; location of stallion; complete animal identification; anatomical locations sampled; and date and time of sample collection. (See Appendix 2 of the “Laboratory Submission and Reporting Protocol for Active Breeding Stallions”.)
- e. Swabs must be shipped with ice packs, by an overnight service provider as identified by the VS Area Office or VS Regional Office, to an APHIS-approved CEM laboratory in the State of sample collection (see Appendix 1, Approved CEM Laboratories). If no CEM laboratory exists in the State of sample collection, samples may be shipped to an APHIS-approved CEM laboratory in another State or to the NVSL (shipping address: National Veterinary Services Laboratories, Diagnostic Bacteriology Laboratory (DBL), 1920 Dayton Ave., Ames, IA 50010). Prior to submission of culture samples to the NVSL, contact Alan Aalsburg (515-337-7957) or Dr. Matt Erdman (515-337-7565 or cell: 515-231-4147).
- f. All samples must reach the lab and be plated within 48 hours after collection. If swabs are delayed, the sample collection must be repeated.
- g. Incubation for *T. equigenitalis* takes a minimum of 7 days.
- h. For suspect positive samples being shipped from a CEM laboratory to the NVSL for confirmation, fax the original VS Form 10-4 from the submission to the approved CEM laboratory and the 10-4 submission for confirmation testing to 515-663-7043. Include both 10-4 forms in with the samples when shipped.
- i. A swab of suspect colonies should be sent to the 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.
- j. Web link to list of laboratories approved by APHIS to process CEM (*T. equigenitalis*) cultures:
http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/ApprovedLabs_CEM.pdf

VI. Enrollment

Enrollment in the national testing project has at least three potential benefits to the owner of an active breeding stallion. First, this is an opportunity to determine that a stallion likely does not have the bacterium that causes CEM and that he is not spreading it to other horses. Second, a breeding stallion owner may need a negative test result for a stallion in order to have him collected or bred at a commercial facility this year. If so, enrollment offers a lower cost way to get testing done (i.e., with no sample shipping costs or laboratory diagnostic costs). Third, if an owner has any reason to suspect that a stallion is positive, then this is a chance to find out with

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minimal financial impact. Any stallion found positive as part of this national testing effort will be treated and retested at USDA's expense. If they wait, however, and he is found positive in the future, for example, as part of an investigation of another CEM-positive horse, the positive stallion's owner will be responsible for the cost of treatment and retesting of the stallion, including the cost of test mares needed to confirm a negative status after treatment.

Stallion owners or accredited equine practitioners interested in participating should contact the VS Area Office or the State Animal Health Official's office to volunteer. Contact information for your nearest office can be found on the following web pages:

http://www.aphis.usda.gov/animal_health/area_offices/
<http://www.aphis.usda.gov/vs/sregs/official.html>

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Table 1. Number of Active Breeding Stallions to be Sampled in Each State			
Eastern Region States	Stallions to be Sampled	Western Region States	Stallions to be Sampled
Alabama	48	Alaska	0
Connecticut	10	Arizona	55
Delaware	5	Arkansas	32
Florida	133	California	187
Georgia	35	Colorado	72
Illinois	59	Hawaii	0
Indiana	77	Idaho	63
Kentucky	333	Iowa	50
Maine	6	Kansas	44
Maryland	32	Louisiana	44
Massachusetts	16	Missouri	82
Michigan	63	Montana	53
Minnesota	62	Nebraska	38
Mississippi	40	Nevada	21
New Hampshire	2	New Mexico	39
New Jersey	35	North Dakota	17
New York	102	Oklahoma	94
North Carolina	49	Oregon	62
Ohio	107	South Dakota	27
Pennsylvania	101	Texas	327
Rhode Island	1	Utah	28
South Carolina	36	Washington	61
Tennessee	91	Wyoming	22
Vermont	4	Total	1,418
Virginia	65		
West Virginia	17		
Wisconsin	53		
Total	1,582		

USDA APHIS VETERINARY SERVICES

FY 2010

CONTAGIOUS EQUINE METRITIS NATIONAL TESTING PROJECT

LABORATORY SUBMISSION AND REPORTING PROTOCOL FOR ACTIVE BREEDING STALLIONS

APPROVED CEM LABORATORIES AND THE NATIONAL VETERINARY SERVICES LABORATORIES

January 2010

I. General Information

The National Veterinary Services Laboratories (NVSL) VS Form 10-4 will be used for submitting all samples collected from active breeding stallions and submitted to either an APHIS-approved contagious equine metritis (CEM) laboratory or to the NVSL.

