



VS Guidance 6701.4

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 or a secondary test only for elk, red deer, white-tailed deer, fallow deer, and reindeer herds. DPP samples may only be submitted to the National Veterinary Services Laboratories (NVSL).

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. Veterinary Services (VS) may make this information 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.

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: 05/01/2023.
- B. This document cancels and replaces Veterinary Services Guidance 6701.3

3. Reason for Reissuance

VS is reissuing this guidance to reflect changes in procedure and structure.

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](#)



VS Guidance 6701.4

B. References:

- [VS Form 6-22, Tuberculosis Test Record \(2/99\)](#)
- [VS Form 10-4, Specimen Submission \(8/09\).](#)
- [VS Form 10-4A, Continuation Sheet for Specimen Submission \(8/09\).](#)
- [NVSL 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*; to direct and/or participate in field epidemiological investigations; and to manage disease control activities in TB-exposed, suspect, and positive animals and herds
- 2) **National Cervid Tuberculosis Disease Specialist:** A VS employee who is a member of the Cervid Health Program 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 to conduct specific program tests and activities. 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 his or her Category II accreditation to expire, the DAV's program certification also expires and the DAV must requalify for the program certification.

5. Audience

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



VS Guidance 6701.4

6. Guidance

A. Test Administration, Ordering, and Payment

- 1) DAVs may perform the single cervical tuberculin skin test (SCT) in cervids. They may also submit serum specimens from captive elk, red deer, white-tailed deer, fallow deer, and reindeer to the NVSL for serologic testing using the DPP. Current DAVs do not need additional training to collect samples for cervid TB serological testing.
- 2) The 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 a gel pack, VS Forms 10-4 (Specimen Submission Form) and 10-4A (Continuation Sheet), shipping instructions, and a prepaid shipping label to the NVSL. DAVs may also submit serum samples using their own shipping box, which should include a frozen gel pack as well as the laboratory submission forms. The Office of Management and Budget has approved VS Forms 10-4 and 10-4A for submitting all diagnostic samples to the NVSL. NVSL prefers to have this form submitted through its web portal; instructions can be found [here](#). Alternatively, submit these forms to NVSL at VS.DB.NVSL.DBRL.Sero.Mgmt@usda.gov.
- 3) The submitting DAV contacts the State animal health official (SAHO) in the State of origin, and State of destination if the test is used for interstate movement, to verify that the State's current animal health regulations allow use of the DPP serologic 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 the NVSL with the samples and submission form. The NVSL does not apply user fees for the secondary DPP retest after 30 days for animals that have 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 red top (clot) tube or a 10-ml serum separation tube labeled with the animal's official identification number.
 - 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.



VS Guidance 6701.4

- 2) Sample processing and handling
 - a. Allow the blood to clot at room temperature.
 - b. Remove the serum from the clot and place it into a new, clean red top (clot) tube. If necessary, centrifuge the clotted blood sample to obtain at least 2 ml of serum. 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 the 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 quickly verify and test the samples.
 - e. Submit a separate red top tube of blood to an approved brucellosis testing laboratory if also testing for brucellosis. The NVSL does not perform routine brucellosis surveillance testing.
- 3) Completing submission forms
 - a. Complete the 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, 10-4A continuation form, record the official identification (and all other ID), species of cervid, age, and gender for each animal tested. Alternatively, record the required information on an attached list.
 - b. Complete the VS Form VS 6-22 (Tuberculosis Test Record) accurately and completely, identifying the cervid species tested, ID, age, and gender.
 - c. Submit the VS Form 6-22 to the SAHO or VS Area Veterinarian in Charge (AVIC) in the State within 5 business days of sample collection.
- 4) Sample submission to NVSL
 - a. Package the serum sample tubes in a shipping container with frozen gel packs. Prevent direct contact between the gel packs and the sample tubes.
 - b. Ship the samples, with the completed form VS 10-4/10-4A, to the NVSL.



VS Guidance 6701.4

- c. Ship serum samples using overnight or 2-day delivery. Samples should be shipped on the day of collection, but must be shipped such that they arrive at NVSL no later than 5 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 the NVSL.
 1. Ship samples so the NVSL receives them Monday through Friday. Do not ship so they could arrive on weekends or holidays.
 2. Sample submission forms must be accurate and complete, and must include a sample collection date.
 3. Samples received by the NVSL more than 5 days after the sample collection date will be classified as invalid.
- d. Ship the serum samples to the NVSL at:

National Veterinary Services Laboratories
1920 Dayton Avenue
Ames, Iowa 50010

C. Reporting Results

- 1) The NVSL tests serum samples using the DPP following test kit instructions and internal NVSL standard operating procedures.
- 2) The NVSL freezes serum samples when DPP kits to perform the testing are not available, and notifies the submitter of the delay.
- 3) The NVSL considers an optical density value below the established cutoff value a negative DPP test result. The NVSL considers an optical density 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) The NVSL reports DPP primary test results to the submitting accredited veterinarian, the respective SAHOs, the respective VS AVICs, and the Cervid Health Program.

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 30 days after collecting the primary sample date. Do not retest a captive cervid that has non-



VS Guidance 6701.4

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 30 days) as reactors.
 - e. Classify samples received by the NVSL more than 5 days after sample collection date as invalid. Samples received on the weekend will be considered to have been received on the following Monday; classify these as invalid if this exceeds 5 days from the collection date. The NVSL considers samples received on a Federal holiday as received the following day; they are classified invalid if this exceeds 5 days from the collection date.
 - f. Classify samples submitted to the NVSL without a collection date or other required information as invalid until the submitting accredited veterinarian provides a corrected copy of the VS10-4 to the SAHO, AVIC, and the 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) The DAV may test groups of animals within a herd using different methods (i.e., test bucks with the DPP and does with the SCT) in routine herd testing. When using different test methods the DAV must complete a separate VS Form 6-22 for each group of animals. Animals or groups that are tested with the DPP as the primary test and are classified suspect or non-negative must be retested with the DPP. Animals or groups that are tested with the SCT as the primary test and are 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.



VS Guidance 6701.4

7. Inquiries

Please direct any inquiries to:

USDA APHIS Veterinary Services

Ruminant Health Center – Cervid Health Program

vs.sprs.cervid.health@aphis.usda.gov

http://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/