

Chronic Wasting Disease

Program Standards

UNITED STATES DEPARTMENT OF
AGRICULTURE

Chronic Wasting Disease Program Standards

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

**Chronic Wasting Disease (CWD)
Program Standards**

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Introduction

The goal of the CWD Herd Certification Program (HCP) is to provide a consistent, national approach to control the incidence of CWD in farmed cervids and prevent the interstate spread of CWD. Achieving this goal will ultimately result in several important long-term outcomes, including:

- 1) Healthy cervids (both farmed and wild populations) with a reduced risk of CWD.
- 2) Increased confidence that HCP-certified herds are low risk for CWD infection.
- 3) Strong trade of cervid animals and products (increased market confidence).
- 4) Reduced risk of transmission from, and environmental contamination by, CWD-positive herds.

The HCP is a cooperative effort between the Animal and Plant Health Inspection Service (APHIS), State animal health and wildlife agencies, and farmed cervid owners. APHIS coordinates with these State agencies to encourage cervid owners to certify their herds and comply with the CWD Herd Certification Program Standards.

This goal is accomplished through the establishment of the national CWD herd certification program and interstate movement requirements for CWD-susceptible cervids found in title 9 of the *Code of Federal Regulations* (CFR) parts 55 and 81. These regulations are written as performance-based regulations that describe the legally required outcomes.

The Program Standards provide detailed descriptions of acceptable methods for complying with the legal requirements in 9 CFR parts 55 and 81:

Part A, Herd Certification Program, describes acceptable methods to meet the minimum requirements to certify farmed cervid herds for interstate movement.

Part B, Guidance on Response to CWD, describes acceptable methods to meet the minimum requirements to respond to the finding of CWD in farmed cervid herds.

The methods in these Program Standards have been approved by the APHIS Administrator. Alternatively, States may propose other methods/approaches to meet the regulatory requirements. These alternative proposals should be submitted in writing to APHIS for approval. States may also have additional or stricter requirements that exceed the minimum requirements described in the CWD regulations.

These Program Standards will be reviewed regularly by APHIS and, as appropriate, representatives of the cervid industry and State and Federal agencies. A notice will be published in the *Federal Register* to inform stakeholders of any revisions APHIS plans to the Program Standards.

Definitions

Accredited Veterinarian: A veterinarian approved by the Administrator in accordance with 9 CFR part 161 to perform functions required by cooperative State-Federal disease control programs specified in title 9 CFR.

Administrator: The Administrator of the Animal and Plant Health Inspection Service, or any person authorized to act for the Administrator.

Animal: Any farmed or captive deer, elk, or moose.

Animal Identification Number (AIN): A numbering system for the official identification of individual animals in the United States that provides a nationally unique identification number for each animal. The AIN consists of 15 digits with the first 3 being the country code (840 for the United States or a unique country code for any U.S. territory that has such a code and elects to use it in place of the 840 code).

Animal and Plant Health Inspection Service (APHIS): The Animal and Plant Health Inspection Service of the United States Department of Agriculture.

Annual Removals: All adults (12 months or older) removed or lost from inventory *for any reason* since the previous annual inventory.

APHIS Employee: Any individual employed by APHIS who is authorized by the Administrator to do any work or perform any duty in connection with the control and eradication of disease.

Approved State: A State determined by the Administrator to have an Approved State CWD Herd Certification Program per 9 CFR part 55.

Approved State CWD Herd Certification Program: A program operated by a State government for certification of cervid herds with respect to CWD the Administrator has determined meets the requirements of 9 CFR part 55.

Approved Laboratory: A diagnostic laboratory approved by the Administrator to conduct official tests for CWD in accordance with 9 CFR 55.8.

Assistant District Director (AD): The APHIS veterinary official assigned by the Administrator to supervise and perform the official APHIS animal health work in the APHIS District and corresponding State or States.

Certified Herd: A Certified herd that has enrolled in a Herd Certification Program and met the minimum requirements defined in 9 CFR part 55.

Certified CWD Sample Collector: An individual who has completed appropriate training and is certified by his or her State to perform collection, submission, and preservation of samples for CWD testing in farmed cervids.

Cervid: All members of the family Cervidae and hybrids, including deer, elk, moose, caribou, reindeer, and related species. For the purposes of this document, the term “cervid” refers specifically to cervids susceptible to CWD. These are animals in the genera *Odocoileus*, *Cervus*, *Alces*, and their hybrids, i.e. deer, elk, and moose.

NOTE: APHIS proposes to amend the CFR in the future by removing the list of susceptible species from the definition of “cervid” and instead listing the genera APHIS considers susceptible to CWD. In anticipation of this change, we are adding a definition of “CWD-susceptible cervid species” to this revision of the Program Standards. These changes will give APHIS more flexibility to change the list of species considered susceptible to CWD as evidence becomes available.

Chronic Wasting Disease (CWD): A transmissible spongiform encephalopathy of cervids. Clinical signs in affected animals include, but are not limited to: Loss of body condition, behavioral changes, excessive salivation, increased drinking and urination, depression, and eventual death.

Commingled, Commingling: Animals are commingled if they have direct contact with each other, have less than 10 feet of physical separation, or share equipment, pasture, or water sources/watershed (i.e., indirect contact). Animals are considered to have commingled if they have had such contact with a CWD-positive animal or contaminated premises within the last 5 years.

CWD-Exposed Animal: An animal that is part of a CWD-positive herd, or that has been exposed to a CWD-positive animal or contaminated premises within the previous 5 years.

CWD-Exposed Herd: A herd in which a CWD-positive animal has resided within 5 years prior to that animal’s diagnosis with CWD, as determined by an APHIS employee or State representative.

CWD Herd Certification Program: This program, established in 9 CFR part 55.

CWD-Positive Animal: An animal that has had a diagnosis of CWD established through official confirmatory CWD testing conducted by the National Veterinary Services Laboratories (NVSL).

CWD-Positive Herd: A herd in which a CWD-positive animal resided at the time it was diagnosed and which has not been released from quarantine.

CWD Source Herd: A herd identified through testing, tracebacks, and/or epidemiological evaluations to be the source of CWD-positive animals identified in other herds.

CWD-Susceptible Cervid Species: APHIS identifies CWD-susceptible species based on scientific evidence of natural infection or experimental infections through intranasal and/or oral routes. This includes animals in the genera *Odocoileus*, *Cervus*, and *Alces* and their hybrids, i.e. deer, elk, and moose. Specifically, the following are considered to be susceptible to CWD: White-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*), and any associated subspecies. It also includes North American elk or wapiti (*Cervus canadensis*), red deer (*Cervus elaphus*), and Sika deer (*Cervus nippon*).

NOTE: APHIS proposes to amend the definition of “cervid” in the CFR in the near future by removing the list of susceptible species from the definition. To accommodate this future change, we are adding the definition of “CWD-susceptible cervid species” to this revision of the Program Standards. In the future, APHIS anticipates adding the genera *Rangifer* and *Muntiacus* to the list of CWD-susceptible species when the CFR is amended.

CWD-Suspect Animal: An animal for which an APHIS employee or State representative has determined that unofficial CWD test results, laboratory evidence, or clinical signs suggest a diagnosis of CWD, but for which official laboratory results have been inconclusive or not yet conducted.

CWD-Suspect Herd: A herd for which unofficial CWD test results, laboratory evidence, or clinical signs suggest a diagnosis of CWD, as determined by an APHIS employee or State representative, but for which official confirmatory laboratory results have been inconclusive or not yet conducted.

Deer, Elk, and Moose: All animals in the genera *Odocoileus*, *Cervus*, *Alces*, and hybrids of these species.

Deputy Administrator: The Veterinary Services (VS) Deputy Administrator or any other official to whom the Administrator has delegated authority to act as the Deputy Administrator.

Designated CWD HCP Coordinator: The epidemiology officer designated by the State to coordinate CWD HCP activities in the State, in accordance with 9 CFR 55.23. The coordinator may be a State representative selected by the State or an APHIS employee identified in consultation with APHIS.

Enrollment Date: The enrollment date for any herd that joins the CWD Herd Certification Program after August 13, 2012 will be the date the herd is approved for participation unless an exception listed in 9 CFR 55.22(a)(1) applies.

Epidemiologically-Linked Herd: A herd in which a CWD-exposed animal resided (or currently resides) and CWD testing has not been completed on the CWD-Exposed Animal.

Epidemiology Officer: A State representative or APHIS employee with epidemiology training or experience who is designated to:

- 1) Make decisions about the use and interpretation of CWD diagnostic test results.
- 2) Participate in field epidemiological investigations.
- 3) Manage disease control activities in CWD-exposed, suspect, positive, and source herds.
- 4) Ensure data quality and accuracy for the CWD program in their State or District.

Farmed or Captive: Privately or publicly maintained or held for economic or other purposes within a perimeter fence or confined area, or captured from a free-ranging population for interstate movement and release.

Herd: One or more animals that are:

- 1) Under common ownership or supervision and are grouped on one or more parts of any single premises (lot, farm, or ranch) or
- 2) All animals under common ownership or supervision on two or more premises which are geographically separated but on which animals have been interchanged or had direct or indirect contact with one another (i.e. commingled).

Herd Inventory: A herd owner's written or electronic record of all of the animals belonging to a herd including each animal's species, date of birth, age, sex, date of acquisition and source (for animals not born into the herd), date of disposal and destination (for animals removed from the herd), and all individual identification numbers (from tags, tattoos, electronic implants, etc.). A physical herd inventory refers to the process by which an APHIS employee, State representative, or accredited veterinarian reconciles a herd owner's records with the animals and their identifications physically present in the herd.

Herd Plan: A written herd and/or premises management agreement developed by APHIS in collaboration with the herd owner, State representatives, and other affected parties. The herd plan will not be valid until it has been reviewed and signed by the Administrator, the State representative, and the herd owner. A herd plan sets out the steps to be taken to control spread of CWD from a CWD-positive herd, to control the risk of CWD in a CWD-exposed or CWD-suspect herd, or to prevent introduction of CWD into that herd or any other herd. A herd plan will require specified means of identification for each animal in the herd; regular examination of animals in the herd by a veterinarian for clinical signs of disease; reporting to a State or APHIS representative of any clinical signs of a central nervous system disease or chronic wasting condition in the herd; maintaining records of the acquisition and disposition of all animals entering or leaving the herd, including the date of acquisition or removal, name and address of the person from whom the animal was acquired or to whom it was disposed; and the cause of death, if the animal died while in the herd.

A herd plan may also contain additional requirements to prevent or control the possible spread of CWD, depending on the particular circumstances of the herd and its premises, including but not limited to depopulation of the herd, specifying the time for which a premises must not contain cervids after CWD-positive, -exposed, or -suspect

animals are removed from the premises; fencing requirements; selective culling of animals; restrictions on sharing and movement of possibly contaminated livestock equipment; premises cleaning and disinfection requirements; or other requirements. A herd plan may be reviewed and changes to it suggested at any time by any party signatory to it, in response to changes in the situation of the herd or premises or improvements in understanding the nature of CWD epidemiology or techniques to prevent its spread. The revised herd plan will become effective after it is reviewed by the Administrator and signed by the Administrator, the State representative, and the herd owner.

Herd Status: The status of a herd assigned under the CWD Herd Certification Program in accordance with 9 CFR 55.24. Herd status is based on the number of years of monitoring without evidence of the disease and any specific determinations that the herd has contained or has been exposed to a CWD-positive, -exposed, or -suspect animal.

Hunt Facility: A privately owned ranch or other premises selling commercial hunts.

Limited Contact: Any brief, incidental contact between cervids from different herds such as occurs in sale or show rings and alleyways at fairs, livestock auctions, sales, shows, and exhibitions. Limited contact does not include penned animals having less than 10 feet of physical separation or contact through a fence; or any activity where uninhibited contact occurs such as sharing an enclosure, a section of a transport vehicle, sharing equipment, food, or water sources; or contact with bodily fluids or excrement. Pens at fairs, livestock auctions, sales, shows, and exhibitions should be thoroughly cleaned and all organic material removed after use and before holding another animal.

Location-Based Numbering System: The location-based number system combines a State- or Tribal-issued location identification (LID) number or a premises identification number (PIN) with a producer's unique livestock production numbering system to provide a nationally unique and herd unique identification number for an animal.

Official Animal Identification: A device or means of animal identification approved for use by APHIS to uniquely identify individual animals. Examples of approved official animal identification devices are listed in 9 CFR 55.25. The official animal identification must include a nationally unique animal identification number that adheres to one of the following numbering systems:

- 1) NUES (the CWD program allows the use of either the eight-character or nine character format for cervids);
- 2) AIN;
- 3) Premises-based number system, which combines an official PIN with a producer's livestock production numbering system (both must appear on the official tag) to provide a unique identification number; or
- 4) Any other numbering system approved by the Administrator for the identification of animals in commerce.

