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

Approval of and Requirements for Laboratories to Conduct Tests for Contagious Equine Metritis

1. Purpose and Background

This document outlines the procedures for approval and maintenance of approval of laboratories to conduct tests for contagious equine metritis (CEM) and the requirements for those laboratories when performing CEM tests.

CEM is a highly transmissible venereal disease of horses caused by the bacterium *Taylorella equigenitalis*. CEM is considered a foreign animal disease (FAD). Although not currently considered a cause of CEM, *Taylorella asinigenitalis*, a related bacterium that may be found in the United States, is also covered by this document because of the importance of properly identifying and differentiating the two species.

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 VS, VS employees may not deviate from the directions provided herein without appropriate justification and supervisory concurrence.

2. Document Status

A. Valid through 8/30/2019.

B. This document replaces VSG 15202.1, which is rescinded.

3. Reason for Reissuance

This document has been updated to correct errors and clarify requirements.

4. Authority and References

A. Authorities (*Code of Federal Regulations* (CFR)):
- 7 CFR 371.4

B. References:
- VS Form 10-4, Specimen Submission (AUG 2009)

5. Audience

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

6. Guidance

A. Steps for Laboratory Approval

1) State and private veterinary diagnostic laboratories may request approval to conduct USDA-authorized diagnostic procedures for CEM. A written request must be made to the appropriate VS Assistant District Director. The decision to consider the request for approval will be made through consensus by the State Animal Health Official, the Assistant District Director, and the National Veterinary Services Laboratories (NVSL). The following actions will be taken if the request for approval is accepted for consideration:

   a. A Federal Veterinary Medical Officer (VMO) will review the regulatory and technical responsibilities and criteria for conducting and reporting official CEM tests with the laboratory official. The Laboratory Director or his or her designee will receive a copy of the inspection checklist and standards (see Attachment 1).

   b. The laboratory seeking approval will send the individual or individuals designated to conduct CEM testing to the NVSL CEM training course.

   c. A Federal Animal Health Official will inspect the physical facilities of the laboratory and record results on the laboratory inspection checklist (Attachment 1).

   d. The laboratory official will sign the Agreement to Conduct Contagious Equine Metritis (CEM) Testing (Attachment 2) to acknowledge that he or she understands the regulatory and technical responsibilities of the laboratory.

2) After the procedures in section 6.A.1 have been completed, the Assistant District Director and the State Animal Health Official may make their recommendations regarding approval of the laboratory. They must seek concurrence from the NVSL Director by forwarding (through the appropriate District Director) a jointly signed memorandum and the completed documents (Attachments 1 and 2) to: Director, NVSL, 1920 Dayton Ave, Ames, IA, 50010.

   a. The NVSL Director will notify the laboratory of the decision and will send appropriate copies to the Director of Animal Health Programs.

   b. Questions regarding the laboratory approval process should be directed to the NVSL Diagnostic Bacteriology Laboratory by calling (515) 337-7565 or by sending a faxed request to (515) 337-7569.

B. Qualifications and Training of Personnel to Conduct CEM Culture Tests

1) VS must certify laboratory personnel performing CEM culture tests. The Assistant District Director and the State Animal Health Official must recommend personnel for training and NVSL approval. The minimum required training is as follows:

a. Laboratory personnel assigned to perform isolation and identification of *Taylorella* isolates must have a bachelor's degree in microbiology or related life science or a minimum of two years' experience in a veterinary bacteriology laboratory isolating and identifying aerobic and microaerophilic organisms,. These individuals must also complete a training course focused on the isolation and identification of *Taylorella* spp. at the NVSL site in Ames, IA. The requesting laboratory will pay all training costs.

b. If the NVSL adopts new technology for isolating and identifying *Taylorella* in specimens, additional training at the NVSL may be required if the approved laboratory wishes to adopt said technology.

c. Laboratory personnel assigned to conduct CEM serological procedures must have a minimum of 2 years' experience conducting serological assays of the same format.

2) Trained personnel must take and pass periodic proficiency tests when offered by the NVSL to continue to conduct testing. Personnel who do not take the proficiency test when offered lose approval to perform testing as of the date the results of the proficiency test are released. Personnel may be reinstated after meeting the requirements listed below.

a. NVSL will supply samples for personnel to use in proficiency testing and will evaluate the results. Personnel who fail the NVSL proficiency test are subject to appropriate, corrective action as determined by the NVSL. Options include removal, additional training, and additional proficiency testing. Personnel who fail the proficiency test twice in 1 year will be removed.

b. Laboratories must order proficiency tests, report proficiency test results, and respond to inquiries regarding the number of tests performed or other requested information in a timely manner. VS charges a user fee for proficiency tests.

c. Previously trained and certified personnel who have had a lapse in testing clinical samples of greater than 1 year will be required to retake and pass a proficiency test panel before resuming CEM testing.

d. Personnel who have a lapse in testing clinical samples of greater than 2 years must attend another NVSL training course. User fees apply.

