

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 one of two official serum tests used for cervids only in the U.S. bovine tuberculosis (TB) eradication program. It detects *Mycobacterium bovis* antibodies in cervid serum. Veterinarians may use the DPP test as either a primary or a secondary test for official TB testing only of elk, red deer, white-tailed deer, fallow deer, and reindeer herds. The National Veterinary Services Laboratories (NVSL) conducts the DPP.

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. 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.

2. Document Status

- A. Review date: April 20, 2021.
- B. This document cancels and replaces Veterinary Services Guidance 6701.2.

3. Reason for Reissuance

VS is reissuing this document after routine review and updating.

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 (2/99) (Attachment on page 7).
 - [VS Form 10-4, Specimen Submission \(8/09\).](#)
 - [VS Form 10-4A, Continuation Sheet for Specimen Submission \(8/09\).](#)

C. Definitions:

Epidemiology Officer: For the purposes of this Guidance, an epidemiology officer is a VS or State employee with epidemiology 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 reactors; to direct and/or participate in field epidemiological investigations; and to manage disease control activities in TB-exposed, suspect, and positive animals and herds.

National Cervid Tuberculosis Disease Specialist: A VS employee who is a member of the Cervid Health Commodity Team with epidemiology 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 cervid herds.

Qualified accredited veterinarian (QAV): A qualified accredited veterinarian, also referred to as a designated accredited veterinarian (DAV), 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. VS will not permit accredited veterinarians without program certifications to perform accredited duties related to that certification. If a QAV allows his or her Category II accreditation to expire, the QAV's program certification also expires and the QAV must requalify for the program certification.

5. Audience

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

6. Guidance

A. Test administration, ordering, and payment:

- 1) Designated accredited veterinarians 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 serological tests such as the DPP. Current DAVs do not need additional training to draw samples for cervid TB serological testing.
- 2) The NVSL can provide serum submission kits if needed; call 515-337-6200 or email NVSL_userfee@aphis.usda.gov. VS does not require the serum submission kit to complete testing. Each kit contains shipping instructions, an ice pack, VS Forms 10-4 (Specimen Submission Form) and 10-4A (Continuation Sheet), and a prepaid

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shipping label to the NVSL. DAVs may also submit serum samples using their own shipping box, which should include ice or gel packs 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. (See the VS Forms 10-4 and 10-4A at

http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf.)

- 3) Before drawing blood samples, the accredited veterinarian will:
 - a. Call the VS Assistant Director (AD) in the animals' State of location to verify that the State's current animal health regulations allow use of the DPP serological tests for bovine TB and confirm completion and submission of all necessary paperwork.
 - b. If testing for interstate movement, verify that the receiving State will also accept the negative serological test results for entry.
- 4) The submitting DAV must pay 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 made out to NVSL with the samples and submission form. VS does not charge for the secondary DPP test after 30 days on animals that have primary positive test results.

B. Sample collection, processing, and shipping for DPP VetTB Assay testing at NVSL:

- 1) Collecting samples:
 - a. Collect blood samples in a 10-ml red top (clot) tube or 10 ml serum separation tube.
 - b. Collect 10 ml of whole blood to obtain 2 ml of serum to submit for testing.
 - c. DO NOT place freshly collected tubes directly on ice.
- 2) Sample processing and handling:
 - a. Allow the blood to clot at room temperature.
 - b. Once clotted, remove the serum from the clot and place it into a new, clean tube. If necessary, centrifuge the clotted blood sample to obtain at least 2 ml of serum. The serum must contain minimal hemolysis. Place the serum tubes in a refrigerator until shipped to the NVSL. Do not freeze the serum, as the test kit instructions state not to do so.
 - c. Number and label submitted tubes with official identification so laboratory personnel can easily correlate the samples to the animal's official ID as listed on

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the submission forms. This helps laboratory personnel verify receiving all the samples and correlate the results to individual animals.