Official CWD Test: Any test for the diagnosis of CWD approved by the Administrator and conducted in a laboratory approved by the Administrator in accordance with 9 CFR 55.8.

Owner: An individual, partnership, company, corporation, or other legal entity that has legal or rightful title to an animal or herd of animals.

Premises: A location where livestock or poultry are housed or kept.

Premises identification number (PIN): A nationally unique number assigned by a State, Tribal, and/or Federal animal health authority to a premises that is, in the judgment of the State, Tribal, and/or Federal animal health authority, a geographically distinct location from other premises. The premises identification number is associated with an address, geospatial coordinates, and/or location descriptors which provide a verifiably unique location. The premises identification number may be used with a producer's own livestock production numbering system to provide a unique identification number for an animal. It may also be used as a component of a group/lot identification number. The premises identification number may consist of:

- 1) The State's two-letter postal abbreviation followed by the premises' assigned number or
- 2) A seven-character alphanumeric code, with the right-most character being a check digit. The check digit number is based on the ISO 7064 Mod 36/37 check digit algorithm.

Quarantine (or Hold Order): An order issued by a State restricting movement of animals from or onto a premises for a given period of time.

State Representative: A person regularly employed in the animal health work of a State and who is authorized by the State to perform the function involved. This could include a wildlife agency official.

Status Date: The day, month, and year on which the respective State or APHIS employee approves a change in the status of a herd in regard to CWD.

Suspect Positive ELISA: The result of a CWD ELISA when either of the duplicate wells from repeat testing at an approved laboratory of the initial reactor ELISA homogenate by the Bio-Rad ELISA is above the prescribed cutoff OD values. These results must be confirmed by NVSL.

Suspect Positive Immunohistochemistry (IHC) Test: The result of an approved CWD IHC test conducted at an approved laboratory in which the presumptive identification of abnormal protease resistant prion protein (PrPres) has been detected in the tissue samples and that result must be confirmed positive by NVSL.

Suspended Status: A temporary status given to a herd that is being epidemiologically assessed for CWD-exposure.

Traceback Herd: A herd in which a CWD-positive animal formerly resided.

Trace-Forward Herd: A herd that has received exposed animals from a CWD-positive herd within 5 years prior to the diagnosis of CWD in the positive herd or from the identified date of entry of CWD into the positive herd.

Veterinary Services (VS): The APHIS unit authorized to conduct prevention, control, and eradication programs for diseases of livestock and poultry.

Part A. Herd Certification Program

1. State Participation

1.1 Participating Approved State: Application and Requirements

States must submit an application, including a completed VS Form 11-2 and supporting documentation, describing their ability to meet the national CWD HCP requirements. In reviewing a State's eligibility to be designated as an Approved State, the Administrator or designee will evaluate the State statutes, regulations, and policies pertaining to the State agency responsible for farmed or captive cervids, as well as relevant reports and publications of the State animal health and/or wildlife agencies. The Administrator or designee will also review a written statement from the State representative describing their CWD control and cervid herd certification activities in farmed or captive cervids. When assessing whether the State program qualifies, the Administrator or his or her designee determines whether the State:

- 1) Has the authority, based on State law or regulation, to quarantine and restrict intrastate movement of all CWD-positive, CWD-suspect, and CWD-exposed animals.
- 2) Has the authority, based on State law or regulation, to require the prompt reporting of any animal suspected of having CWD; and to forward test results for any animals tested for CWD to APHIS employees and State representatives.
- 3) Has signed a Memorandum of Understanding (MOU) with APHIS that delineates the respective roles of each party in CWD HCP implementation. A link to the MOU template can be found in Appendix I.
- 4) Has placed all known CWD-positive, CWD-exposed, and CWD-suspect animals and herds under movement restrictions, allowing movement only for destruction with appropriate carcass disposal, or under permit.
- 5) Has effectively implemented policies to:
 - A. Promptly investigate all animals reported as CWD-suspect animals within 7 business days of official notification to the State.
 - B. Designate herds as CWD-positive, CWD-exposed, or CWD-suspect and promptly restrict movement of animals from such herds after an APHIS employee or State representative determines that the herd contains or has contained a CWD-positive animal.
 - C. Remove herd movement restrictions only after completion of a herd plan.

- D. Conduct an epidemiological investigation of CWD-positive, CWD-exposed, and CWD-suspect herds that includes the designation of suspect and exposed animals and that identifies animals to be traced in accordance with 9 CFR part 55 and Part B of these CWD Program Standards).
 - E. Initiate and conduct tracebacks of CWD-positive animals in affected herds and trace-outs of CWD-exposed animals.
 - F. Report, within 45 business days following notification of a CWD-positive animal, any out-of-State traces to the appropriate State representative and APHIS employee.
 - G. Conduct tracebacks based on slaughter sampling. Investigation should be initiated promptly following notification of a CWD-positive animal at slaughter.
- 6) Effectively monitors and enforces State quarantines or hold orders and State reporting laws and regulations for CWD, documenting any noncompliance with quarantines, hold orders, or reporting.
 - 7) Has designated at least one State representative to coordinate CWD HCP activities in the State.
 - 8) Has programs to educate those engaged in the interstate movement of farmed or captive cervids regarding the identification and recordkeeping requirements of 9 CFR part 81.
 - 9) Requires, based on State law or regulation, official identification of all animals in herds participating in the CWD herd certification program, effectively enforces this requirement, and documents any noncompliance with this requirement.
 - 10) Maintains the following information in a State database recognized by the Administrator as meeting the following data requirements in an accurate and timely manner:
 - A. Premises information, assigned premises numbers, and owner information (location, address, and contact information) for all farmed or captive cervid herds participating in the CWD HCP in the State.
 - B. Program status of all enrolled herds.
 - C. Any restrictions to herd statuses including designation as a CWD-positive, exposed, suspect or epidemiologically linked to a positive herd.
 - D. All program actions such as changes to herd status, depopulation, and adoption of herd plans.

- E. Individual animal information on all farmed or captive cervid herds participating in the CWD HCP in the State.
 - F. Individual animal information on all out-of-State farmed or captive cervids to be traced.
- 11) Requires that tissues from all CWD-exposed and suspect animals from affected herds that die or are depopulated or are otherwise killed be submitted to a laboratory authorized by the Administrator to conduct official CWD tests.
 - 12) Requires appropriate disposal of the carcasses of CWD-positive, CWD-exposed, and CWD-suspect animals.
 - 13) Enforces all testing and disposal requirements, and documents any noncompliance.
 - 14) Ensures that herds comply with program requirements including physical herd inventories at least every 36 months, annual herd and premises inspections, and verification of required CWD surveillance.

1.2 Provisional Approval

Provisional approval may be granted to States that do not meet all the national CWD HCP minimum requirements on application to the program. APHIS and the State will work to develop a plan with an appropriate time frame to meet program requirements.

1.3 Annual HCP Reports from Approved States

Comprehensive annual reports of HCP status and activities of enrolled herds are provided to the respective APHIS District Office for review and endorsement for the year beginning July 1 through June 30. The report will be submitted along with an application for Chronic Wasting Disease HCP approval, renewal or reinstatement of a state (VS Form 11-2). The annual report and VS 11-2 will be reviewed and signed by the Assistant Director and a designated State representative and submitted to the Cervid Health program staff. The reports will be used to monitor compliance with HCP program requirements and disease control efforts in Approved States.

The Cervid Health Program staff will provide guidance to States on annual reporting formats prior to the end of the reporting period. The following data will be included in the Annual HCP reports:

- 1) Enrolled herds—by State and certification status, species, number of animals in each herd, and number inspected.
- 2) CWD samples and tests—number of animals tested during the reporting period, species, herd type (breeder, hunting operation, etc.) and test results. CWD-

positive herds—under quarantine, depopulated and released from quarantine, not under quarantine, under herd plans, number of animals in each herd.

- 3) CWD-exposed herds—under quarantine, depopulated and released from quarantine, not under quarantine, under herd plans, number of animals in each herd.
- 4) Epidemiological information—traceouts initiated, traceouts pending, and traceouts completed.

1.4 Review of Approved State HCP

APHIS may periodically review an Approved State's CWD HCP program. States may be reviewed on request by APHIS or the Approved State. Review activities may include:

- 1) Evaluating State program activities to verify compliance with Federal requirements and identifying opportunities for program improvement.
- 2) Evaluating enrolled herd owner compliance with HCP requirements including reviewing laboratory reports, herd inventories, surveillance sampling, and other records and documents.
- 3) Reviewing reports and records related to epidemiological investigations of CWD-positive, CWD-exposed, or CWD-suspect herds.
- 4) Assessing compliance and completeness of data entered into an approved State database.
- 5) Conducting site visits as necessary.

APHIS will issue a summary report to the Approved State that will include the findings of the review including recommendations to achieve compliance with the National HCP Program or to improve the overall effectiveness and efficiency of the program in the State. APHIS will work with States to develop a plan to respond to the findings, and a specified period of time to complete any proposed actions.

1.5 Withdrawal of State Approval

APHIS may withdraw State approval if the State's action plan to achieve compliance is not completed or not completed during the specified period of time agreed on by APHIS and the State. The State may reapply for State approval once they can meet all the national CWD HCP minimum requirements.

2. Herd Participation

2.1 Participating Herd: Requirements for Enrollment

The requirements for participation in the national CWD HCP are found in 9 CFR part 55 subpart B.

- 1) Herd owners already participating in an Approved State CWD HCP will maintain the same enrollment date for the National CWD HCP as the first date that the herd participated in the Approved State program.
- 2) Herd owners enrolled in the Approved State CWD HCP agree to maintain their herds in accordance with the following requirements:
 - A. Each animal in the herd must be identified before reaching 12 months of age using means of identification described in Section A 3.2 of these Program Standards.
 - B. The herd premises must have perimeter fencing adequate to prevent ingress or egress of cervids. This fencing must comply with any applicable State regulations, and follow the guidance provided in Section A 4 of these Program Standards.
 - C. The owner must report all deaths of farmed or captive cervid aged 12 months or older (including animals killed on premises maintained for hunting, and animals sent to slaughter) to a State or to an APHIS employee in a prompt and timely manner. States may routinely allow up to 1 week for reporting mortalities. However, State representatives or APHIS employees may approve mortality reporting schedules other than immediate notification when herd conditions warrant it in the opinion of both APHIS and the State.
 - D. Carcasses of animals must be made available for tissue sampling and testing in accordance with instructions from the State representative or APHIS employee.
 - E. Herd inventory records should be updated and reconciled at least annually and submitted to the Approved State representative.
 - F. The owner must report in a prompt and timely manner from time of discovery any animals that escape, disappear, or are otherwise missing from the premises to a State representative or an APHIS employee. States may routinely allow up to 72 hours for reporting such incidents. This also may allow time for the herd owner to recapture the animal and work with the Approved State for decisions on disposition of the animal or animals. Likewise, entry of any wild cervids into the facility should also be reported as above.

- G. Records, including a complete inventory of animals, must be kept in accordance with Section A 3.3 of these Program Standards. Herd owners must make animals and records available to accredited veterinarians, APHIS employees, or State representatives for inspection. Owners are responsible for assembling, handling, and restraining animals for physical herd inventories or other inspections under conditions that will allow the accredited veterinarian, APHIS employee, or State representative to safely read all identification on the animals. The owners are responsible for the costs that may be incurred to present the animals for inspection and must agree that any liability or injury to the animals during handling rests with the owner.

Farmed cervids commingled (see definition) with other farmed cervids assume the status of the lowest program status animal in the group. If an owner wishes to maintain two or more separate herds (see definition), he or she must maintain separate herd inventories, records, working facilities, water sources, equipment, and land use. There must be a buffer zone or geographic zone of at least 30 feet between the perimeter fencing around the separate herds, and no commingling of animals may occur. Movement of animals between herds must be recorded as if they were separately owned herds.

- H. New animals may be introduced into the herd only from other herds enrolled in the CWD herd certification plan and under the conditions outlined in Section A 2.3.

Failure to comply with any of the listed HCP requirements will affect the herd status and could result in suspension or removal from the national CWD HCP.

2.2 Herd Owner Enrollment and Advancement

The enrollment date will be the day, month, and year in which an owner's herd is officially enrolled in the HCP. This date is important because it will be used to calculate when herds may advance to a higher herd status under the HCP after completing successive years without CWD being diagnosed in the herd. For a herd that only adds animals from herds with the same or greater status, the enrollment and status dates will remain the same. However, if a herd adds animals from a herd with a lesser status the enrollment and status dates for the receiving herd will reflect the lowest status date. The enrollment date is a fixed date, while the status date may change based on herd additions or status progress.