3) Laboratories approved to conduct CEM culture tests must inform the Assistant District Director and NVSL when any personnel certified by the NVSL to conduct the tests are no longer available to conduct testing. VS will revoke the laboratory's approval if qualified certified personnel are not available to conduct the tests.

C. Laboratory Standards for Performing CEM Culture Testing

1) Collection and Transport of Diagnostic Specimens:

a. Specimens of U.S. origin for testing must be collected and submitted by an accredited veterinarian, Federal veterinarian, or State animal health official. If the laboratory staff has questions about the accreditation status or any other information on a submission, they should contact the assistant district director or State animal health official for clarification.

b. Sample collection protocols may vary slightly depending on purpose (e.g. import, export, trace back). The Assistant District Director has the latest information on culture procedures. Laboratories are encouraged to distribute current information to their clients.

c. Current NVSL guidance on specimen collection:

1. Small diameter swabs must be used for clitoral sinus sampling. Rayon is the material of choice. Swabs made from calcium alginate (Calgiswabs) must not be used and swabs made of polyester should not be used as they tend to dissolve in the transport medium. If a swab has dissolved during transit the sample must be reported as invalid and the submitter advised to re-collect with a rayon swab. Undissolved calcium alginate and polyester swabs may be tested. However, the submitter should be advised to use a rayon swab.

2. Cervical or endometrial swabs must be collected with an appropriately sized guarded uterine swab. Standard sized swabs should be used for all other sites.

3. Specimens (swabs) submitted for culture must be placed in Amies transport medium with charcoal and sent to the laboratory with sufficient ice packs to keep the specimens cold (4° C) on arrival. Overnight shipment is the method of choice as specimens must be set up in the laboratory no more than 48 hours after collection. Samples arriving after 48 hours from collection will not be tested and new samples should be requested.

2) Specimen Identification

a. Specimens for culture must be individually and uniquely identified and must be accompanied by a completed submission form (VS 10-4 or equivalent). Information on the specimen container must match that on the submission form.

b. Specimens must include equine identification matching that on the paperwork, collection date and time, and anatomical sites of sample collected. Sample collection time may be waived if samples arrive at the laboratory the day following collection. For each specimen, the submission form must include:

1. Name, address, and telephone number of the submitting veterinarian.

2. Signature of the submitting veterinarian.

3. Name of the owner or person responsible for the equine. The submitter must be able to provide contact information for this person.

4. Location (including county) of the equine sampled.

5. Unique identification of the equine sampled, which may include a combination of name, age, breed, sex, color, markings, and any registration, tattoo, or microchip or microchip number.

6. Sample collection date and time.

7. Anatomical sites of sample collection.

3) Conditions that preclude testing of culture specimens:

a. Specimens not properly identified or in poor condition will not be tested.

b. Specimens will also not be tested if/when:

1. Received more than 48 hours after collection.

2. Received in broken containers.

3. Submitted in expired or degraded media or in any media type other than Amies transport medium with charcoal.

4. Not cold on arrival.

5. Contain more than one swab/site per transport medium.

6. Swabs dissolved during transit.

7. The information specified in C.2 cannot be obtained.

8. Not collected and submitted by an accredited, State, or Federal veterinarian.

c. Each submission will be assigned a unique accession or identification number to allow identification in the laboratory.

4) Isolation and presumptive identification of *Taylorella* spp.

   a. Approved laboratories must follow the most current NVSL CEM testing standard operating procedures (SOP). NVSL will distribute updated SOPs to approved laboratories when available.

   b. For suspect colonies:

      1. All *Taylorella* suspect isolates as defined in the NVSL CEM testing SOP must be sent immediately to NVSL for confirmation.

      2. Approved labs shall not use their own confirmatory testing when deciding to send suspect isolates to NVSL or report final results. *Taylorella* confirmation tests performed by NVSL are described in the NVSL CEM testing SOPs.

      3. Suspect isolates should be sent to NVSL on swabs in Amies transport medium with charcoal and with ice packs, using the most expedient shipping option possible. Multiple suspect colonies should be sent when more than one suspect colony is present. Parafilm-sealed plates with pure culture should also be sent if available. Additionally, original samples from the suspect accession should be sent in the same shipment.