Culture Submissions.

1. There are 15 APHIS-approved CEM laboratories for bacteriological cultures of *Taylorella equigenitalis* as trained by the NVSL and following NVSL standard operating procedures (i.e., SOPTA-BACT-0001.07). A list of approved CEM laboratories, including addresses for sample shipment, can be found in Appendix 1.
2. All submissions for culture must go to an approved CEM laboratory or to the NVSL.
3. Samples collected in a State with an approved CEM laboratory should be submitted to that laboratory, unless the submitter is instructed otherwise. If no approved CEM laboratory exists in the State of sample collection, samples may be shipped to an APHIS-approved CEM laboratory in another State or to the NVSL.
4. VS Form 10-4 must be used for all submissions, whether to an approved CEM laboratory or to the NVSL. A separate 10-4 should be used for each stallion sampled; see Appendix 2 for an example of a specimen submission form for active breeding stallions. (A fillable version of VS Form 10-4, specifically for use with active breeding stallion submissions for the CEM national testing project, may be accessed at: http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/VS_Form10-4_CEM-SURV.pdf.)

Laboratory Submission and Reporting Protocol for Active Breeding Stallions
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5. The accredited equine veterinarian collecting the samples must fax a copy of the VS Form 10-4 for each stallion sampled to the AVIC for the State in which the samples were collected.
6. The VS Form 10-4 for each stallion sample submission to an approved CEM laboratory must be faxed to the NVSL (515-663-7043) by the approved CEM laboratory within a day or two of receipt.
7. All suspect positive colonies found at an approved CEM laboratory must be submitted immediately to the NVSL for confirmation.
8. The correct address for submitting culture samples to the NVSL:

National Veterinary Services Laboratories (NVSL)
Diagnostic Bacteriology Laboratory (DBL)
1920 Dayton Ave., Ames, IA 50010
9. Prior to submission of culture samples to the NVSL, contact Alan Aalsburg (515-337-7957 or 515-520-2114) or Dr. Matt Erdman (515-337-7565 or cell: 515-231-4147).
10. In addition, fax the original 10-4 from the submission to the approved CEM laboratory and the new 10-4 for confirmation testing to 515-663-7043. Include both 10-4 forms in with the samples when shipped.

II. Submission Requirements for Approved CEM Laboratories for Culture

1. The State or Federal animal health official authorizing the submission of samples must contact the selected approved CEM laboratory prior to shipping the samples to ensure testing capacity is available and to confirm any specific submission requirements for the laboratory (e.g., notification required). Points of contact for approved CEM laboratories and their addresses are provided in Appendix 1.
2. Samples for culture must be shipped to reach the lab and be plated within 48 hours after collection or they will not be tested. Overtime, weekend and holiday work is not expected at approved CEM laboratories or at the NVSL, so plan accordingly in the collection and shipment of samples.
3. Sample collection:
 - a. Culture swabs must be placed in Amies transport medium with charcoal (one swab per tube) and refrigerated. (This transport media is provided at no cost by the NVSL through APHIS-VS Area Offices; the contact for Area Offices to obtain this media is Alan Aalsburg (515-337-7957 or 515-337-7565).)
 - b. The swabs that come in the Amies tubes are adequate for collection of stallion samples.

