



United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

Sample Collection Protocol
Dual Path Platform (DPP) test evaluation in Mule and Sika Species

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) is evaluating the effectiveness of the Dual Path Platform (DPP) as an official primary test and secondary test for bovine tuberculosis (TB) program use in captive and free ranging mule deer (*Odocoileus hemionus*) and sika deer (*Cervus nippon*).

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Sample collection procedures for Mule and Sika DPP Evaluation

A. Test administration, ordering, and payment:

- 1) Designated accredited veterinarians (DAV's) may perform the DPP sample collection for Mule and Sika deer
- 2) The National Veterinary Services Laboratory (NVSL) can provide serum submission kits if needed; call 515-337-6200 or email NVSL_userfee@aphis.usda.gov. The serum submission kit is not required 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 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 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/V_S_Form10_4.pdf.)
- 3) Before drawing blood samples, the accredited veterinarian will:
 - a. Contact 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 in Mule or Sika deer.
 - b. Contact the State Animal Health Official (SAHO) if testing is for interstate movement. Verify the receiving State will accept the DPP serologic test results for entry.

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

- 1) Sample collection:
 - 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



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serum must contain minimal hemolysis. Place the serum tubes in a refrigerator until shipped to the NVSL. Do not freeze the serum.

- 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 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) Sample submission forms and TB test Record:

- 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 http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf.)
 1. Include official identification (and all other ID), species of cervid tested (Mule, Sika), 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 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 (Mule or Sika), 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. 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 shipping. Do not freeze the serum.
- 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. The submitting veterinarian is responsible for payment of DPP tests conducted at NVSL. Three options are available for payment: call the business office 515-337-6200 and set up

an account, provide a credit card number on the submission form, or include a check made out to NVSL with the samples and submission form. Diagnostic test rates can be found at

https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/AmesDiagnosticTestingCatalog.pdf

VS does not charge for the secondary DPP test after 30 days on animals that have primary positive test results.

- e. Ship the serum samples to the NVSL at the following address:

**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, the NVSL will freeze serum samples and notify the submitter of the delay.
- b. The NVSL will report an optical density value below 200 a negative DPP test result. The NVSL will report an optical density value equal to or above 200 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.

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