      4. NVSL should be immediately notified of the suspect isolates and provided the shipping tracking number.

5) Reporting results of isolation and identification of *Taylorella*

   All tests and results are official and must be reported. “Unofficial” tests may not be performed to determine the status of an animal. Test results must be made available to the NVSL on request.

   a. Test results must be sent to:

      1. Appropriate State or Tribal officials in the State where the equine is located.

      2. Appropriate Federal officials.

      3. Veterinarian submitting the sample.

   b. The official test results must be reported as follows:

      1. Indicate “*Taylorella* was not isolated” if that is the case. Similar language such as “not recovered” or “not detected” is also acceptable. Do not report results as “negative.”

      2. Indicate that *Taylorella* was isolated/recovered/detected and the species isolated if that is the case.

c. All positive and suspect isolates must be sent immediately to NVSL for confirmation. Approved laboratories may not send out a positive *Taylorella* result until NVSL has completed confirmation.

d. NVSL will notify the following of confirmation of a positive sample by e-mail, telephone, or fax within 6 business hours of test completion:

i. State or Tribal Animal Health Officials in the State or States where the animal and laboratory are located.
ii. Federal officials for the State or States where the animal and laboratory are located.

e. Upon completion of all testing, NVSL will issue a final report.

f. If no test was performed, report as "not tested" and give the reason (refer to the list of reasons in C.3.). Notify submitter that new samples need to be collected and submitted.

g. If the result is invalid due to either overgrowth or no growth, report as "invalid due to no growth" or "invalid due to overgrowth." Notify submitter that new samples must be collected and submitted.

D. Serological Testing

1) Laboratory personnel who conduct serologic tests to detect antibodies against *Taylorella* must follow NVSL protocol.

2) Serum samples submitted for serology testing must be clear (non-hemolyzed) and uncontaminated. Preferred samples are sera that have been separated from the blood clots and poured off into plastic snap-capped tubes. Centrifuged serum separator tubes are acceptable. Samples must be shipped with ice packs to help preserve the sera.

3) The laboratory must include known positive and negative controls with each *Taylorella* serological test.

4) All results of *Taylorella* serological tests are official and must be reported. The report must include the following information:

a. Name, address, and telephone number of the owner.

b. Location of the horse (including city, county, and State).

c. Official horse identification (see C(2)b.5 above).

d. Test result (positive, negative, suspect).

e. Serum dilution at which the test result was interpreted (Ex.: Pos at 1:16, Neg at 1:4).

5) All positive test results must be reported to the submitter, Assistant District Director, and the NVSL within 6 business hours.

6) NVSL must confirm all positive test results.

7) NVSL will offer a CEM serology proficiency test.

E. Maintaining Approval

1) The Assistant District Director and the NVSL must be informed of any change of address or other contact information (e.g. telephone number), or a change in Laboratory Director. The new Laboratory Director must sign a new copy of the agreement.

2) Each approved laboratory must pass periodic proficiency tests (one for each person approved to conduct CEM testing). Refer to Section B(1) for additional information.

3) Continued approval will require an official inspection conducted by Federal personnel at least every 2 years. Additional inspections may be conducted at the discretion of the Assistant District Director if there are performance or compliance concerns.

4) Official inspections of the laboratories are subject to user fees.

5) Inspection by VS (Federal) personnel does not absolve laboratories from other requirements established by local or State agencies.

6) The Federal inspecting official will verify the following:

a. Compliance with this Guidance Document.

b. Conducting tests according to the current versions of official protocols.

c. Certification of personnel conducting the tests to perform CEM testing.

d. Documentation of sample referral and test reporting for compliance with regulatory obligations.

e. Verification that the appropriate number of laboratory tests are being performed and reported annually.

7) An approved laboratory may be inspected by Federal or State Animal Health Officials at any time during the laboratory's normal business hours. Laboratories with deficiencies in compliance or procedures are subject to corrective actions, as

appropriate. Laboratories with severe or uncorrected deficiencies will be recommended for removal.

8) Each laboratory must show active involvement in serving the CEM program, compliance with the specifications of this Guidance Document, and successful completion of a proficiency panel. Laboratories that perform fewer than 100 CEM tests per year are subject to approval withdrawal. Exceptions to the minimum testing expectation must be justified to APHIS by letter from the Assistant District Director and State Animal Health Official.

F. Removal of Laboratory Approval

1) The Deputy Administrator may remove laboratory approval when any of the criteria for approval have not been met. In all cases, the laboratory will receive written notification of removal or removal recommendation from the APHIS Administrator or his or her designee.