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- c. Specimens should be identified with the name, address and phone/fax number of owner and submitting veterinarian (State or Federal animal health official); location of horse; complete animal identification; anatomical location of each swab; and date and time of collection.
Note that the minimum information to be written on each tube is sample ID number, horse number or name, and anatomical location, since space on tube is limited.
- d. Swabs must be shipped with ice packs, by an overnight service provider as identified by the VS Area Office or VS Regional Office, to an approved CEM laboratory in the State of sample collection.
- e. Requests for shipping boxes should be directed to:
- NVSL Shipping
Fax Number: 515-663-7378
Email: NVSLShipping@aphis.usda.gov
Phone Number: 515-337-7530 (prefer fax or email orders if possible)
- f. The accredited equine veterinarian collecting the samples must fax a copy of the VS Form 10-4 for each stallion sampled to the AVIC for the State in which the samples were collected.
- g. Incubation for *T. equigenitalis* takes a minimum of 7 days.

III. VS Form 10-4 Requirements for Culture Submission

Submissions from Active Breeding Stallions – Submit to an approved CEM laboratory or to the NVSL:

A separate 10-4 should be used for each stallion sampled; see Appendix 2 for an example of a specimen submission form for active breeding stallions.

- Box 1 Name of Submitter** – The State or Federal animal health official **authorizing the sample submission**; include email address of submitter in the mailing address section in addition to the mailing address, phone and fax.
- Box 4 Location of Animals** – Enter location of stallion at time of sampling, including premises ID number, if applicable.
- Box 5 Payment Method** – Leave blank.
- Box 9 Examinations Requested** – Enter “CEM Culture” and record the time of sample collection.
- Box 10 Collected by** – The accredited veterinarian who actually collected the sample.
- Box 12 Authorized by** – AVIC of submitting State.
- Box 13 Purpose of Submission** – Check “Surveillance.”

- Box 15 Referral Number** – Must contain the reference “CEM-SURV;” add any referral number or local reference number, as needed.
- Box 21 Identification** – List each sample collection site separately under “Sample ID” – collection sites (one swab each) are: distal urethra, urethral sinus, and fossa glandis; under “Animal ID” give the name of the stallion, in addition to any other names or identification available; indicate breed, age, and sex in the appropriate columns.
- Box 22 Additional Data** – Record the exact registered name, or other full name, of the breeding stallion, plus his breed registration number (if applicable). Indicate “CEM surveillance stallion.” Indicate priority category(ies) of stallion sampled: (1) collected/bred with other stallion(s) on same day in '08 or '09; (2) collected/bred on multiple days in '08 or '09; (3) collected/bred in both '08 and '09; (4) potentially collected/bred outside USA.

IV. Submission of Suspect Positive Samples to the NVSL for Confirmation from Approved CEM Laboratories

A swab of suspect colonies should be sent to the 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 the NVSL. Prior to submission of the samples to the NVSL, contact Alan Aalsburg (515-337-7957 or 515-337-7565) or Dr. Matt Erdman (cell: 515-231-4147). In addition, fax the original 10-4 from the submission to the approved CEM laboratory and the 10-4 submission for confirmation testing to 515-663-7043. Include both 10-4 forms in with the samples when shipped.

V. Reporting of CEM Results from Approved CEM Laboratories

1. Approved CEM laboratories provide negative results to the submitter (who will be a State or Federal animal health official), State Veterinarian, and the AVIC of the submitting State by the route that they normally use in that State (email or fax). The submitter is responsible for notification of results to the sample collector (accredited veterinarian) and the stallion owner.
2. Approved CEM laboratories promptly report out suspect positive results **by email** to the submitter, State Veterinarian, and AVIC of the submitting State. See above for immediate contact requirement with the NVSL for submission for confirmation.
3. Normal reporting protocols are to be followed for notifications to their State veterinarians for samples originating from other States for both negative and suspect positive results.

VI. Reporting of CEM Culture Results from the NVSL

1. Negative and positive results are emailed to:
 - State Veterinarian of submitting State (and designees)
 - AVIC of submitting State (and designees)
 - Submitter (State or Federal animal health official)
 - Approved CEM laboratory that submitted suspect positive
 - VS EM&D PIC
 - VS ERMT (if Eastern)
 - CEM POC for ER (if Eastern)
 - VS WRMT (if Western)
 - CEM POC for WR (if Western)
 - Thomas Bunn
 - Paul Hauer
 - Beth Lautner

2. The submitter is responsible for notification of results to the sample collector (accredited veterinarian) and the stallion owner.