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VS Guidance 6701.5

Primary and Secondary Serological Test for Diagnosing Bovine Tuberculosis (TB) in Farmed and Captive Cervids

1. Purpose and Background

The Dual Path Platform VetTB Assay (DPP) is an official serum test used only for cervids in the U.S. bovine tuberculosis (TB) eradication program. The DPP test detects antibodies to *Mycobacterium bovis* in cervid serum. Veterinarians may use the DPP as a primary and, when required, as a supplemental test for elk, red deer, white-tailed deer, fallow deer, reindeer, and mule deer. In addition, veterinarians may use the DPP as a primary or secondary test in sika deer and axis deer through an existing pilot project to evaluate the effectiveness of the DPP as an official primary and supplemental/secondary test for TB in these species. DPP samples may only be submitted to the National Veterinary Services Laboratories (NVSL) for testing.

This guidance document represents the Animal and Plant Health Inspection Service's (APHIS) position on this topic and is intended solely as guidance. It does not have the force and effect of law, does not create or confer any rights for or on any person, and does not bind the U.S. Department of Agriculture (USDA) or the public. Language suggesting that this guidance is mandatory (e.g., "shall," "must," "required," or "requirement") should not be construed as binding unless the terms quote from a statutory or regulatory requirement. The information this document contains may be made available to the public. While this document provides guidance for users outside Veterinary Services (VS), VS employees may not deviate from the directions provided herein without appropriate justification and supervisory concurrence.

Pursuant to the Congressional Review Act (5 U.S.C. 801 et seq.), the Office of Information and Regulatory Affairs designated this rule as a non-major rule, as defined by 5 U.S.C. 804(2).

2. Document Status

A. Review date: 8/30/2027.

B. This document replaces Veterinary Services Guidance 6701.4.

3. Reason for Reissuance

VS is reissuing this guidance to reflect changes in collection and testing procedures.

4. Authority and References

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

- 7 CFR 371.4
- 9 CFR 77.33
- 9 CFR 77.34
- 9 CFR 86.1
- 9 CFR 161.5

B. References:

- VS Form 6-22, Tuberculosis Test Record
- VS Form 10-4, Specimen Submission
- VS Form 10-4A, Continuation Sheet for Specimen Submission
- NCAH Portal

C. Definitions:

1) Epidemiology Officer: For the purposes of this guidance, an epidemiology officer is a VS or state employee with epidemiological training or experience designated to make decisions about using and interpreting TB diagnostic test results. Epidemiology officers are qualified to classify cervids as *suspect* or *reactor*; direct and/or participate in field epidemiological investigations; and manage disease control activities in TB-exposed, suspect, and positive animals and herds.

2) National Cervid Tuberculosis Disease Specialist: A VS employee on the Cervid Health Commodity Team with epidemiological training or experience designated to coordinate and oversee decisions about using and interpreting TB diagnostic test results. National Cervid Tuberculosis Disease Specialists are qualified to coordinate and oversee decisions regarding classifying cervids as *suspect* or *reactors* and to take part in field epidemiological investigations and disease control activities in TB-exposed, suspect, and positive animals and herds.

3) Designated Accredited Veterinarian (DAV): A designated accredited veterinarian is specially trained and approved by the state or VS to conduct specific program tests and activities, including the single cervical tuberculin skin test (SCT) and the DPP. VS may grant this certification to Category II accredited veterinarians once they complete an additional orientation or training program in the specific area for which the veterinarian seeks program certification. The accredited veterinarian may have to pay for orientation or training. VS will not permit accredited veterinarians without program certifications to perform accredited duties related to that certification. If a DAV allows their Category II accreditation to expire, the DAV's program certification also expires, and they must requalify for the program certification.

5. Audience

VS employees, other federal and state agencies, and accredited veterinarians.

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6. Guidance

A. Test Administration, Ordering, and Payment

1) DAVs perform the SCT in cervids. They can also submit serum specimens from elk, red deer, white-tailed deer, fallow deer, reindeer, mule deer, sika deer, and axis deer to NVSL for serological testing using the DPP. Current DAVs require additional training to collect samples for cervid TB serological testing.