2) Approval will be removed in any of the following situations:

a. The laboratory requests removal.

b. The laboratory no longer has personnel approved to conduct CEM testing.

c. The NVSL Director recommends removal.

d. The NVSL Director receives written recommendation for removal by concurrence of the Assistant District Director and the State Animal Health Official.

e. The laboratory fails to meet the proficiency testing or inspection requirements.

f. The laboratory does not meet the annual testing numbers as determined by the NVSL, the assistant district director, and the State Animal Health Official.

3) Laboratories may be reinstated if they provide sufficient evidence to the NVSL Director, Assistant District Director, and State Animal Health Official that the conditions leading to removal of approval have been ameliorated. Laboratories applying for reinstatement must go through the entire approval process. On reinstatement, trained and approved personnel must meet all requirements before testing resumes.

G. List of Approved Laboratories

The NVSL Director will maintain a current list of laboratories approved to conduct tests for CEM. The list will be updated regularly and will be available on the APHIS Website.

7. Inquiries

If you need additional information about implementing this guidance document, please contact the National Veterinary Services Laboratories, Diagnostic Bacteriology Laboratory, Bacterial Identification Section or Serology Section at 1920 Dayton Ave, Ames IA, 50010. You may also use the NVSL general e-mail: NVSL_Concerns@aphis.usda.gov. Note whether your inquiry is related to CEM culture or serology. Questions may also be addressed to the following phone numbers:
Bacteriology: (515) 337-7565
Serology: (515) 337-7563

Attachments

ATTACHMENT 1

Laboratory Inspection for Contagious Equine Metritis (CEM) Testing

Laboratory Name _____

Telephone # _____Fax # _____

Laboratory or Director email address _____

Laboratory address (physical address – not P.O. Box)
_____
_____

Mailing address (if different from above)
_____
_____

Shipping address for supplies and proficiency tests (if different from above)
_____
_____

Name of laboratory staff escort for the inspector
_____

Personnel currently conducting CEM tests at the laboratory and date each certified

Name Date Certified
_____
_____
_____
_____
_____
_____
_____
_____

Inspector _____ Title _____

Inspector telephone # _____

Laboratory number _____ (to be assigned by NVSL)

Laboratory Inspection for Contagious Equine Metritis (CEM) Testing

CEM Laboratory Inspection

Section	Item	Yes	No	Notes
I. Laboratory	Separate room or portion of room is used for testing.			
	Laboratory should not be accessible to the general public during testing.			
	Adequate open bench space (at least 3 to 5 feet) and lighting is available to perform test.			
	Sink is available in same area with hot and cold running water.			
	Laboratory and laboratory equipment is clean and properly stored.			
	Appropriate disposal facilities are available in accordance with local rules and regulations.			
	Only laboratory equipment and supplies are within the laboratory. No eating, drinking, applying cosmetics, handling contact lenses, or storage of food is allowed in the lab.			
II. Laboratory Equipment	**The following equipment must be available and functioning properly:**			
	Incubator capable of maintaining a temperature of 37 C° and a carbon dioxide level of 5-10 percent.			
	Tank containing carbon dioxide (technical grade, at least 99.5 percent pure) with a system to measure gas delivered. The tank must be secured to the wall.			
	Second incubator capable of maintaining a temperature of 37 C°. (Aerobic, no CO2.)			
	Microscope with oil immersion lens and light source adequate to read gram stains.			
	Covered metal pans or similar container to hold discarded specimens and test media.			
	Autoclave of adequate size to handle the discarded pans.			
	Refrigerator with enough shelf space to hold CEM specimens and media until completion of tests.			
	Non-defrost -20 C° or -80 C° freezer for storage of *Taylorella* control strains.			

III. Laboratory Supplies	**The following supplies must be available:**			
	Eugon agar base (BBL) with 10 percent chocolate horse blood. The shelf life of Eugon agar is listed in the NVSL SOP.*			
	Eugon agar base (BBL) with 10 percent chocolate horse blood and selective inhibitors (Modified Timoney-Shin Medium): amphotericin B (5 µg/ml) clindamycin (5 µg/ml) Trimethoprim (1 µg/ml) The shelf life of the Timoney-Shin medium is listed in the NVSL SOP.*			
	Amies transport medium with charcoal. This media must be stored according to manufacturer's instructions until used. It should be discarded if it is expired, the charcoal is not evenly distributed, or if there is evidence of dehydration.*			
	Catalase test reagent (hydrogen peroxide, 3%).			
	Cytochrome oxidase test reagent.			
	Gram stain reagents.			
	Microbiological laboratory supplies such as inoculating loops, microscope slides, petri dishes, etc.			
	Trypticase Soy Agar with 5 percent sheep blood or equivalent non-selective blood agar plates. These plates must not support growth of *Taylorella* spp. when incubated at 37 C° without supplemental CO2.			
	The following apply to serology testing:			
	NVSL antigen for CEM serologic test.			
	NVSL control sera for CEM serologic test.			
	Serology laboratory supplies such as pipettors, test tubes, microtiter plates, etc.			