When initially enrolled in an Approved State CWD HCP all herds will be placed in First Year status. Each year, on the anniversary of the enrollment date or status date (whichever is later) of meeting the HCP requirements, the herd status is upgraded by 1 year; i.e., Second Year status, Third Year status, Fourth Year status, and Fifth Year status. After 5 continuous years of compliance (the end of the Fifth status year) with no findings of CWD in the herd, the herd status is changed to Certified. The herd remains

in Certified status as long as continuous enrollment is maintained in the program and the herd continues to meet all of the program requirements. Enrolled herds that have achieved Certified status are eligible to move interstate in accordance with 9 CFR 81.3.

Herds that are established and sourced solely from other Certified herds will be enrolled as Certified herds and must continue to demonstrate compliance with program requirements to maintain Certified status.

Eligibility for advancement from one status to the next is based on compliance with program requirements, including the submission of surveillance samples. Should the herd owner not be in compliance with 9 CFR part 55, State representatives and APHIS employees may withhold advancement, lower, suspend, or revoke the status.

2.3 Additions of Animals to a Herd: Effects on Status

A herd may add animals from herds with the same or a greater status in the national CWD HCP with no negative impact on the status of the receiving herd.

If animals are acquired from a herd with a lesser status, the receiving herd reverts to the lower status. If a herd participating in the program acquires animals from a nonparticipating herd, the receiving herd reverts to First Year status with a new status date listed as the date of acquisition of the animal. The enrollment date in the national CWD HCP would remain unchanged but the herd status level would be modified (and modification date recorded).

If a herd acquires animals from herds with a lower or nonparticipating status, the owner must notify a State representative or APHIS employee within 5 business days of such acquisition. New herds assembled from multiple sources will be assigned the status date of the lowest status herd.

Other sources of equivalent or higher status animals may include cervid herds enrolled and at an appropriate level in a CWD HCP in another country where APHIS recognizes the HCP to be at least equivalent to the APHIS national CWD HCP.

2.4 Additions of Genetic Material (Germplasm) to a Herd: Effects on Status

There is currently no scientific evidence that germplasm may transmit CWD.

2.5 Inspections and Inventories

Inspections and physical herd inventories ensure herd compliance with HCP requirements. Herds may not advance in status until the annual inspections have been completed, submitted, reconciled, and approved. Inspections are conducted annually and physical herd inventories are conducted at least every 3 years. Records and inventories will be reconciled during all annual inspections.

The annual inspection must verify that the herd premises has perimeter fencing adequate to prevent ingress or egress of cervids. This fencing must also comply with any applicable State regulations.

An initial complete physical herd inventory must be conducted in which all animals within the herd are visually inspected. Individual identification must be verified and recorded for each animal at the time a herd is initially enrolled in the CWD HCP. APHIS will accept a complete physical herd inventory performed by a State representative, an accredited veterinarian, or an APHIS employee not more than 12 months before the herd's date of enrollment in the CWD HCP as fulfilling the requirement for an initial inventory.

Thereafter, a physical herd inventory must be performed for all enrolled CWD HCP herds no more than 3 years after the last complete physical herd inventory. Physical inventories may be conducted on unrestrained animals as long as it is possible to visually inspect and verify at least one CWD HCP required identification number and device on each animal, and that information is matched to herd written or electronic records. Animals may be temporarily gathered in pens or other means used for viewing. Any animals in which ID cannot be visually inspected will need some form of restraint for confirmation.

2.6 Loss of Certification Status

Herds may lose national herd certification status if the Administrator or a designee, in consultation with the respective Approved State representative, determines that the herd owner failed to comply with any of the program requirements.

2.7 Relocation of a Herd

If a herd moves, either within a State or to another State, it must meet all Approved State intrastate or Federal interstate movement requirements. In addition, the appropriate State representative or APHIS employee administering the Federal CWD rule should be notified of the relocation within 30 days.

2.8 Cancellation of Participation

Mandatory Cancellation

The Administrator, in concurrence with the Approved State, may cancel the enrollment of a herd by giving written notice to the herd owner. The Administrator may cancel enrollment after determining that the herd owner failed to comply with any HCP requirements.

Before enrollment is canceled, an Approved State representative or an APHIS employee will inform the herd owner of the reasons for the proposed cancellation and of the 10-day appeal deadline. The herd owner may appeal the proposed cancellation in

writing to the Administrator within 10 business days after being notified. The appeal must include all of the reasons and supportive evidence with documentation needed to challenge the proposed cancellation. The Administrator may grant or deny the appeal in writing as promptly as circumstances permit, stating the reason for his or her decision. If there is a conflict as to any material fact, a hearing will be held to resolve the conflict. The Administrator sets the rules of practice concerning the hearing.

In the event of cancellation, the herd owner may reapply to enroll in the national CWD HCP but will not reach Certified status until 5 years after APHIS approves the herd owner's new application for enrollment regardless of the status of the animals in the herd.

Voluntary Cancellation

An owner may decide to cancel participation in the CWD HCP at any time unless otherwise required by State regulations or a signed herd plan. The cancellation should be in writing to a State representative or APHIS employee. Owners who voluntarily cancel their participation may re-enroll at any time as a First-Year status herd and will receive a new enrollment and status date.

3. Registration, Identification, and Recordkeeping

The regulatory authority for registration, recordkeeping, and identification for each animal within enrolled herds is found in 9 CFR 55.23.

3.1 Premises Identification

All participating premises must have a unique Premises Identification Number (PIN).

3.2 Animal Identification

In accordance with 9 CFR 55.25, all animals in the herd must be identified with two unique animal identification numbers for each individual. One of these animal identifications must be a nationally unique official animal identification.

The official animal identification must be a device using an APHIS-approved animal identification numbering system that uniquely identifies individual animals. Information on official animal identification and devices can be found on [the APHIS Traceability Web site](#).

The official animal identification device must be approved by APHIS, and must be a legible ear tattoo, tamper-resistant eartag, electronic implant, legible flank tattoo, or other approved device. If a microchip is used and the animals are slaughtered under State or Federal meat inspection it should be used in compliance with applicable State or Federal regulations.

The official animal identification must be linked to that animal and herd in a State database. The second animal identification must be unique for the individual animal within the herd and also must be linked to the same animal and herd in the State database. The unique Animal Identification Number may be used on two separate identification devices on the same animal to fulfill the identification requirements if desired.

Natural additions to the herd must be identified before 12 months of age. However, all animals regardless of age must be properly identified as described in this section to move interstate.

If, at the time of enrollment in the Approved State CWD HCP, identification of animals in a herd does not meet the above criteria, the herd owner must bring the herd and animal identifications into compliance as soon as possible and no later than the next inventory on a schedule specified by the State representative or APHIS employee.

APHIS recommends that all animal identification devices be visible on the animal from an appropriate distance to allow visual verification of the identification number on the device without animal restraint. Any animals in which identification cannot be visually inspected will need some form of restraint for confirmation during herd inventories.

All animals from enrolled herds that are sent to hunt facilities under the same ownership must retain official identification for surveillance testing.

In accordance with 9 CFR 86.4, removal of official identification devices is prohibited except at the time of slaughter, at any location upon the death of an animal, or as otherwise approved by the State or Tribal animal health official, or a VS Assistant Director when a device needs to be replaced.

The Food and Drug Administration (FDA) Center for Veterinary Medicine regulates the marketing of implantable transponder devices (electronic identification devices/EID) for use in animals. Please contact the FDA or the manufacturer or distributor for information on approved EIDs. USDA's Food Safety and Inspection Service (FSIS) should be contacted regarding anatomic placement of the EIDs in animals that may be presented for slaughter in official slaughter facilities to determine if these devices pose a potential physical food safety hazard.

3.3 Owner Records: Herd Inventory

Each owner must maintain a current complete herd inventory which must include, at a minimum, the following information and records for each animal:

- 1) All identification devices (tags, tattoos, electronic implants, etc.).
- 2) Age.
- 3) Species.
- 4) Sex.
- 5) The date of acquisition and source of each animal that was not born into the herd (owner name, city, State).
- 6) The date of removal and destination of any animal removed from the herd (owner name, city, State).
- 7) Birth date.
- 8) Date of death (and cause, if known) for animals dying within the herd.
- 9) Date of CWD sample submission, submitter, owner, premises, and animal information, and official CWD test results from NVSL or approved laboratory for samples required by the program.

All records, electronic or written, must be kept for 5 years after the cervid has left the herd or has died. Records must be made available to an APHIS employee or State

representative at their request and presented at the time of each annual inspection or inventory.

4. Fencing Requirements

The regulatory authority for fencing requirements of enrolled herds is found in 9 CFR 55.23(b)(2). Fencing alone does not delineate individual herds, which must be separated by a distance of 30 feet or greater, as described in 9 CFR 55.23(b)(5).

APHIS considers perimeter fencing with the following characteristics to be adequate to prevent ingress or egress of cervids:

- 1) Structurally sound.
- 2) Maintained in good repair.
- 3) Of sufficient construction to contain the animals.
- 4) Compliant with any other existing State regulations or requirements.

NOTE: For herds established after the effective date of the CWD rule (August 13, 2012), the fence should be a minimum of 2.4 meters (8 feet) high.

Cervid producers enrolled in the HCP may voluntarily elect to use additional barriers and/or other biosecurity measures to minimize escapes and/or to mitigate disease transmission risks associated with direct contact between free-ranging and farmed cervids.

State representatives have the discretion to require the use of additional barriers and/or other biosecurity measures deemed necessary to mitigate the risks of CWD transmission.

In the case of CWD-positive, suspect, trace-back exposed, and trace forward herds, APHIS and the State representative will assess the risk of CWD transmission between farmed and free-ranging cervids on a case-by-case basis. They may include requirements for additional barriers and/or other biosecurity measures deemed necessary to mitigate the risks of CWD transmission in the herd plan.

5. Surveillance and Sampling

The regulatory authority for surveillance and sampling of animals in enrolled herds is found in 9 CFR 55.23(b)(3).

To achieve certified status, farmed cervid herds must conduct CWD surveillance on all deaths of cervids aged 12 months or older, including animals in the enrolled herd, animals that are slaughtered on premises, and animals under the same ownership killed on a premises maintained for hunting and/or sent to slaughter, for at least 5 consecutive years, unless:

- 1) The herd owner purchases or assembles a herd of animals from herds with certified status and concurrently enrolls the resulting herd in a State HCP.
- 2) The enrolled herd owner can document that he or she has maintained a closed herd of animals for at least 5 years; had no on-farm mortalities; had no “missing” animals; and did not move any animals off the premises during that time for any reason without testing them for CWD.

If the enrolled herd does not have any animal deaths meeting surveillance criteria for the year, the herd is considered to be in compliance with surveillance requirements for the year.

5.1 CWD-Suspect Animals

The owner must immediately report to a State representative, accredited veterinarian, or an APHIS employee all suspected cases of CWD. These are to include any animal exhibiting signs of a neurological or wasting disease as described below. These animals should be euthanized or closely monitored until death and the carcasses must be made available for tissue sampling and testing. Clinical CWD suspects that die or are euthanized should be tested for CWD regardless of age. Animals with non-negative results on an unofficial test are also considered to be CWD-suspect animals and must be reported.

The clinical signs associated with CWD are nonspecific and could be caused by other diseases affecting farmed or captive cervids; thus, laboratory confirmation is required for CWD diagnosis. Not all animals display all clinical signs of disease. Duration of clinical signs varies from a few days in unusual cases to as long as a year, but is most often 2 to 3 months.

Usually, the earliest clinical signs displayed are behavioral changes which may include alterations in interaction with humans and members of the herd. These subtle changes are often only recognized by caretakers familiar with the individual animal. With disease progression, behavioral and physical changes may be noted including periods of stupor and depression, altered stance, and progressive weight loss. At the terminal stage of disease, animals are emaciated and may exhibit increased drinking and urination, excessive salivation, lack of coordination, and trembling. However, concurrent disease, especially aspiration pneumonia, may cause an affected animal to die while still in good to fair body condition.

Animals with progressive neurological disease or wasting syndromes that are not responsive to treatment should be considered CWD clinical suspects and consequently be euthanized and tested. If an owner of a clinical suspect declines to allow euthanasia, the animal should be tested in accordance with program requirements after it dies.

5.2 Mortality Reporting and Routine Surveillance

To achieve and maintain herd certification status, enrolled herd owners are required to conduct CWD testing as described in 9 CFR 55.23(b)(3). Herd owners must report and make the following animals available for sample collection and CWD testing:

- 1) All on-farm deaths of farmed or captive deer, elk, and moose aged 12 months or older.
- 2) All animals 12 months or older that are slaughtered on the farm.
- 3) All animals under their ownership that are sent to hunting operations and/or slaughter facilities.

