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

**1. Purpose and Background**

The CervidTB Stat-Pak® test was approved for use as a primary test for TB in cervids in 2012 with the Dual Path Platform VetTB Assay (DPP) as a secondary test after 30 days on those animals with a primary positive test result. The manufacturer has since stopped producing the Stat-Pak for cervids. Veterinary Services (VS) is approving the DPP as both the primary test and subsequent secondary test for official program testing to diagnose TB in farmed and captive elk, red deer, white-tailed deer, fallow deer, and reindeer when the test is conducted at the National Veterinary Services Laboratories (NVSL) or an APHIS-approved laboratory.

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 March 21, 2017
- B. This document replaces VS Guidance 6701.1, which is cancelled.

**3. Reason for Reissuance**

Stat-Pak production has been discontinued by its manufacturer. Thus, the DPP VetTB Assay® will replace Stat-Pak as the primary test for bovine TB in cervids. It will continue to serve as the secondary test after 30 days.

**4. Authority and References**

- A. Authorities (*Code of Federal Regulations* (CFR) and U.S. Code (U.S.C.)):
  - [7 U.S.C. 8301–8317](#)
  - [7 CFR 2.22](#)
  - [7 CFR 2.80](#)
  - [7 CFR 371.4](#)
  - [9 CFR 77.33](#)

**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)

**5. Audience**

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

**6. Guidance**

**A. Test Administration, Ordering, and Payment**

- 1) Accredited veterinarians who receive supplemental training and are certified as Designated Accredited Veterinarians (DAV) (VS-accredited veterinarians specially trained and approved to conduct specific tuberculosis program tests and activities) may perform the single cervical tuberculin skin test (SCT) in cervids. They are also authorized to 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) Cervid DPP serum submission kits may be ordered from NVSL by calling 515-337-6200 or emailing NVSL\_userfee@aphis.usda.gov. Each kit contains shipping instructions, an ice pack, VS Forms 10-4 (Specimen Submission Form) and 10-4A (Continuation Sheet), and a prepaid shipping label to 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. VS Forms 10-4 and 10-4A have been approved by the Office of Management and Budget for submitting all diagnostic samples to NVSL.
- 3) Before drawing blood samples, the veterinarian will:
  - a. Call the APHIS VS Assistant District Director (ADD) in the State where the animals are located to verify that the State's current animal health regulations allow the DPP serological tests for bovine TB to be used and confirm that all necessary paperwork is being completed and submitted.
  - 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. This may be done by setting up an account with the NVSL business office by calling 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. There is no charge for the secondary DPP test after 30 days on those animals that have primary positive test result.

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- 5) Definition: National Cervid TB Epidemiologist – for the purposes of Cervid TB testing, a national cervid TB epidemiologist is a Federal or State veterinary epidemiologist designated by APHIS to evaluate and interpret cervid TB test results (serology and skin testing) and to classify cervids as suspects or reactors.
- B. Sample Collection, Processing, and Shipping for DPP VetTB Assay® Testing at NVSL
- 1) Collecting samples
    - a. Blood samples are to be collected in a 10-ml red top (clot) tube or 10 ml serum separation tubes.
    - b. 10 ml of whole blood needs to be collected to obtain 2 ml of serum to be submitted 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. It is essential that the serum contains minimal hemolysis. Place the serum tubes in a refrigerator until shipped to NVSL. Do not freeze the serum.
    - c. Submitted tubes should be numbered and labeled with official identification so they can be easily correlated to the animal's official ID as listed on the submission forms. This helps laboratory personnel verify all the samples were received and correlate the results to individual animals.
    - d. Ship serum samples by either first- or second-day delivery. NVSL recommends shipping the sample as quickly as possible (ideally 24-48 hours), but no more than 72 hours after collection.
    - e. If also testing for brucellosis, submit a separate red top tube of blood to an approved brucellosis testing laboratory.
  - 3) Completing submission forms and paperwork
    - a. Fill out the VS Form 10-4 and VS Form 10-4A accurately and completely. (See the attached sample VS Forms 10-4 and 10-4A. These forms can also be found at [http://www.aphis.usda.gov/library/forms/pdf/VS\\_Form10\\_4.pdf](http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf).)