* If the special media required for *Taylorella* culture is not available at the time of inspection, laboratory personnel should be able to demonstrate they know what media must be obtained and the source they will use to obtain it, or demonstrate they can make the required media and have the appropriate cultures for performing quality control testing on the media as described in the current version of the NVSL protocol.

Section	Item	Yes	No	Notes
IV. Control of Specimens and Reporting	Laboratory assigns a unique accession number to each sample. The accession number is recorded on the official reporting form.			
	Specimens not appropriately identified are not tested.			
	Specimens are received with proper submission form (with name of the owner, name and address of the submitting veterinarian, location of animal at the time the test sample was obtained, unique animal identification, and signed by the submitting veterinarian).			
	The specimen identity is maintained on the worksheets and on the culture plates.			
	The test results for each sample are recorded on a worksheet which should be made available for review.			
	The results are recorded on the reporting form with a copy kept in the laboratory. Results are reported as described in the Guidance Document.			
	All tests are reported regardless of results. No unofficial CEM tests are performed to determine the status of the animals before the "official" test is performed. **All CEM tests are official tests.**			
	Only testing as described in the current version of the NVSL SOP produces official reportable results.			
	Official test results are reported to the States and/or Federal animal health officials within the time specified by these officials.			
	An official result of "*Taylorella* was isolated" is only issued after completing and reporting confirmatory testing by the NVSL.			
	Specimens are held refrigerated or frozen for at least 2 weeks after results are reported and preferably for at least 30 days.			
	Taylorella spp. is listed on the laboratory's permit (VS 16-16A) to receive controlled organisms.			
	The laboratory has viable cultures, including frozen parent stock, of the control strains required by the current version of the NVSL test protocol.			
V. Test Procedure	**The current version of the NVSL test protocol must be followed.**			

Section	Item	Yes	No	Notes
VI. Building and Cleanliness	The building is in good repair and provides a professional appearance inside and outside.			
	Adequate doors, windows, and screens are provided and are in good repair.			
	The laboratory is separated from the office, storeroom and unused areas by partitions with doors.			
	Adequate lighting is available.			
	Restrooms are available, clean, and in good repair.			
	Laboratories are free of rodents, insects, and other pests.			
	All refuse is placed in proper containers and removed frequently.			
	Clean laboratory clothes (coats) are worn.			

ADDITIONAL REMARKS

Laboratory is: Satisfactory Unsatisfactory (circle one)

_____ _____

Inspector's signature Date

_____

Inspector's name (printed)

_____ _____

Laboratory escort's signature Date

_____

Laboratory escort's name (printed)

- o Copy of completed inspection form given to laboratory
- o Copy of original inspection form filed at area office

Attachment 2

Agreement to Conduct Contagious Equine Metritis (CEM) Testing

I,_____, have read and understand Veterinary Services (VS) Guidance Document 15202.2. I understand my responsibilities as outlined in the Document and agree to abide by the guidelines therein. The guidelines and requirements include, but are not limited to, the following:

1. The individual or individuals responsible for conducting CEM tests must have completed training in the proper techniques. The training will be conducted at the National Veterinary Services Laboratories (NVSL) in Ames, Iowa.

2. All testing must be conducted in accordance with the current version of the official protocol for the test as provided by the NVSL.

3. Periodic laboratory proficiency tests must be completed satisfactorily and in a timely manner for the laboratory to maintain approval.

4. All results of CEM tests conducted must be signed appropriately and reported to the State and/or Federal animal health officials in the State where the laboratory is located and in the State in which the animals were sampled.

5. Laboratory officials must comply with inspections and VS animal health official requests regarding CEM testing activities.

6. The laboratory will lose its approval if personnel trained to conduct CEM tests are no longer available to conduct CEM testing or any of the listed requirements are not followed.

Signature of Laboratory Director _____

Laboratory Name _____

Laboratory email address _____

Laboratory address _____

Telephone _____ Date _____