State representatives or APHIS employees may approve mortality reporting schedules other than immediate notification when herd conditions warrant it. Herd inventory records should be updated at least annually and reconciled to include mortalities and testing results for samples submitted.

5.3 Sample Collection and Submission Procedures

It is the owner's responsibility to ensure complete, good quality tissue samples are collected and all required samples are submitted. Failure to comply with the surveillance requirements in this section may result in loss of program status or other actions applicable under Approved State or Federal regulation.

Tissue samples may only be collected by State officials, APHIS employees, accredited veterinarians, or certified CWD sample collectors. Alternatively, owners may remove and submit the entire head with all attached identification devices to an approved CWD laboratory for tissue collection. Samples should be submitted to an approved laboratory within 7 days of collection.

Detailed instructions regarding sample collection and submissions can be found in Appendix V.

The obex and retropharyngeal lymph node should be collected regardless of sample condition (e.g. autolyzed, frozen, etc.) and submitted to the approved laboratory to comply with the routine herd surveillance requirement. However, there may be circumstances when only one tissue sample can be collected from an animal. In those circumstances, the producer should notify the Approved State official to explain the reason. If that single sample submission is determined by the laboratory to be unsuitable or untestable, then it will be recorded as a missed sample (not tested) and that animal will not be counted in the mortality surveillance for herd certification status. A positive IHC or ELISA test result on any sample submitted to the approved laboratory will be considered a CWD-suspect test result to be confirmed by IHC at NVSL.

5.4 Consequences of Poor Quality and Missing Samples

Surveillance of all animal mortalities in a herd is the key to increasing our confidence that HCP-certified herds are at low risk for CWD infection. Poor quality samples and missing samples undermine our ability to assess the CWD status of the herd.

Poor quality samples include samples that are severely autolyzed, from the wrong portion of the brain, the wrong tissue, or not testable for other reasons. Approved laboratories should closely monitor sample quality. They should provide timely feedback to the producer, certified sample collector, State officials, and APHIS employees regarding the receipt of poor quality samples. Approved State officials should provide oversight on sample collection by certified sample collectors and address any skill inadequacies which may require additional training or loss of certification as a sample collector.

Missing samples occur when samples from any animal 12 months of age or older in an enrolled herd that dies, is slaughtered, escapes, or is lost are not submitted for diagnostic testing for CWD.

Approved States (in consultation with APHIS) should develop risk-based assessments to implement consequences for poor quality/incomplete samples and missed samples of test-eligible animals in enrolled herds. If *neither* the obex or the retropharyngeal lymph node in a test-eligible animal can be tested due to being missing or of poor quality, then consequences should include:

- 1) A requirement to replace missed or poor quality samples with testable post-mortem samples from an equal number of animals of the same sex and species that resided in the herd for at least as long as the untested animals; or
- 2) A reduction in herd status date (with loss, reduction, or delay in herd certification); or

- 3) A direct suspension of herd status for some period of time.

The following tables are provided as **examples** of adjustments that could be made to CWD herd status to account for poor quality, incomplete, or missing samples. This example considers the current status of the enrolled herd, the number of poor quality/missing samples, and the percentage of annual removals from the herd. Annual Removals are defined as all adult animals (12 months or older) that were removed or lost from inventory for **any** reason since the previous annual inventory. When animals are removed from a herd, they are lost to surveillance testing.

In this example, status reduction may be avoided if the owner elects to remove and perform post-mortem CWD testing on an animal of the same sex and species that has resided in the herd for at least as long as the untested animal with the poor quality or missing sample.

NOTE: In the National Animal Health Monitoring Service [Cervid 2014: Health and Management Practices on U.S. Farmed Cervid Operations, 2014](#), the average removal rate (sales, hunt-harvest, slaughter, etc) was 21.3 percent per year, with deer operations at 22.3 percent and elk operations at 20.3 percent.

Herds without Certified Status: HCP herd status will be reduced for *each* animal that dies, is slaughtered or hunt-harvested, escapes, or is lost and is not tested for CWD (including due to poor quality, incomplete, or missed samples) as follows:

% Annual Removals from Herd	Status Reduction
0 to 20%	1 year
21 to 40%	1.5 years
41% or more	2 years

Herds with Certified Status: HCP herd status will be reduced for *each* animal that dies, is slaughtered or hunt-harvested, escapes, or is lost and is not tested for CWD (including due to poor quality, incomplete, or missed samples) as follows:

% Annual Removals from Herd	Status Reduction
0 to 20%	0.5 year
21 to 40%	1 year
41% or more	1.5 years

Examples:

- 1) A certified herd with a 10 percent annual removal rate fails to test an animal that died in the herd. The owner also declines to euthanize and test a comparable animal from the herd as a replacement for the missed sample. In this case, the herd would be reduced to uncertified status and would be unable to move animals interstate for 0.5 year. The herd inventory would be repeated after the 0.5 year (6 months) and the herd could regain certified status assuming it continued to comply with program requirements.
- 2) A certified herd with a 10 percent annual removal rate fails to test 3 animals that died in the herd. They also decline to euthanize and test comparable animals from the herd as a replacements for the missed samples. In this case, the herd would be reduced to uncertified status and would be unable to move animals interstate for 1.5 years. The herd inventory would be repeated after 1.5 years (18 months) and the herd could regain certified status assuming it continued to comply with program requirements.
- 3) A certified herd with a 50 percent annual removal rate fails to test an animal that died in the herd. They also decline to euthanize and test a comparable animal from the herd as a replacement for the missed samples. In this case, the herd would be reduced to uncertified status and would be unable to move animals interstate for 1.5 years. The herd inventory would be repeated after 1.5 years (18 months) and the herd could regain certified status assuming it continued to comply with program requirements.
- 4) A non-certified herd with a 15 percent annual removal rate fails to test 2 animals that died in the herd. They also decline to euthanize and test comparable animals from the herd as replacements for the missed samples. In this case, the herd would be reduced in status by 2 years.
- 5) A non-certified herd with a 15 percent annual removal rate fails to test 2 animals that died in the herd. They agree to euthanize and test 2 comparable animals

from the herd as replacements for the missed samples. In this case, the herd would retain their status as long as the test results are “not detected”.

States may choose to develop their own risk-based approach for consequences for poor quality or missing samples, subject to APHIS approval.

5.5 Exceptions

Exceptions to the testing requirement may be granted by APHIS or the Approved State Official for extenuating circumstances beyond the control of the herd owner as follows:

CWD sample collections may be limited to two animals per occasion when APHIS or the Approved State Official determines that the animals died from a mass casualty/mortality event (where numerous animals die over a short period of time from the same apparent cause) such as during a natural disaster or an infectious disease outbreak (such as epizootic hemorrhagic disease), or from a known zoonotic disease where sample collection would pose a public health risk. In these cases, the certified sample collector will sample the animals believed to be at higher risk for CWD. Higher-risk animals would include older animals, males preferentially over females, or those animals having any known pre-existing health conditions or in poor body condition.

5.6 Tissue for DNA Testing

APHIS recommends that an eartag with a piece of fresh (not in formalin) ear tissue attached be included with each sample that is submitted for CWD testing.

An enrolled herd owner may request DNA comparison testing to confirm that the sample submitted for CWD testing originated from the same animal as the submitted ID device. Identity verification testing is performed at the owner’s expense. The herd owner may request DNA comparison testing, in writing, to the Assistant Director (AD) in the corresponding District for the State. The AD will notify NVSL of the request. NVSL will extract DNA from the test specimens and associated skin tissue, and forward the extracts to a laboratory selected by NVSL for DNA comparison testing.

It is critical that consistent documentation and sample security ensure that the samples remain appropriately linked to the source animal from the time of sample collection to the end of the testing process. All specimen containers must be clearly and permanently marked to include official identification of the animal, name of owner, name of collecting official, and date. Laboratory tracking numbers must be included with all corresponding documents. If part of the ear cannot be removed (e.g., for taxidermy purposes), then a new identification tag could be affixed to the hide skin and recorded in the animal’s official record, and the tagged hide section submitted with the diagnostic specimens. This practice will also allow APHIS to conduct genotype testing associated with susceptibility to CWD (e.g., codon 96 testing in white-tailed deer) if the animal tests positive.

6. Diagnostics

The regulatory authority for official CWD tests and laboratory approval is found in 9 CFR 55.8.

6.1 Testing Authority and Approved Laboratories

Testing Authority

Laboratories will be approved by NVSL, as designated by the APHIS Administrator, to conduct official CWD testing in accordance with 9 CFR 55.8. All suspect positive test results must be confirmed by NVSL.

Approved Laboratories

Only laboratories that are members of the National Animal Health Laboratory Network (NAHLN) will be approved to conduct official CWD diagnostic testing. Requirements for laboratory approval and a [list of laboratories approved to conduct CWD testing](https://www.aphis.usda.gov/animal_health/nahln/downloads/cwd_elisa_lab_list.pdf) can be found on the NAHLN Web Site (https://www.aphis.usda.gov/animal_health/nahln/downloads/cwd_elisa_lab_list.pdf).

Not all laboratories are approved to perform all officially recognized types of CWD assays. The VS Cervid Health staff, the NVSL Director, and the NAHLN Coordinator will maintain a list of officially recognized CWD assays and when appropriate the tissues approved for laboratories that conduct these tests for CWD. The list will be available on request to all interested parties.

6.2 Official CWD Tests

An official CWD test is approved by the Administrator in accordance with 9 CFR 55.8. To be considered as an official test for CWD, a test method must be:

- 1) Licensed by the Center for Veterinary Biologics (CVB), if required (i.e., ELISA tests, etc).
- 2) Performed by APHIS-approved laboratories, at NVSL, or at another laboratory to which NVSL has referred a case for confirmatory testing.
- 3) Performed following NVSL protocols.

The following are considered official tests for CWD when used as described in these Program Standards:

Approved CWD Test Method	Tissue Tested	Approved Use
Immunohistochemistry (IHC) test	Medial retropharyngeal lymph node (MRPLN) and obex collected post-mortem and preserved in formalin ¹	<ul style="list-style-type: none"> • Routine herd surveillance • Testing in conjunction with epidemiological investigations and herd plans for trace back, CWD-exposed herds, and trace forward herds
Immunohistochemistry (IHC) test	Ante-mortem biopsy of white-tailed deer rectoanal-associated mucosa-associated lymphoid tissue (RAMALT)	<p>This is an official test in white-tailed deer only when outlined in a herd plan and:</p> <ul style="list-style-type: none"> • Genotype at codon 96 is established • Used as a whole herd test as indicated in herd plans for trace back, CWD-exposed herds, and trace forward herds as described in Part B and • Performed at NVSL
Immunohistochemistry (IHC) test	Ante-mortem biopsy of white-tailed deer MRPLN	<p>This is an official test in white-tailed deer only when outlined in a herd plan and:</p> <ul style="list-style-type: none"> • Genotype at codon 96 is established • Used as a whole herd or individual test as indicated in herd plans for trace back, CWD-exposed herds, and trace forward herds as described in Part B and • Performed at NVSL
Enzyme-linked immunosorbent assay (ELISA) by Bio-Rad	Fresh medial retropharyngeal lymph node (MRPLN) and obex collected post-mortem ¹	<p>This is an official HCP test only when used for:</p> <ul style="list-style-type: none"> • Slaughter surveillance in farmed cervids; or • Carcass segregation for disposal; or

¹ Although medial retropharyngeal lymph nodes (MRPLNs) may be early CWD detection sites in deer and elk, it is not uncommon to find elk that are obex-positive and MRPLN-negative. Therefore, confidence in CWD detection is increased when **both** obex and MRPLNs are tested.

		<ul style="list-style-type: none"> • Other purpose as approved in advance by APHIS and • Is performed at NVSL or at a NAHLN laboratory approved to conduct the ELISA <p>Many States use the ELISA to conduct wildlife surveillance. This use is not subject to APHIS approval.</p>
Western blot	Fresh medial retropharyngeal lymph node (MRPLN) and obex collected post-mortem ¹ .	This is an official test only when performed at NVSL

6.3 Approval of Official Diagnostic Tests

Prior to evaluation for official use, the manufacturer should obtain a product license from the CVB, if needed.

Companies/researchers are encouraged to contact the Cervid Health Team to review preliminary data and discuss additional data needs for candidate tests prior to submission.