1. 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 must be included.
  2. Animal identification does not need to be written on the submission form as long as there is an attached 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 NVSL as part of the sample submission described in Section 6 (B)(4) of this guidance document.
  - 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 ADD in your State within 5 business days of sample collection.
- 4) Sample submission to NVSL
- a. Organize the serum tubes into a tube box in the same order as animals are listed on the submission form or attached list. This allows the receiving laboratory personnel to quickly verify and test the submitted samples. If sample submission kits from NVSL are needed, they should be obtained before collecting the blood samples.
  - b. Make sure that submitted sample tubes are packed with frozen gel packs, but not in direct contact with the frozen packs in the container used to ship the samples to NVSL. Refrigerate all serum samples until they are shipped. Do not freeze the serum.
  - c. Ship the samples along with the completed form VS 10-4/10-4A to NVSL as described in this guidance document. If shipping materials are needed, please contact NVSL at 515-337-6200 or [NVSL\\_userfee@aphis.usda.gov](mailto:NVSL_userfee@aphis.usda.gov) to order a serum submission kit.
  - f. Serum samples should be shipped using overnight or 2-day delivery. NVSL recommends shipping the sample as quickly as possible (ideally 24-48 hours), but no more than 72 hours after collection. 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. Do not ship the samples so they will arrive at NVSL on the weekend or a holiday. The U.S. Postal Service does not deliver directly to NVSL.

5) Sample testing

- a. Serum samples will be tested using the DPP test following test kit instructions and internal NVSL standard operating procedures.
- b. If the DPP primary test result obtained is above the optical density reader value cutoff point that has been established, the test will be called positive.
- c. Results of the DPP primary test will be reported to the submitting accredited veterinarian, the respective State animal health official, ADD, and District epidemiologist of the State where the animals are located. The District epidemiologist will report to the National Cervid TB epidemiologist.
- d. The District Epidemiologist or National Cervid TB Epidemiologist will classify the animals.
  1. Animals negative on the DPP primary test should be classified as negative.
  2. Animals non-negative on the DPP primary test should be classified as suspect unless the District or National Cervid TB staff epidemiologist determines that a reactor classification is warranted.
  3. Animals classified as suspect by a DPP primary test may be retested with the DPP secondary test with a new blood sample drawn no sooner than 30 days after the initial sample was obtained.
  4. Animals testing negative on the DPP secondary test should be classified as negative.
  5. Animals that are non-negative on two successive DPP tests (primary and secondary after 30 days) should be classified as reactor.
- d. Any exceptions to reactor classification must be justified by the District epidemiologist in writing and have the concurrence of the National Cervid TB epidemiologist.
- e. The animal is then handled according to its classification following TB program regulations as stated in the 1999 TB UM&R and 9 CFR part 77, subpart C.

**C. TB Program Testing Protocol**

- 1) If an animal tests non-negative to the DPP primary serological test, it must be retested after 30 days using the DPP serological test as the secondary test.
- 2) If an animal tests non-negative to the SCT, it must be retested using the comparative cervical tuberculin skin test (CCT) as the secondary test.
- 3) The CCT will not be used as a primary test for any animals.
- 4) If parallel testing is performed with the DPP and SCT tests, it must be completed with permission from and consultation with the District epidemiologist and the National Cervid TB epidemiologist. The testing protocol, timing of different tests, interpretation of the tests, classification of the animals, and disposition of the animals must be determined in the protocol before the testing. Secondary tests to any non-negative primary test must follow instructions 1) and 2) above.
- 5) In routine herd testing, different groups of animals within a herd may be tested using the different methods (i.e., bucks tested serologically and does tested via the skin test) if a different VS Form 6-22 is used for each group of animals. However, individual animals testing non-negative to a primary test must be followed up with a secondary test of the same test method as required in 1) and 2) above.
- 6) In affected herds or herds under investigation, a testing protocol using serological and skin tests separately or in series or parallel may be devised and used by the designated or District epidemiologist and National Cervid TB epidemiologist in consultation.