2) NVSL can provide serum submission kits if needed; call (515) 337-6200 or email APHIS-NVSLUserfee@usda.gov. VS does not require the serum submission kit to complete testing. Each kit contains shipping instructions, a gel pack, VS Forms 10-4 (Specimen Submission Form) and 10-4A (Continuation Sheet), and a prepaid shipping label to NVSL. DAVs can also submit serum samples using their own shipping box, which should include a frozen gel pack and the laboratory submission forms. Submit the VS Forms 10-4 (Specimen Submission Form) and 10-4A (Continuation Sheet) through the National Center for Animal Health (NCAH) web portal; see instructions at NCAH Portal Guidance for NVSL Submitters. If you can't access the portal, include hard copies of the VS 10-4 and 10-4A with the samples and send an Excel spreadsheet of the sample and animal identifications to VS.DB.NVSL.DBRL.Sero.Mgmt@usda.gov.

3) The submitting DAV contacts the state animal health officials in the state of origin, and state of destination to verify that the state's current animal health regulations allow the use of the DPP serological test for bovine TB.

4) The submitting DAV pays for routine screening DPP tests by setting up an account with the NVSL business office at (515) 337-6200, providing a credit card number on the submission form, or including a check to NVSL with the samples and submission form. NVSL does not apply user fees for the secondary DPP after thirty (30) days for animals with primary positive test results.

B. Sample Collection, Processing, and Shipping for DPP Testing at NVSL

1) Collecting samples

a. Obtain adequate sample packaging supplies before collecting the blood samples.

b. Collect blood samples in a 10 ml serum separation tube or a 10 ml red top (clot) tube labeled with the animal's official identification number. VS strongly recommends collecting blood samples in a serum separation tube.

c. Collect 10 ml of whole blood to obtain a minimum of 2 ml of serum.

d. Use a sterile needle for each animal. Do not reuse needles.

2) Sample processing and handling

a. Allow the blood to clot at room temperature.

b. Centrifuge the clotted blood sample to obtain at least 2 ml of serum. Remove the serum from the clot and place it into a new, clean red top tube. The serum must contain no or minimal hemolysis (samples that appear red or pink must be discarded, and the animals resampled). Place the serum tubes in a refrigerator until shipped to NVSL. Do not freeze the serum.

c. Number and label tubes to be submitted with the animal's official identification number. Proper sample labeling facilitates sample verification and correlation to individual animals by laboratory personnel.

d. Organize the serum tubes into a tube box in the same order animals are listed on the submission form or attached list. This allows laboratory personnel to verify and test the samples quickly.

e. Submit a separate red top tube of blood to an approved brucellosis testing laboratory if also testing for brucellosis. NVSL does not perform routine brucellosis surveillance testing.

3) Completing submission forms

a. Complete the original VS Forms 10-4 and, when needed, 10-4A, as part of the sample submission package described in Section 6 B.4) of this guidance. On the VS Form 10-4 and, if needed, the 10-4A continuation form, record the official identification (and all other ID), species of cervid tested, age, and gender for each animal. Alternatively, record the required information on an attached list.

b. Complete the submission portion of the VS Form 6-22 (Tuberculosis Test Record) accurately and completely, identifying the cervid species tested, ID, age, and gender.

c. Send the VS Form 6-22 with the submission portion completed to the state animal health official or VS Area Veterinarian in Charge (AVIC) for the state (USDA APHIS | VS Contacts: Area Offices) within 5 (five) business days of sample collection. Check with the state animal health official to determine if your state has additional requirements for VS Form 6-22 submission, such as adding or attaching the NVSL results to the VS Form 6-22. NVSL generally sends result reports to the submitter, to the AVIC, and state officials.

4) Sample submission to NVSL

   a. Package the serum sample tubes in a shipping container with frozen gel packs. Prevent direct contact between the sample tubes and the frozen gel packs.

   b. Ship the samples along with the packing slip from the portal submission or the completed form VS 10-4/10-4A with the submission portion completed to the NVSL.

   c. Ship serum samples using overnight or two (2)-day delivery. Ship samples on the day of collection or to arrive at NVSL no later than ten (10) calendar days after the sample collection date. Do not use the U.S. Postal Service (USPS) as a delivery method. The USPS does not deliver directly to NVSL.