The test manufacturer should submit an application package containing the following information to the Cervid Health Team:

- 1) A standardized protocol that includes a description of the test, sample type, all methods associated with preparing the sample and conducting the test, reagent specifics, required materials and equipment, and control and quality assurance measures.
- 2) A description of the proposed use of the test in the CWD HCP program and the suitability of the test for the stated purpose. Specifically include cervid species, post- or ante-mortem use, and conditions for use (e.g., whole herd versus individual animal, routine surveillance testing versus use in herds under epidemiological investigation, etc.)
- 3) Data/scientific evidence to demonstrate:
 - A. Diagnostic sensitivity of the test evaluated in a range of infected animals including:

1. Animals early in the clinical progression, such as:
 - a. Animals that are MRPLN-only positive,
 - b. Elk that are obex-only positive, or
 - c. Animals of all three genetic polymorphisms (96 for white-tailed deer, 132 from elk).
 2. Animals late in the clinical progression, such as:
 - a. Animals that are MRPLN- and obex-positive, or
 - b. Animals of all three genetic polymorphisms (96 for white-tailed deer, 132 from elk).
 3. Data provided should include the genotype (96 for white-tailed deer, 132 from elk) and complete post-mortem testing results for IHC on obex and MRPLN for each animal.
 4. Description of the calculation.
- B. Diagnostic specificity in animals believed to be non-infected based on HCP herd certification status and results from mortality testing from at least the last 5 years.
- C. Repeatability of the test result. This refers to the ability of a test to repeatedly produce the same result on a given sample. Evidence to demonstrate repeatability includes detailed information about the collection of the data, including controls and control data.
- D. Reproducibility of the test results at other laboratories. This refers to the ability of a test to repeatedly produce the same result on a given sample when the test is performed at multiple laboratories by multiple people. In addition to the supporting data, a letter of support and certification of test results from participating laboratories is suggested.
- 4) Other data and documentation, as requested by APHIS.
 - 5) Field trials and/or pilot projects using the test may be recommended/required prior to final approval.

The Cervid Health Team will coordinate with NVSL, NAHLN, CVB and other scientific experts within APHIS and USDA to review the application package and evaluate the test based on, but not limited to, the criteria described in 9 CFR 55.8. APHIS may approve the new test methods or request additional data, including results from field trials.

APHIS may limit use of the test to certain species or types of animals or for use in specific situations. APHIS will clearly describe the conditions for official use of the approved test.

6.4 Test Results

As described in Section A 5.6, sections of brainstem/obex, MRPLN, and RMALT are evaluated by an official test in an approved laboratory to demonstrate the presence of the infectious CWD prion. Samples in which the infectious CWD prion is detected in testing at approved laboratories are considered to be CWD suspect pending confirmatory testing at NVSL. All suspect diagnostic test results from an approved laboratory must be confirmed by NVSL to establish a diagnosis of a CWD positive animal.

Brainstem or lymph tissues from an animal in which CWD prions are not detected by an official test does not mean absence of infection, only that prion was not detected in those tissues from that animal at the time of testing. Based on current transmissible spongiform encephalopathy research and pathogenesis studies, it is possible to have CWD prions present at levels below the analytical sensitivity of the test. CWD prions may be present in tissues other than those that were examined. Hence, “not detected” test results may not indicate the true status of the animal if it is in the early stages of the infection.

6.5 Rejected Samples

Samples may be rejected as unsuitable for diagnostic purposes for a wide variety of reasons. These poor quality samples will not contribute to required herd surveillance and may result in the consequences described in Section 5.9. Common examples of rejected samples include:

- 1) No identification submitted with the sample.
- 2) Incorrect tissue type.
- 3) Autolyzed (degraded) samples.
- 4) Samples where the tissue is unidentifiable.
- 5) Brain samples that do not include the obex.
- 6) Sample of insufficient size.
- 7) Sample contains an insufficient number of lymphoid follicles.

The reason for rejected samples can be described on official laboratory reports as follows:

- 1) ISF: Insufficient follicles (<6 follicles and no positive staining present).

- 2) LOC: Location (used for CNS exclusively, no DMNV (Dorsal Motor Vagus Nucleus) identifiable, wrong brain region).
- 3) ISF: Loc: (RB (Rectal Biopsy); <6 follicles and >50 percent squamous epithelium, rather than rectal mucosa).
- 4) U: Unsuitable (no significant lymphoid tissue, e.g. salivary gland).
- 5) S: Suspect (NAHLN lab sees suspicious stain).
- 6) NT: Not tested (not tested because unnecessary).
- 7) UNA: Unacceptable (poor quality sample).

6.6 Reporting of Results

Positive test results are to be reported by NVSL to the submitting NAHLN lab, State animal health official, the Assistant Director in the State where the herd resides, and the National Cervid Health program staff.

All other test results are to be reported by the testing laboratory to the submitter with copies provided to the corresponding Approved State Official for farmed cervids in the State where the herd resides.

7. Interstate Movement

The requirements for interstate movement of live cervids with regard to CWD are described in 9 CFR 81.2 and 81.3. These requirements apply to both farmed cervids and wild-caught cervids that are moved interstate to eventually be released back into the wild.

The following conditions must be met for live farmed cervids to be eligible for interstate movement:

- 1) The animals are enrolled and the herd has achieved Certified status in an approved State CWD HCP.
- 2) Each animal in the shipment must have at least two forms of unique identification attached, one of which must be an official animal identification with a nationally unique identification number, as described above in Section (3.2) Animal Identification.
- 3) A certificate of veterinary inspection (CVI) must be issued for interstate movement. It must contain the following information:
 - A. All identification numbers of each animal in the shipment.
 - B. Total number of animals covered by the certificate.
 - C. Purpose for which the animals are to be moved.
 - D. Consignor and herd of origin with complete addresses.
 - E. Consignee and point of destination with complete addresses.
 - F. A statement by the issuing accredited veterinarian or State or Federal veterinarian that the animals in the shipment have achieved Certified status in the CWD HCP and that the animals were not exhibiting clinical signs associated with CWD at the time of examination. The consignor or owner should contact the State representative in the State of destination to determine if there are any additional requirements.

Cervids eligible to move interstate in accordance with CWD regulations, and meeting the conditions specified in 9 CFR 81.5, can transit States en route to their destination. The regulations at 9 CFR 81.5 (only) preempt State and local laws or regulations.

9 CFR 81.3 identifies specific exemptions to these requirements, including exemptions for:

- 1) Animals moved directly to a recognized slaughter establishment. The consignor or owner also should contact the State representative in the State of destination to determine if they meet all import requirements.
- 2) Research animals.
- 3) Interstate movements approved by the Administrator on a case-by-case basis.

States or Tribes may transport wild-caught cervids (elk, deer, moose, or other cervidae) from one State or Tribal location to another for release to establish new or augment existing free-ranging herds. The movement is subject to approval by the animal health officials of the receiving State and APHIS. [VS Guidance 8000 “Surveillance and Testing requirements for Interstate Transport of Wild Caught Cervids”](#) establishes a uniform process of disease risk assessment and recommended minimum standards for testing to help prevent the spread of CWD, bovine tuberculosis (TB) and brucellosis when wild cervids are captured for interstate movement and release.

Transport of game meat and other products derived from farmed cervids for purposes of interstate commerce is regulated by the Food and Drug Administration and is not addressed in the APHIS CWD regulations or these Program Standards. Similarly, transport of carcasses and other parts derived from hunt-harvested wild cervids is regulated by appropriate State agencies and is not addressed in the APHIS CWD regulations or these Program Standards.

Part B. Guidance on Responding to CWD

The CWD regulations in 9 CFR part 55 describe minimum requirements for States in response to the finding of a CWD-positive animal. These Program Standards describe acceptable methods to meet these minimum regulatory requirements. The methods in these Program Standards have been approved by the APHIS Administrator.

Alternatively, States may propose other methods/approaches to meet the regulatory requirements. These alternative proposals should be submitted in writing to APHIS for approval.

1. Epidemiological Investigations

The purpose of the investigation is to identify animals and herds that were exposed to the CWD-positive animal during the last 5 years. Quarantines and/or movement restrictions limit the potential for further spread of the infection until the infection status of the exposed animal or herd can be assessed.

Upon NVSL confirmation of a CWD-positive animal, the Approved State, in cooperation with APHIS, should conduct an investigation to determine the locations where the CWD-positive animal resided during the last 5 years. The investigation should start within 7 business days of the laboratory confirmation.

All out-of-State traces should be promptly reported to the appropriate State authorities within 45 days following notification of a CWD-positive animal. All notification should be provided in writing to the respective State or States and a copy provided to the AD in the corresponding District Office even if the initial contact was verbal.

In addition to tracing movements of animals, other factors should be considered in the epidemiological investigation. These factors are addressed in Appendix III, CWD Epidemiology Investigation and Report Templates. They may include, but are not limited to: The genetics of CWD-positive animal or animals, the tissue or tissues that tested positive, the length of time the CWD-positive animal or animals spent in the herd or herds, and the testing history of the traceout herds.

Ideally, the investigation will determine the source of infection; however, this is not always possible. If the investigation determines the likely source of infection, then herds that are not epidemiologically linked may be released from quarantine at State discretion.

2. Quarantine

The State representative should issue quarantine or hold orders for CWD-positive and CWD-exposed herds. CWD-exposed animals must remain on the premises where they currently reside unless a State or Federal permit for movement (such as VS Form 1-27) has been obtained.

If a quarantined herd is not depopulated, the herd should remain in quarantine for 60 months (5 years) from the last case, as otherwise stipulated in the herd plan (e.g. following 2 whole-herd ante-mortem tests), or at the discretion of the State representative for a period of time as determined by a risk evaluation based on the findings of the epidemiological investigation. State representatives may also modify a quarantine to permit cervid movement onto a CWD-positive quarantined premises, such as a hunting facility, where all cervids harvested must be tested for CWD.

Quarantine may be released only after all herd plan requirements have been met and completed, or as determined by the State representative.

3. Classification of Animals and Herds During an Epidemiological Investigation

Any CWD-susceptible cervid that has, by definition, commingled with the CWD-positive animal in the last 5 years is considered to be CWD-exposed. All herds that contain CWD-exposed animals will immediately be placed in Suspended status until further epidemiology can be assessed. The Suspended herds will then be classified as follows (also see Appendix VI):

3.1 CWD-Positive Herd

The herd where the CWD-positive animal resided upon diagnosis is considered a CWD-positive herd and will immediately lose HCP herd status. The herd may reenroll in the HCP only after entering into a herd plan.

Options for responding to a CWD-positive herd:

- 1) Complete depopulation and post-mortem CWD testing of the herd. Depopulation may include hunter harvesting and/or slaughter with movements under permit, or
- 2) Quarantine for 5 years since last CWD-positive case, with or without selective culling of animals. The herd will remain under Suspended status until a herd plan is developed and implemented (see Herd Plan section below). Ante-mortem CWD testing and genotyping using NVSL protocol and APHIS-approved procedures may be included in the herd plan for disease management purposes (see Appendix II).

3.2 Trace Back, CWD-Exposed Herd(s)

If the epidemiological investigation determines that the CWD-positive animal resided in another herd (or multiple herds) within the last 5 years, then the herds are considered trace back, CWD-exposed herds and will immediately lose HCP status. The herd may reenroll in the HCP only after entering into a herd plan.

Options for responding to a trace back, CWD-exposed herd:

- 1) Complete depopulation and post-mortem CWD testing of the herd. Depopulation may include hunter harvesting and/or slaughter with movements under permit, or
- 2) Quarantine for 5 years since the last exposure to a CWD-positive animal, with or without selective culling of animals. The herd will remain under Suspended status until a herd plan is developed and implemented (see Herd Plan section below). Time in quarantine may be lessened for:
 - A. Time spent in an Approved State HCP since the last CWD-positive animal was removed from the herd, so long as the herd has maintained compliance with all HCP requirements.
 - B. If the CWD-exposed herd contains only white-tailed deer – Whole herd ante-mortem IHC RAMALT CWD testing and genotyping using NVSL protocol and APHIS-approved procedures as included in the herd plan (see Appendix II).
 - C. If the CWD-exposed herd contains only white-tailed deer – Whole herd ante-mortem IHC MRPLN biopsy CWD testing and genotyping using NVSL protocol and APHIS approved procedures as included in the herd plan (see Appendix II).
 - D. At the discretion of the State representative for a period of time as determined by a risk evaluation based on the findings of the epidemiological investigation.

3.3 Epidemiologically-Linked Herds

If the epidemiological investigation determines that CWD-exposed animals that resided with a CWD-positive animal within 5 years prior to the diagnosis of CWD have since moved to or through other herds, then those herds are considered to be epidemiologically linked.