**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  
Phone: 970-494-7317  
[http://www.aphis.usda.gov/animal\\_health/animal\\_diseases/tuberculosis/](http://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/)

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

VS Guidance

6701.2

Date 03/21/14

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 0579-0090, 0101, and 0212. The time required to complete this information collection is estimated to average 5 hours per response for 0579-0090, 1 hour per response for 0579-0101, and 333 hours per response for 0579-0212, 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  
0579-0090, 0579-0101,  
and 0579-0212

<b>UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE NATIONAL VETERINARY SERVICES LABORATORIES P.O. BOX 844, 1920 DAYTON AVENUE, AMES, IA 50010 (515) 337-7514</b>		<b>SPECIMEN SUBMISSION</b>			PAGE OF				
<b>INSTRUCTIONS: Use a separate form for each species and each owner/broker. See "Instructions for Completing VS Form 10-4" for definitions.</b>									
1. SUBMITTER NAME (including Business Name)		2. NVSL SUBMITTER ID	3. NAME OF OWNER		<input type="checkbox"/> Check if wildlife (no owner)				
EMAIL ADDRESS		OWNER CITY		STATE/COUNTRY					
PHONE NO.		FAX NO.		<b>4. LOCATION OF ANIMALS</b>					
MAILING ADDRESS (Street, City, State, ZIP Code)		PREMISES ID		COUNTY					
				STATE/COUNTRY					
<b>5. PAYMENT METHOD</b>									
<input type="checkbox"/> USER FEE ACCOUNT NO.		<input type="checkbox"/> CHECK/MONEY ORDER (Enclosed, payable to USDA in US dollars)		<input type="checkbox"/> CREDIT CARD					
				Number: Exp. Date:					
6. HERD/FLOCK SIZE	9. EXAMINATIONS REQUESTED			10. COLLECTED BY					
7. NO. IN HERD/FLOCK AFFECTED				11. DATE COLLECTED					
8. NO. IN HERD/FLOCK DEAD				12. AUTHORIZED BY					
13. PURPOSE OF SUBMISSION (See instructions for definitions)				14. COUNTRY OF ORIGIN/DESTINATION					
<input type="checkbox"/> Interstate Movement	<input type="checkbox"/> Import	<input type="checkbox"/> TB	<input type="checkbox"/> Reagent Evaluation	15. REFERRAL NUMBER					
<input type="checkbox"/> Export	<input type="checkbox"/> FAD/EP Diagnostic	<input type="checkbox"/> General Diagnostic	<input type="checkbox"/> NVSL Intralab						
<input type="checkbox"/> Pre-Import	<input type="checkbox"/> Surveillance	<input type="checkbox"/> Developmental Research							
<b>16. PRESERVATION</b>									
<input type="checkbox"/> None <input type="checkbox"/> Ice Pack <input type="checkbox"/> Dry Ice <input type="checkbox"/> Formalin <input type="checkbox"/> Borax <input type="checkbox"/> Alcohol <input type="checkbox"/> Other (Specify)									
17. SPECIMENS SUBMITTED ("X" applicable item(s))					18. TOTAL NUMBER OF SPECIMENS SUBMITTED				
<input type="checkbox"/> Blood	<input type="checkbox"/> Feces	<input type="checkbox"/> Parasite	<input type="checkbox"/> Serum	<input type="checkbox"/> Tissue (specify)	<input type="checkbox"/> Whole Animal				
<input type="checkbox"/> Culture	<input type="checkbox"/> Feed	<input type="checkbox"/> Plant	<input type="checkbox"/> Soil	<input type="checkbox"/> Urine	<input type="checkbox"/> Fetus				
<input type="checkbox"/> Extract	<input type="checkbox"/> Milk	<input type="checkbox"/> Semen	<input type="checkbox"/> Swab (specify)	<input type="checkbox"/> Water	<input type="checkbox"/> DNA/RNA				
19. SPECIES OR SOURCE ("X" ONLY one)					20. NUMBER OF ANIMALS SAMPLED				
<input type="checkbox"/> Cattle	<input type="checkbox"/> Goat	<input type="checkbox"/> Chicken	<input type="checkbox"/> Bison	<input type="checkbox"/> Fish	<input type="checkbox"/> Other (specify)				
<input type="checkbox"/> Swine	<input type="checkbox"/> Horse	<input type="checkbox"/> Turkey	<input type="checkbox"/> Deer (specify)	<input type="checkbox"/> Environment					
<input type="checkbox"/> Sheep	<input type="checkbox"/> Donkey	<input type="checkbox"/> Other bird (specify)	<input type="checkbox"/> Elk	<input type="checkbox"/> Reagent					
<b>21. IDENTIFICATION (See instructions &lt;250 samples per form)</b>									
<b>IDENTIFICATION</b>			<b>IDENTIFICATION</b>						
Sample ID	Animal ID	Breed	Age	Sex	Sample ID	Animal ID	Breed	Age	Sex
22. ADDITIONAL DATA (History, clinical signs, post mortem findings, remarks, tentative diagnosis, special instructions. Use additional sheets if necessary).									
23. SIGNATURE OF SUBMITTER AND DATE					<b>NVSL USE ONLY</b>				
X									
<b>NVSL USE ONLY</b>									
CONDITION	PRIORITY	DISTRIBUTION	RECEIVED BY						