- d. Organize the serum tubes into a tube box in the same order you listed the animals on the submission form or attached list. This allows the receiving laboratory personnel to quickly verify and test the submitted samples. If you need additional sample submission kits from the NVSL, obtain them before collecting the blood samples.
- e. If also testing for brucellosis, submit a separate red top tube of blood to an approved brucellosis testing laboratory. The NVSL does not perform routine brucellosis surveillance testing.

3) Completing submission forms and paperwork:

- a. Fill out the VS Form 10-4 and VS Form 10-4A accurately and completely. (See the VS Forms 10-4 and 10-4A at http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf.)
 1. Include official identification (and all other ID), species of cervid tested (e.g., elk, red deer, white-tailed deer, fallow deer, or reindeer), age, and gender for each animal.
 2. You do not have to write animal identification on the submission form as long you attach a list with all of the above information. If preferred, attach to the VS Form 10-4 a spreadsheet or printout of the animals with official ID corresponding to their blood tube numbers or attach a copy of the completed (all above information) VS Form VS 6-22 (Tuberculosis Test Record) with the corresponding blood tube numbers.
- b. Submit the original VS Forms 10-4 and 10-4A to the NVSL as part of the sample submission described in Section 6 (B)(4) of this Guidance.
- c. Fill out the VS Form VS 6-22 (Tuberculosis Test Record) accurately and completely, identifying the cervid species tested (e.g., elk, red deer, white-tailed deer, fallow deer, or reindeer), ID, age, and gender.
- d. Submit the VS Form 6-22 to the State Veterinarian or VS AD in the State within 5 business days of sample collection.

4) Sample submission to NVSL:

- a. Make sure you pack submitted sample tubes with frozen gel packs. Do not put the tubes directly on the frozen packs in the container used to ship the samples to the NVSL. Refrigerate all serum samples until you ship them. Do not freeze the serum as the test kit indicates not to do so.

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- b. Ship the samples along with the completed form VS 10-4/10-4A to the NVSL.
- c. Ship serum samples using overnight or 2-day delivery. The NVSL recommends shipping the samples as quickly as possible (ideally within 24 to 48 hours), but no more than 72 hours after collection. Do not ship the samples so they will arrive at NVSL on the weekend or a holiday. VS prefers that you use a shipper such as Federal Express or United Parcel Service if not using prepaid NVSL shipping containers, as they deliver directly to the NVSL facility. The U.S. Postal Service does not deliver directly to the NVSL.
- d. Ship the serum samples to the NVSL at:

National Veterinary Services Laboratories
1920 Dayton Avenue
Ames, Iowa 50010

5) Result interpretation and classification of animals:

- a. The NVSL will test serum samples using the DPP following test kit instructions and internal NVSL standard operating procedures.

If the NVSL does not have enough DPP test kits to perform the testing, it will freeze serum samples and notify the submitter of the delay.

- b. The NVSL will consider an optical density value below the established cutoff value a negative DPP test result. The NVSL will consider an optical density value equal to or above the established cutoff value a non-negative DPP test result.
- c. The NVSL will report DPP primary test results to the submitting accredited veterinarian, the respective State animal health officials, the respective State VS AD, and the Cervid Health Commodity Team.
- d. An epidemiology officer will classify the animals based on the DPP test results:
 - 1. Classify animals with a negative result on the DPP test as negative.
 - 2. Classify animals with a non-negative test result to the DPP primary test as suspect and retest with the DPP as a secondary test. Draw a new blood sample from the suspect animal no sooner than 30 days after drawing the initial sample. Do not retest a captive cervid that has non-negative test results to the DPP test using the single cervical tuberculin test (SCT) or comparative cervical tuberculin test (CCT).
 - 3. Classify animals with a negative test result on the DPP secondary test as negative.