Options for responding to an epidemiologically-linked herd:

- 1) If all of the CWD-exposed animals have died, were tested for CWD, and had “not detected” results, then the epidemiologically-linked herd is removed from Suspended status and maintains its original HCP status, including time spent in Suspended status.
- 2) If CWD-exposed animals are still present in the herd, then those animals may be euthanized and tested for CWD. If all CWD-exposed animals are accounted for and no samples tested positive for CWD, then the herd is removed from Suspended status and maintains its original HCP status, including time spent in Suspended status.
- 3) If any of the CWD-exposed animals have died and were not tested for CWD, or if the CWD-exposed animals no longer reside on the premises, or if the CWD-

exposed animals are still present in the herd, but the owner does not agree to euthanasia and testing, then the herd will remain under Suspended status until a herd plan is developed and implemented (see Herd Plan section below). The herd should be quarantined for 5 years since the exposure to a CWD-exposed animal, with or without selective culling of animals. Time in quarantine may be lessened for:

- A. Time spent in an Approved State HCP since the last CWD-exposed animals arrived in the herd, so long as the herd has maintained compliance with all HCP requirements.
- B. If the herd contains only white-tailed deer – Whole herd ante-mortem CWD testing and genotyping using NVSL protocol and APHIS approved procedures as included in the herd plan (see Appendix II).
- C. If the herd contains only white-tailed deer – Ante-mortem IHC MRPLN biopsy testing and genotyping of all CWD-exposed deer using NVSL protocol and APHIS approved procedures as included in the herd plan (see Appendix II).
- D. At the discretion of the State representative for a period of time as determined by a risk evaluation based on the findings of the epidemiological investigation.

4. Reporting

Sharing accurate, timely, complete information about ongoing CWD epidemiological investigations among Federal and State animal health officials helps to control the spread of CWD by quickly and accurately identifying exposed animals and placing movement restrictions on animals and herds. It also provides State animal health officials with information they may use to release or reduce quarantines for herds under investigation, as appropriate.

Appendix III provides a template that States may use to report findings from their epidemiological investigation to APHIS and other State representatives. States are required to submit both a preliminary and a final report for herds enrolled in the HCP. Additionally, States must submit these reports for any herd that requests Federal indemnity. This reporting requirement will be included in the herd plan. States should submit a preliminary report for a newly identified CWD-infected herd to APHIS within 7 business days of NVSL confirmation of the CWD-positive animal. States should submit a final report for CWD-positive herds as part of their annual HCP report.

APHIS may request clarification or additional information on CWD-positive herds as needed for risk assessments, indemnity requests, or other reasons.

5. Herd Plans

A herd plan describes in detail the actions to be taken to control the spread of CWD from and within CWD-positive, exposed, or suspect herds. It is a herd and/or premises management agreement based on a risk evaluation of the affected premises and herd and developed by APHIS in collaboration with the herd owner, State representatives, and other affected parties. The herd plan is not valid until it has been signed by the Assistant Director, the State representatives, and the herd owner. Herd plans should be signed within 60 days of a confirmed diagnosis of CWD.

A written, signed herd plan is required for herds to receive Federal indemnity. Quarantined herds must complete the requirements described in a herd plan before quarantines are released.

At a minimum, the herd plan should include:

- 1) Specified means of identification for each animal in the herd.
- 2) Regular examination (time period as determined by a State official or APHIS employee) of animals in the herd by a veterinarian for signs of disease.
- 3) Reporting to a State official or APHIS employee of any signs of central nervous system or wasting disease in herd animals.
- 4) Maintaining records of births and deaths as well as of the acquisition and disposition of all animals entering or leaving the herd, including the date of acquisition or removal, name and address of the person from whom the animal was acquired, and the cause of death, if the animal died while in the herd.
- 5) Testing of all mortalities, regardless of age (9 CFR 55.24 (2)(ii)). Records should be maintained for all samples submitted for CWD testing.

A herd plan may also contain additional requirements to prevent or control the possible spread of CWD, depending on the particular condition of the herd and its premises, including, but not limited to:

- 1) Depopulation of the herd if funds for indemnity are available. Depopulation also may be accomplished by moving animals from CWD-positive, suspect, and exposed herds (by permit and under seal) to a slaughter facility or to an appropriate hunt facility at the discretion of the State officials.
- 2) Specifying the time for which a premises must not contain cervids after CWD-positive, CWD-exposed, or CWD-suspect animals are removed from the premises.

- 3) Removal of CWD-exposed or CWD-suspect animals from the premises if funds for indemnity are available or at the discretion of State officials.
- 4) Fencing requirements and time period for regular inspection of fences.
- 5) Selective culling of animals.
- 6) Restrictions on use and movement of possibly contaminated livestock equipment.
- 7) Procedures for cleaning and decontamination of premises, including the use of bleach and/or lye for EPA required reporting.
- 8) Whole herd ante-mortem CWD testing and genotyping using NVSL protocol and APHIS-approved procedures.
- 9) Requirement to provide information needed to complete the preliminary and final epidemiology reports (see Appendix III).
- 10) Current Centers for Disease Control and Prevention (CDC) guidelines for prevention of potential human exposure to CWD.
- 11) Other requirements.

A herd plan may be reviewed and changes proposed at any time by any signatory party in response to changes in the situation of the herd or premises. The plan may also be changed if the understanding of the nature of CWD epidemiology, or techniques to prevent its spread, improves. However, any proposed changes must be reviewed and approved by all signatories before they are adopted.

Additional information on CWD environmental contamination and recommended procedures for cleaning and decontamination of premises that may be included in herd plans for CWD-positive herds is provided in Appendix IV.

6. Federal Indemnity

6.1 Eligible Animals

Federal indemnity may be available for the purchase, destruction, and disposal of CWD-positive, exposed, and suspect animals.

APHIS will pay reasonable costs for destruction and carcass disposal for animals that are indemnified.

Once the animals are euthanized, the carcasses become the property of APHIS, and APHIS may collect tissue samples as desired.

At the State's discretion, a person may remove the skull plate with antlers attached and cleaned of all soft tissue and blood from the premises if the material is being moved to a taxidermist for processing and after the animal is tested "not detected" for CWD.

6.2 Appraisals

An appraisal must be conducted by a government or a private appraiser (VS Memorandum 534.1) The appraisal report and detailed supporting documentation must be submitted to the Cervid Health Team for review.

6.3 Indemnity Requests

The Assistant Director responsible for the State in which the animals reside should provide the following to the Cervid Health Team when submitting a request for Federal indemnity:

- 1) Completed indemnity request form signed by the Assistant Director.
- 2) The appraisal report with detailed supporting documentation, such as:
 - A. The white-tailed deer appraisal calculator.
 - B. Pedigrees.
 - C. Sale receipts or invoices.
 - D. Documentation of antler scores.
- 3) VS Form 1-23 and a herd plan signed by the herd owner and the Assistant Director.
- 4) Preliminary epidemiological report (see Appendix III).

6.4 Evaluation and Prioritization of Requests for Federal Indemnity Funds

Whole-herd depopulation and post-mortem testing of all cervids on the premises is often the preferred response to control the spread of CWD within and from CWD-positive and exposed herds. A limited amount of Federal indemnity funding is available to compensate producers and encourage depopulation. In recent years, the amount of available Federal indemnity funding has been insufficient to depopulate all CWD-positive herds identified in a single year. Further, indemnity funds have not been available to remove CWD-exposed animals for diagnostic testing to determine their infection status and the exposure status of specific herds involved in epidemiological investigations.

In light of these financial constraints, it is increasingly important for APHIS to prioritize how limited funds are used to provide indemnity in a way that:

- 1) Reduces the potential for disease transmission and environmental contamination.
- 2) Strategically removes CWD-exposed animals to inform risk evaluation and decision making regarding movement restrictions and other risk mitigations.
- 3) Encourages participation and compliance in the HCP.

APHIS will consider requests for Federal indemnity for CWD-positive, -exposed, and suspect animals and herds on a case-by-case basis. APHIS, in consultation with State representatives, will consider a number of interrelated factors as we comprehensively evaluate each case to make a decision about providing Federal indemnity. The factors we will consider and the relative priority of possibilities within each factor include (but are not limited to):

- 1) Availability of funds for indemnity.
- 2) Herd size (as it is related to the availability of funding).
- 3) Herd Status (CWD-positive herd >> Whole herd depopulation for herds with only CWD-exposed or suspect animals).
- 4) Type of Herd (Breeding herd >> Hunt preserve).
- 5) HCP Status (Enrolled and compliant >> Not enrolled or Enrolled but not compliant).
- 6) CWD detection in the local area (CWD not detected in wildlife or farmed cervids >> CWD detected in farmed cervids only >> CWD detected in wildlife).
- 7) Cervid density in local area (High >> Moderate >> low density).
- 8) Value of post-mortem testing of animals to understand epidemiology and inform decision making (Animal removal will likely impact knowledge/decisions about multiple herds >> will only inform knowledge/decisions about herd animal is residing in).

7. Carcass Disposal

Destruction or inactivation of infectious prions is difficult and few methods have been documented as completely successful. In addition, there are currently no quality assurance or quality control methods to ensure prion inactivation.

Carcasses from CWD-positive, suspect, or exposed animals or herds should be disposed of in compliance with all Federal, State, and local regulations. Additional information about State requirements for carcass disposal is available on the [Veterinary Compliance Assistance Web site](#). APHIS, upon request, can provide technical support and guidance to assist in identifying and implementing a local disposal plan.

Carcasses must be carefully transported to treatment or burial sites to prevent environmental contamination. Precautions should be taken to prevent ashes, blood, tissues, or feces from leaking from transport vehicles. All vehicles should be cleaned and disinfected after each use as described in Appendix IV.

The following list describes acceptable options for the disposal of carcasses from animals euthanized as part of a diagnostic or depopulation effort for CWD. Incineration, alkaline digestion, disposal of materials in appropriate landfills, and onsite burial, or a combination of these methods, are generally the most suitable options. These options are based on the available science of CWD inactivation. Changes to the list of options may be made as new information becomes available.

7.1 Incineration

Carcasses may be incinerated in an Environmental Protection Agency (EPA)-approved conventional incinerator, air curtain incinerator, or cement kiln. Prions can be destroyed through incineration provided the incinerator can maintain a temperature of 900° F for 4 hours. Incineration of animals onsite with a mobile incinerator is an option as it presents the least risk of spreading contaminated materials by moving carcasses. However, mobile incinerators require large amounts of fuel to maintain an even, high temperature appropriate for prions.

After incineration, ashes should be buried in an active, licensed landfill at a depth that meets local and State regulations to prevent scavenging or contamination of groundwater.

7.2 Alkaline hydrolysis

Carcasses of infected animals can be destroyed in a sterile alkaline solution using an alkaline hydrolysis digester. This consists of an insulated steam-jacketed stainless steel vessel which operates at up to 70 psi and 300° F into which sodium hydroxide and water is added, heated, and continuously circulated. This process degrades proteins and the temperature, together with alkali concentrations, deactivates prions.

After digestion, treated material may be buried in an active, licensed landfill at a depth that meets local and State regulations.

7.3 Landfill

Carcasses may be buried in a licensed, active landfill that meets local and State regulations for animal carcass disposal. However, this method will NOT inactivate the prions.

The definition of infectious waste varies among States, which could affect the standards associated with collection, handling, and disposal of waste that can include tissue, body parts, heads, and carcasses as well as contaminated laboratory materials. Consult with local and State authorities when pursuing this option.

In addition, individual animals could be tested for CWD using an ELISA with carcass disposal delayed until results are obtained. Subsequently, carcasses from positive animals can be disposed of with incineration or alkaline hydrolysis with burial of the treated materials. Carcass burial in a landfill in compliance with local and State regulations may be used for other animals with “Not Detected” results.

7.4 Onsite Burial

Carcasses may be buried onsite at a depth that meets local and State regulations for animal carcass disposal. However, this method will NOT inactivate the prions.

In addition, individual animals could be tested for CWD using an ELISA with carcass disposal delayed until results are obtained. Subsequently, carcasses from positive animals can be disposed of with incineration or alkaline hydrolysis with burial of the treated materials. Carcass burial onsite in compliance with local and State regulations may be used for other animals with “Not Detected” results.

Appendix I: Links to Forms and Documents

Forms and templates for application to the Approved State CWD Herd Certification Program include:

- [VS Form 11-2 \(Application for Chronic Wasting Disease Herd Certification program \(CWD HCP\) approval, renewal, or reinstatement of a State\)](#)
- [MOU Between State and APHIS for CWD HCP](#)

The Final CWD Rule:

- [9 CFR part 55](#)
- [9 CFR part 81](#)

[A list of Approved State CWD HCPs](#)

[VS Form 10-4 Laboratory Submission Forms](#)

[VS Form 10-4A Additional Page for Sample Submissions](#)

[CWD Program – “CWD Sample Collection Guidance”](#)

[Additional information about the Cervid Health Program](#)

Appendix II: Guidelines for Use of Whole Herd Ante-Mortem Testing of Herds that Contain or Contained CWD-Exposed Animals

Biopsy of the medial retropharyngeal lymph node (MRPLN) or the rectal anal mucosal associated lymphoid tissue (RAMALT) for the detection of the abnormal prion protein (protease resistant misfolded prion) associated with CWD is an official test *only in white-tailed deer*, and **only** when:

- 1) Genotype at codon 96 is established;
- 2) Used with herd plans for trace back, CWD-exposed herds, and epidemiologically-linked herds as described in Part B. , **and**
- 3) When performed at NVSL.