VS FORM 10-4  
AUG 2009



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

VS Guidance

6701.2

Date 03/21/14

According to the Paperwork Reduction Act of 1995, no persons are 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 0579-0084. The time required to complete this information collection is estimated to average .3 hours per response, including the time for reviewing instructions, search existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

STATE	<b>ALL INCOMPLETE RECORDS WILL BE RETURNED FOR COMPLETION</b> COOPERATIVE STATE - FEDERAL TUBERCULOSIS ERADICATION PROGRAM	FORM APPROVED OMB NO. 0579-0084
<b>TUBERCULOSIS TEST RECORD</b>		

COUNTY	TWP	SEC	HERD OWNER'S NAME - LAST	FIRST	MI	PREVIOUS TEST DATE	VET CODE	TOTAL	REA	SUB
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HERD NUMBER	HERD OWNER'S COMPLETE ADDRESS	CERTIFICATION FOR PAYMENT <input type="checkbox"/> State/Federal Expense <input type="checkbox"/> Owner's Expense	DATE LISTED
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LESION	TEST	D-B	U	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 the appropriate spaces. 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.							
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COUNTY	TOWNSHIP OR DISTRICT	SEC.	FARM NO.	SUMMARY NEG-ATIVE SUB-PECT REAC-TOR TOTAL				PRACTITIONER'S SIGNATURE	TELEPHONE NO
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REASON FOR TEST		COMPLETE HERD TEST OF ALL ELIGIBLE ANIMALS				PRACTITIONER'S NAME (Please print)		AGREE CODE
AREA	1	RETEST	8	<input type="checkbox"/> YES <input type="checkbox"/> NO    NO. ELIGIBLE ANIMALS IN HERD		PRACTITIONER'S SIGNATURE		AGREE CODE

HERD (RE) ACCREDIT	2	TRACING REG. KILL	7	KIND OF HERD		INJECTION	DATE	HOUR
				<input type="checkbox"/> DEER <input type="checkbox"/> ELK	<input type="checkbox"/> CATTLE <input type="checkbox"/> BISON <input type="checkbox"/> OTHER			

MILK ORDINANCE	3	TRACING REACTORS	8	METHOD OF TEST		OBSERVATION	DATE	HOUR
				<input type="checkbox"/> CAUDAL FOLD (CFT)	<input type="checkbox"/> SNG CERVICAL (CST) (Cervid)			

SALE-SHOW	4	TRACING EXPOSED	9	METHOD OF TEST		REACTORS TAGGED AND BRANDED	DATE	SIGNATURE	AGREE CODE
				<input type="checkbox"/> CERVICAL (CT) (Bovine)	<input type="checkbox"/> OTHER				

IMPORTED	5	OTHER	10	AGE	BREED	SEX	RESULTS	REACTOR TAG NO.	1	IDENTIFICATION NUMBER	AGE	BREED	SEX	RESULTS	REACTOR TAG NO.
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1	IDENTIFICATION NUMBER	AGE	BREED	SEX	RESULTS	REACTOR TAG NO.	1	IDENTIFICATION NUMBER	AGE	BREED	SEX	RESULTS	REACTOR TAG NO.
					SIZE    NRS							SIZE    NRS	
1							16						
2							17						
3							18						
4							19						
5							20						
6							21						
7							22						
8							23						
9							24						
10							25						
11							26						
12							27						
13							28						
14							29						
15							30						

RT - Retag NA - Natural Addition PA - Purchased Addition	N - Negative S - Suspect R - Reactor	I hereby acknowledge receiving a copy of this record which I have examined and find correct. DATE _____ OWNER'S SIGNATURE _____	THIS AUTHORIZATION TO TEST EXPIRES: _____
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