      1. Ship samples so NVSL receives them Monday through Friday. Do not ship them so they could arrive on weekends or holidays.

      2. Sample submission forms must be accurate, complete, and must include a sample collection date.

      3. Samples received by NVSL more than ten (10) calendar days after the sample collection date do not meet the test submission requirements.

   d. Ship the serum samples to NVSL at:

      National Veterinary Services Laboratories
      1920 Dayton Avenue
      Ames, IA 50010

C. Reporting Results

1) NVSL tests serum samples using the DPP and follows test kit instructions and internal NVSL standard operating procedures.

2) NVSL freezes serum samples when DPP kits to perform the testing are not available and notifies the submitter of the delay.

3) NVSL considers a DPP reader value below the established cutoff value a negative DPP test result. NVSL considers a DPP reader value equal to or above the established cutoff value a non-negative DPP test result. Non-negative animals are reported as positive on the accession.

4) NVSL reports DPP test results to the submitting accredited veterinarian, the respective state animal health official, the respective state's AVIC, and the Cervid Health Team.

D. Result Interpretation and Classification of Animals

1) An epidemiology officer classifies the animals based on the DPP test results:

a. Classify animals with a negative test result on the DPP primary test as negative.

b. Classify animals with a positive test result to the DPP primary test as suspect and require retest with the DPP as a secondary test. VS recommends collecting a second blood sample from the suspect animal no earlier than thirty (30) days after collecting the primary sample. Do not retest a captive cervid that has non-negative test results to the DPP test with the single cervical tuberculin test (SCT) or comparative cervical tuberculin test (CCT).

c. Classify animals with a negative test result on the DPP secondary test as negative.

d. Classify animals that are non-negative on two successive DPP tests (primary and secondary after thirty (30) days) as reactors.

e. Classify samples received by NVSL more than ten (10) calendar days after the sample collection date as not meeting submission requirements. In such cases, negative results may not be used for official purposes unless the state animal health official of the state of residence determines that there were mitigating factors. For the movement of animals from non-TB accredited herds, the state animal health official of the destination state must also concur. Samples received on the weekend will be considered to have been received on the following Monday; classify these as not meeting submission requirements if this exceeds ten (10) calendar days from the collection date. NVSL considers samples received on a federal holiday as received the following day; they are classified as not meeting submission requirements if this exceeds ten (10) calendar days from the collection date.

f. Classify samples submitted to NVSL without a collection date or other required information as not meeting submission requirements until the submitting accredited veterinarian provides a corrected copy of the VS 10-4 to the State animal health official, AVIC, and NVSL.

2) The epidemiology officer must justify any exceptions to reactor classification in writing with the concurrence of the National Cervid Tuberculosis Disease Specialist.

3) The epidemiology officer manages suspect and reactor animals consistent with the program regulations described in 9 CFR part 77 and the 1999 TB Uniform Methods and Rules or any TB program standards subsequently published.

E. Herd Testing Protocol

1) In routine herd testing, the DAV may test groups of animals within a herd using different methods (e.g., test bucks with the DPP and does with the SCT). When using different test methods, the DAV must complete a separate VS Form 6-22 for each group of animals. Animals or groups tested with the DPP as the primary test and classified suspect or non-negative must be retested with the DPP. Animals or groups tested with the SCT as the primary test and classified responder must be retested with the CCT.

2) In affected herds or herds under investigation, APHIS may develop a testing protocol using serological and skin tests separately, in series, or in parallel with permission from and in consultation with the epidemiology officer and the National Cervid Tuberculosis Disease Specialist. The testing protocol, timing of the different tests, interpretation of the tests, classification of the animals, and disposition of the animals must be determined before the testing occurs.

7. Inquiries

Please direct any inquiries to: USDA APHIS Veterinary Services Ruminant Health Center – Cervid Health Program at vs.sp.cervid.health@usda.gov.

Please also refer to Bovine Tuberculosis in Cervids for further information.