A case-by-case agreement will outline the specific timing and procedures to be used in a particular situation and will be included in the overall herd plan.

The following is a draft herd agreement for ante-mortem RAMALT testing that could be modified for the specific situation and incorporated into a herd plan:

Draft Herd Agreement for CWD Exposed Herds to Use Rectal Biopsy Testing as a Risk Assessment Herd Management Tool

Preface: Biopsy of rectal anal mucosal associated lymphoid tissue (RAMALT) for the detection of the abnormal prion protein (protease resistant misfolded prion) associated with CWD has a high specificity but a relatively low sensitivity for the detection of CWD in individual animals in comparison to post-mortem testing. Serial, whole-herd testing using RAMALT increases the confidence of detecting at least one positive animal in a potentially exposed herd. Sampling must be conducted by proficient collectors with adequate animal restraint.

The genotype of the animal is known to be associated with the tissue distribution of the abnormal prion over time (GG on codon 96 will have earlier and more extensive tissue distribution than GS on codon 96). The timing of the second whole herd testing will therefore depend on the genetic makeup of the herd. Current research suggests that the dose load and route of infection may also impact the time from exposure to detection.

Assumptions:

- 1) Genotype of codon 96 influences the interpretation of the RAMALT results.
- 2) At least two whole herd CWD tests using RAMALT samples must be conducted in series.
- 3) If more than 10 percent of the animals in a whole herd test have insufficient follicles for diagnostic purposes, then those animals must be resampled until a minimum of 90 percent of the entire herd is successfully sampled. A minimal number of samples with insufficient follicles is inherently accepted as part of the RAMALT technique.

APHIS Approved Procedure:

- 1) Initial whole herd test will be conducted not less than 24 months after the last known exposure to a CWD-positive animal. Whole-herd RAMALT biopsy, and whole blood samples for codon 96 genotyping, will be collected on all animals equal to or greater than 12 months of age as described in Appendix II. Biopsy samples will be sent to NVSL and blood samples will be sent to an APHIS-approved genetics laboratory.
- 2) Timing of the second whole herd RAMALT test will be determined by the results of the herd genotyping.
 - A. The second whole herd test for herds with over 70 percent GG animals will be at least 3 years after the last known exposure and at least 6 months after the initial whole herd test.
 - B. The second whole herd test for herds with 50 percent to 70 percent GG animals will be at least 3.5 years after the last known exposure and at least 6 months after the initial whole herd test.
 - C. Herds with fewer than 50 percent GG animals will not be permitted to use ante-mortem RAMALT testing.
- 3) All sample collection shall be done by a State or Federal veterinarian or a licensed, accredited veterinarian under the supervision of a State or Federal veterinarian, and the samples shall be considered to be the property of USDA.
- 4) All CWD diagnostics shall be performed by NVSL. Genetic testing of whole blood should be performed at an approved laboratory.
- 5) If more than 10 percent of the animals in a whole herd test have insufficient follicles for diagnostic purposes, then those animals must be resampled until a minimum of 90 percent of the entire herd is successfully sampled.

- 6) All costs associated with sample collection, genetic testing, and diagnostic testing are the responsibility of the herd owner.
- 7) The loss of any animal, function, or part of an animal that could arise as a result of handling or sample collection associated with this agreement shall be borne by the herd owner and not by the State or USDA.
- 8) Any method of chemical restraint used for testing shall be performed or administered by a licensed accredited veterinarian approved by the State and USDA.
- 9) The herd owner agrees to be in, and remain in, compliance with the terms of the State CWD HCP, and continue to maintain appropriate licensure with the State. In addition, any animal 6 months of age or older, that dies during the period of the herd plan, must be made available for sample collection.
- 10) If a positive result is found on rectal biopsy, the animal must be surrendered for further testing. There will be no compensation to the owner unless indemnity funds are available. The herd will remain under quarantine and will be designated a CWD-positive herd.
- 11) Notwithstanding paragraph 9, if the herd is negative on both whole herd tests, the State and USDA will evaluate the test results and agreement compliance for quarantine release. If the herd has remained in compliance with all terms of the herd plan, the quarantine will be released.
- 12) If animals that were RAMALT tested are not sampled and tested as described above, then the herd will remain quarantined until 60 months have passed since the last exposure to a CWD-positive animal.

Appendix III: CWD Epidemiology Investigation and Report Templates

Preliminary Epidemiology Report Worksheet

APHIS requests that States provide the following preliminary information to APHIS within 7 business days of NVSL confirmation of a CWD-positive animal in a newly identified CWD-positive herd. APHIS may request clarification or additional information on CWD-positive herds as needed for risk assessments, indemnity requests, or other reasons. Submit the completed worksheet to: vs.sprs.cervid.health@aphis.usda.gov

State _____ County _____ Herd _____

Owner _____

Please complete one form for each CWD-positive herd that you have identified in your State.

Index Case (defined as the first positive case identified in a herd) Check if traced from another positive herd

1. Age at the time of death/euthanasia? ___ Yr ___ Mo
2. Sex? ___ M ___ F
3. Species? _____
4. Was the index case a natural addition? ___ or a purchased addition? ___ (check one)
If natural addition, date of birth ___/___/_____
If purchased, date added to herd ___/___/_____
If purchased, from where? _____ (herd/name)
_____(State)
5. Date of death/euthanasia? ___/___/_____
6. Date CWD samples were taken? ___/___/_____
7. Was the index case exhibiting clinical signs at the time of death/euthanasia?
Y/N/Don't know
8. Obex test result? Positive ___ Not detected ___ Location ___ Not sampled ___
Lymph node test result? Positive ___ Not detected ___ Location ___
Not sampled ___
_____ test result? Positive ___ Not detected ___ Location ___
Genetics testing results? ___@codon___ ___@codon___ Not tested ___

Positive Premises (defined as the premises on which the index case resided at the time of diagnosis)

1. Date cervid herd was established? ___/___/___
2. Type of operation (check all that apply)? ___ Breeding___ Hunting ___ Other
(If Other, specify type _____)
3. Most recent known/reported captive cervid inventory at the time the index case was diagnosed: Date of inventory ___/___/___

Cervid Herd Inventory at the Time of Index Case Diagnosis					
Species	1 year old and over		Under 1 year old		Total Inventory
	Males	Females	Males	Females	
Elk					
White-tailed deer					
Other (_____)					

4. Total size of the area where captive cervids were held? _____ acres
5. Size of the enclosure where the index case was held? _____ acres
6. Were animals from the index herd housed on more than one location?
Y/N/Don't know
If yes, please explain

7. Was the premises double-fenced at the time the index case was diagnosed?
Y/N/Don't know
8. Was the premises managed as a closed herd at the time of diagnosis?
Y/N/Don't know
If yes, for what length of time prior to the index case diagnosis? ___ Yr ___ Mo
If the herd was not managed as a closed herd, how many other herds were cervids sourced from in the 5-year period prior to the index case diagnosis?
In-State sources # of premises _____ # of animals _____
Out-of-State sources # of premises _____ # of animals _____

(Please include any known details of sources)

How many other herds were cervids moved to in the 5-year period prior to the index case diagnosis?

In-State departures	# of premises _____	# of animals _____
Out-of-State departures	# of premises _____	# of animals _____

(Please include any known details of departures)

9. Were any ancillary businesses associated with the positive premises? (e.g. urine collection, taxidermy, wildlife rehabilitation, fawn raising)? Y/N/Don't know
(If Yes, specify type(s))

_____)

10. Was the index herd enrolled in a Herd Certification Program (HCP) at the time that the index case was diagnosed? Y/N If yes, date of enrollment?
____/____/____

If yes, was the herd in compliance with the requirements of the HCP at the time the index case was diagnosed? Y/N/Don't know

If the herd was not in HCP compliance at the time the index case was diagnosed, please explain:

-
11. At the time that the index case was diagnosed, was the index herd located:
Within 10 miles of known CWD positives in **wildlife**? Y/N/Don't know
Between 11 and 50 miles of known CWD positives in **wildlife**? Y/N/Don't know
12. At the time that the index case was diagnosed, was the index herd located:
Within 10 miles of known CWD positives in other **captive cervids**? Y/N/Don't know
Between 11 and 50 miles of known CWD positives in other **captive cervids**? Y/N/Don't know
13. What is the wild cervid population density outside of the positive premises?
14. Any other known risk factors or important information regarding the positive herd?

Final Epidemiology Report Worksheet

A final report of the epidemiological investigation is required for all HCP-enrolled CWD-infected herds and for all herds that receive APHIS indemnity funds. Ideally, States will submit final epidemiology reports from all CWD-positive herds to facilitate future disease mitigation efforts. States should submit the final report for CWD-positive herds as part of their annual HCP report.

State _____ County _____ Herd _____

Owner _____

Please complete one form for each CWD-positive herd that you have identified in your State.

Index Case (defined as the first positive case in a herd) Check if traced from another positive herd

1. Age at the time of death/euthanasia? ___ Yr ___ Mo
2. Sex? ___ M ___ F
3. Species? _____
4. Was the index case a natural addition? ___ or a purchased addition? ___
(check one) If natural addition, date of birth ___/___/____
If purchased, date added to herd ___/___/____
If purchased, from where? _____ (herd/name)
_____ (state)
5. Date of death/euthanasia? ___/___/____
6. Date CWD samples were taken? ___/___/____
7. Was the index case exhibiting clinical signs at the time of death/euthanasia?
Y/N/Don't know
8. Obex test result? Positive ___ Not detected ___ Location ___ Not sampled ___
Lymph node test result? Positive ___ Not detected ___ Location ___
Not sampled ___
_____ test result? Positive ___ Not detected ___ Location ___
Genetics testing results? ___@codon___ ___@codon___ Not tested ___

Positive Premises (defined as the premises on which the index case resided at the time of diagnosis)

1. Date cervid herd was established? ___/___/____
2. Type of operation (check all that apply)? ___ Breeding___ Hunting ___ Other
(If Other, specify type _____)

3. Most recent known/reported captive cervid inventory at the time the index case was diagnosed: Date of inventory ___/___/_____

Cervid Herd Inventory at the Time of Index Case Diagnosis					
Species	1 year old and over		Under 1 year old		Total Inventory
	Males	Females	Males	Females	
Elk					
White-tailed deer					
Other (_____)					

4. Total size of the area where captive cervids were held? _____ acres
 5. Size of the enclosure where the index case was held? _____ acres
 6. Were animals from the index herd housed on more than one location?
 Y/N/Don't know
 If yes, please explain

7. Was the premises double-fenced at the time the index case was diagnosed?
 Y/N/Don't know

8. Was the premises managed as a closed herd at the time of diagnosis?
 Y/N/Don't know
 If yes, for what length of time prior to the index case diagnosis? ___ Yr ___ Mo
 If the herd was not managed as a closed herd,
 How many other herds were cervids sourced from in the 5-year period prior to the index case diagnosis?
 In-State sources # of premises _____ # of animals _____
 Out-of-State sources # of premises _____ # of animals _____

(Please include any known details of sources)

How many other herds were cervids moved to in the 5-year period prior to the index case diagnosis?
 In-State departures # of premises _____ # of animals _____
 Out-of-State departures # of premises _____ # of animals _____

(Please include any known details of departures)

9. Were any ancillary businesses associated with the positive premises? (e.g. urine collection, taxidermy, wildlife rehabilitation, fawn raising)? Y/N/Don't know
(If Yes, specify type(s))

10. Was the index herd enrolled in a Herd Certification Program (HCP) at the time that the index case was diagnosed? Y/N
If yes, date of enrollment? ___/___/____
If yes, was the herd in compliance with the requirements of the HCP at the time the index case was diagnosed? Y/N/Don't know
If the herd was not in HCP compliance at the time the index case was diagnosed, please explain:

11. At the time that the index case was diagnosed, was the index herd located:
Within 10 miles of known CWD positives in **wildlife**? Y/N/Don't know
Between 11 and 50 miles of known CWD positives in **wildlife**? Y/N/Don't know