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4. Classify animals that are non-negative on two successive DPP tests (primary and secondary after 30 days) as reactors unless VS determines you should use another disease classification.
 - e. The epidemiology officer must justify any exceptions to reactor classification in writing with the concurrence of the National Cervid Tuberculosis Disease Specialist.
 - f. Handle the animal according to its classification, following TB program regulations as stated in 9 CFR part 77, subpart C and the 1999 TB Uniform Methods and Rules.
- C. Herd testing protocol:
- 1) In routine herd testing, you may test different groups of animals within a herd using different methods (i.e., test bucks serologically and does via the skin test). Use a different VS Form 6-22 for each group of animals. Follow up on individual animals testing non-negative to a primary test with a secondary test of the same method (i.e., serological or skin test).
 - 2) In affected herds or herds under investigation, you 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:

National Cervid Tuberculosis Disease Specialist

USDA APHIS Veterinary Services

Sheep, Goat, Cervid, and Equine Health Center – Cervid Health Program

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

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

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0146. The time required to complete this information collection is estimated to average 3 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. **OMB Approved 0579-0146 EXP. 01/2020**

**UNITED STATES DEPARTMENT OF AGRICULTURE
 ANIMAL AND PLANT HEALTH INSPECTION SERVICE
 VETERINARY SERVICES**

**COOPERATIVE STATE - FEDERAL TUBERCULOSIS
 ERADICATION PROGRAM
 TUBERCULOSIS TEST RECORD**

ALL INCOMPLETE RECORDS WILL BE RETURNED FOR COMPLETION

STATE			HERD OWNER - LAST NAME, FIRST MI			K				
COUNTY	TWP	SEC	HERD OWNER COMPLETE ADDRESS			PREVIOUS TEST DATE	VET CODE	TOTAL	REA	SUS
HERD NUMBER						CERTIFICATION FOR PAYMENT			DATE LISTED	
LESION	TEST	D-B	U	<input type="checkbox"/> STATE/FEDERAL EXPENSE <input type="checkbox"/> OWNER EXPENSE			I certify that this test was made and read by me on each of the cattle identified below on the dates and with the results as entered in appropriate spaces, and that when payment is claimed at program expense in accordance with agreement number below, no payment has been or will be received from any other source.			
COUNTY		TOWNSHIP OR DISTRICT		SECTION	FARM NUMBER					

REASON FOR TEST		COMPLETE HERD TEST OF ALL ELIGIBLE ANIMALS		SUMMARY		PRACTITIONER SIGNATURE		TELEPHONE	
AREA	1	RETEST	6	<input type="checkbox"/> YES <input type="checkbox"/> NO NO. ELIGIBLE ANIMALS IN HERD: _____		NEGATIVE		PRACTITIONER NAME (print)	
HERD (RE)ACCREDIT	2	TRACING REG. KILL	7	KIND OF HERD <input type="checkbox"/> DEER <input type="checkbox"/> BISON <input type="checkbox"/> ELK <input type="checkbox"/> OTHER <input type="checkbox"/> CATTLE _____		SUSPECT		INJECTION	
MILK ORDINANCE	3	TRACING REACTORS	8			REACTOR		DATE	
SALE SHOW	4	TRACING EXPOSED	9	METHOD OF TEST <input type="checkbox"/> CAUDAL FOLD (CFT) <input type="checkbox"/> SNG CERVICAL (SCT) (CERVID) <input type="checkbox"/> CERVICAL (CT) (BOVINE) _____		TOTAL		OBSERVATION	
IMPORTED	5	OTHER	10					DATE	
								TUBERCULIN SERIAL NUMBER	

1	OFFICIAL IDENTIFICATION NUMBER	AGE	BREED	SEX	RESULTS		1	OFFICIAL IDENTIFICATION NUMBER	AGE	BREED	SEX	RESULTS	
					SIZE	NBS						SIZE	NBS
	1.							13.					
	2.							14.					
	3.							15.					
	4.							16.					
	5.							17.					
	6.							18.					
	7.							19.					
	8.							20.					
	9.							21.					
	10.							22.					
	11.							23.					
	12.							24.					

I hereby acknowledge receiving a copy of this record which I have examined and find correct.

RT - Retag NA - Natural Addition PA - Purchased Addition	N - Negative R - Reactor S - Suspect	OWNER SIGNATURE	DATE	THIS AUTHORIZATION TO TEST EXPIRES:
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