12. At the time that the index case was diagnosed, was the index herd located:
Within 10 miles of known CWD positives in other **captive cervids**?
Y/N/Don't know
Between 11 and 50 miles of known CWD positives in other **captive cervids**?
Y/N/Don't know

13. What is the wild cervid population density outside of the positive premises?

14. Was this herd depopulated? Y/N
If yes, date of depopulation? ___/___/____
If no, date quarantined? ___/___/____

15. If this herd was depopulated, inventory at the time of depopulation:
Date of inventory ___/___/____
Check box if same as inventory listed in item 12 above:

Cervid Herd Inventory at the Time of Depopulation					
Species	1 year old and over		Under 1 year old		Total Inventory
	Males	Females	Males	Females	
Elk					
White-tailed deer					
Other (_____)					

CWD Test results from the depopulated inventory (rows below should add up to total inventory in item above):

Obex test results? #Positive ____ #Not detected ____ #Location ____

#Not sampled ____

Lymph node test result? #Positive ____ #Not detected ____ #Location ____

#Not sampled ____

_____ test result? #Positive ____ #Not detected ____ #Location ____

#Not sampled ____

16. Did any cervids die prior to depopulation of the herd or while the herd was being held under quarantine (including euthanasia deaths)? Y/N/Don't know
If yes, how many? (please complete the following table):

Number of Cervids that Died or were Euthanized Prior to Depopulation or While Held under Quarantine					
Species	1 year old and over		Under 1 year old		Total
	Males	Females	Males	Females	
Elk					
White-tailed deer					
Other (_____)					

CWD Test results (rows below should sum to total above):

Obex test results? #Positive ____ #Not detected ____ #Location ____ #Not sampled ____

Lymph node test result? #Positive ____ #Not detected ____ #Location ____

#Not sampled ____

_____ test result? #Positive ____ #Not detected ____ #Location ____

#Not sampled ____

17. For **all CWD POSITIVE cervids** (TOTAL herd numbers) that died or were euthanized following the index case diagnosis (during depopulation or otherwise AND including the index case), please provide:
- TOTAL number of CWD-positive animals: _____
 - Of the total number of CWD-positive animals above, how many were:
 - 0-24 months of age? : _____
 - 25-48 months of age? : _____
 - 49+ months of age? : _____
 - Total number of positive males: _____
 - Total number of positive females: _____

- e. Were all positives the same species? Yes / No
If no, please provide the total number of positive:
Elk ____ White-tailed deer ____ Other (_____) ____
- f. Total number of positive natural additions: _____
- g. Total number of positive purchased additions: _____
Were all positive purchased animals from the same place? Yes/No
1. If yes, total number of animals purchased? _____
From herd _____ in State _____
2. If no, number of facilities from which positive animals were purchased?

Provide number of animals purchased from each herd and the State of origin _____
- h. Total number of animals showing clinical signs at time of death:

- i. Genetics testing results on positives? Y/N/Don't know
If yes (WTD), # GG @ codon 96? ____ # GS @ codon 96? ____
SS @ codon 96? ____
If yes (Elk), # LL @ codon 132? ____ # LM @ codon 132? ____
MM @ codon 132? ____
18. How many *trace-forward* cervids were identified in the epidemiological investigation?
In-State trace-forwards # ____ Out-of-State trace-forwards # ____
 Check box if unable to trace due to poor records, etc.
- How many of the identified trace-forward cervids were tested for CWD? ____
Were any trace-forward cervids diagnosed as positive for CWD? Y/N/Don't know
If yes, how many were diagnosed as positive for CWD? ____
19. How many *trace-back* cervids were identified in the epidemiological investigation?
In-State trace-backs # ____ Out-of-State trace-backs # ____
 Check box if unable to trace due to poor records, etc.
- How many of the identified trace-back cervids were tested for CWD? ____
Were any trace-back cervids diagnosed as positive for CWD? Y/N/Don't know
If yes, how many were diagnosed as positive for CWD? ____

Chronic Wasting Disease Program Standards

For the most recent years prior to the index case being diagnosed, please provide:

Number of Years Prior to CWD Index Case Diagnosis	Reported Inventory	# Sold or Transferred from Herd	#Purchases (or Other Non-Natural Additions)	#Slaughtered and/or Hunter Harvested (and # CWD sampled)	# Natural Deaths (and # CWD Sampled)	#Valid Reported CWD Test Results (i.e. do not count location or untestable results)
1 Year Prior				()	()	
2 Yrs. Prior				()	()	
3 Yrs. Prior				()	()	
4 Yrs. Prior				()	()	
5 Yrs. Prior				()	()	

Please include a copy of any epidemiological reports conducted on this herd and copies of any lab test results or other pertinent findings.

Appendix IV: Biosecurity and Decontamination Procedures for Farmed Cervid Facilities

Chronic wasting disease (CWD) is an infectious disease of cervids that can be transmitted directly, animal to animal, and indirectly via contact with the environment and objects within it. The time between CWD exposure, proliferation in the body, and shedding in excreta (saliva, urine, feces, and blood) has yet to be definitively determined in cervids. However, studies using highly sensitive amplification assays have shown that infectious material is shed into the environment via these pathways at levels sufficient to cause significant site contamination over time. Once in the environment, prions are highly persistent, and can remain a source of CWD exposure for extended periods of time. Studies with scrapie in sheep suggest long environmental persistence times, greater than 10 years. Because of these factors it is prudent to use basic biosecurity practices, and attempt to decontaminate objects and equipment that may have become contaminated. There are currently no means available to decontaminate soil.

The recommended decontaminated procedures outlined below are believed to reduce the overall CWD burden on objects and equipment on a site. These recommended procedures may change as new scientific information becomes available.

1) Biosecurity: General Principles and Approach

Biosecurity refers to measures or management practices taken to try to stop the spread of harmful biological agents. Although not guaranteed to prevent disease spread, the following suggested measures are believed to reduce potential exposure of captive cervids to CWD and other infectious diseases:

A. Direct Contact: Contact with cervids and other wildlife

1. Monitor and maintain perimeter fences. Repair holes and washouts to prevent the entry of wildlife.
2. Place feeders away from perimeter fences as to not attract wild cervids to the fenceline where direct contact can occur between wild and captive cervids.
3. Reduce or eliminate forage immediately outside the perimeter fence to make fence lines less attractive to captive cervids.
4. Consider installing a strand of electric fence along perimeter fences to discourage contact between captive and wild cervids.
5. If wild birds are a problem at feeders or waterers consult State wildlife agencies to develop deterrent strategies.

6. Remove dead animals from the landscape as soon as they are discovered. Do not form carcass or “dead” piles to dispose of dead animals. The carcasses attract scavengers, which can translocate infectious agents. See section B of this document for proper disposal methods.

B. Indirect Contact: Contact with potentially contaminated objects or materials

1. Store feed and hay so it is not accessible to wild cervids.
2. Personnel working on the site should have designated boots and outerwear that are not worn elsewhere.
3. Delivery vehicles and transport vehicles should be cleaned and decontaminated before and after going onto the site. Instructions for decontamination can be found below.
4. Producer vehicles such as cars, trucks, transport vehicles, tractors, skid loaders, and ATVs should be cleaned and disinfected **prior to, and after**, use on other sites (see recommended procedures in section 2.A. below). A pressure washer is useful to remove mud and feces from wheels and equipment prior to decontamination.
5. Equipment (feeders, water troughs, chutes, buckets, antler removal equipment, bolus guns, multiple-dose syringes, etc) should not be shared between herds.
6. Do not bring cervid carcasses, tissues, or byproducts onto the sites where direct or indirect contact with the captive cervids, or their associated equipment, could occur.

2) Decontamination: Principles and Approach

The recommended decontamination procedures outlined below are believed to reduce the overall CWD burden on objects and equipment on a site with known CWD contamination. Decontamination procedures are directed at items and locations within the facility most likely to harbor the agent. Areas where CWD-positive animals have resided will be the most contaminated. These areas should be evaluated by:

- A. Assessing the facility in detail to document areas of animal congregation or particular movement patterns.
- B. Characterizing the entire facility in terms of concentration of animals over time. This includes identification of fencelines (past and present), pens, corrals or handling facilities, watering and feeding areas (including natural water sources),

points of concentration in a landscape (i.e. sheltered areas, woodlots etc.), drainage areas, and calving areas.

- C. Identifying where known positive animals resided relative to the areas of animal concentration.

3) Recommended Procedures for Decontamination of Premises and Associated Equipment

A. Pastures

Small pastures where CWD-positive animals have resided or particular areas in a pasture where animals are known to have congregated may be treated as follows:

1. If practical, till soil under or do not use area to graze CWD-susceptible animals.
2. Organic material (hay, accumulations of manure, etc.) in congregation areas should be buried. Congregation areas include animal shelters, feeding grounds, and water sources (if applicable).

B. Dry Lot

Where CWD-positive animals have been held in close confinement (this includes but is not limited to corrals, pens, stalls, and alleyways or pathways) may be treated as follows:

1. Remove organic materials (manure, feed, bedding, and other organic material). This material may be buried deeply onsite in areas not accessed by farmed or wild animals, incinerated, or digested by alkaline hydrolysis. Composting may be used to reduce the volume of organic materials. Composted material should be buried deeply, incinerated, or digested by alkaline hydrolysis after composting is complete. Composting alone does not inactivate prions.
2. In addition, removal of the top 1 to 2 inches of soil may help to reduce surface contamination. The soil removed may be buried deeply or incinerated.

C. Earth Surfaces Inside Structures

1. Remove and dispose of the organic material as described for dry lot.
2. When practical, remove the top 1 to 2 inches of soil to help reduce surface contamination. Bury the removed material in areas not accessed by farmed or wild cervids.

D. Nonearth Surfaces

Cement floors, wood, metal, tools, equipment, instruments, grain feeders, hay feeders, panels, chutes, working facilities, transport vehicles, skid loaders, and ATVs may be treated as follows:

1. Remove all organic material and deeply bury the removed material onsite in areas not accessed by farmed or wild cervids.
2. Clean and wash surfaces of items using hot water and detergent. A high-pressure washer after initial manual removal of organic debris and cleaning surfaces is recommended for thorough cleaning of large equipment items.
3. Allow all surfaces, tools, and equipment to dry completely before disinfecting using the following suggested methods below for clean dry surfaces:

E. To Clean Dry Surfaces:

1. Apply a solution of 2 percent available chlorine (equivalent to approximately 20,000 ppm available chlorine at room temperature (at least 18.3° C [65° F]) for 1 hour of wet contact time. This can be achieved by mixing 50 ounces [6 1/4 cups] of household bleach (sodium hypochlorite) with enough water (78 ounces or 9¾ cups) to make 1 gallon of solution. Rinse to remove solution after 1 hour. Multiple applications may be required to ensure the 1 hour contact time. Due to variations in chlorine bleach concentrations, care must be taken to verify that the minimum of 20,000 ppm is achieved. If chlorine bleach is not available, a 1 molar or 4 percent sodium hydroxide (5 ounces sodium hydroxide dissolved in 1 gallon of water) solution may be used at room temperature (at least 18.3° C [65° F]) for at least 1 hour of wet contact time. Rinse to remove solution after 1 hour. Multiple applications may be required to insure the 1 hour contact time.
2. Synonyms for sodium hydroxide (NaOH) are caustic soda, soda lye, and sodium hydrate. Sodium hydroxide is a white, brittle solid that dissolves readily in water to form a strong alkaline and caustic solution and is used as an alkalinizing agent. Sodium hydroxide is very caustic and in solution is **extremely corrosive**. For environmental reasons, only use this disinfection method when the preceding method is not available.

4) Restocking

The premises may be restocked with non-cervid species immediately after decontamination. Generally, restocking with cervids is not recommended. If restocking with cervids occurs, then additional biosecurity practices such as additional fencing or other barriers to minimize CWD exposure should be

considered. The cervid herd should immediately enroll in the Approved State CWD HCP. All mortalities 12 months of age or older must be reported, investigated, and CWD tested.

5) Decontamination Safety Precautions

Professional judgment should be exercised in the choice and use of disinfectants. All disinfectants are hazardous to humans, animals, and the environment in varying degrees. Label directions should be carefully read and followed. If corrosive disinfectants are used directly on metal items, the items must be thoroughly rinsed with fresh water to minimize damage.

Disinfectants, especially in concentrated form, may irritate the skin, eyes, and respiratory systems. Protective equipment such as coveralls, rubber boots, rubber gloves, masks, or respirators as well as eye protection should be worn while mixing and applying disinfectants. If areas of the body are exposed directly to a disinfectant, they should be washed thoroughly with water. Any employee should notify his or her supervisor if excessive human or animal exposure to disinfectants occurs or if there is an accidental release into the environment.

6) Required Reporting of Bleach and Lye Use

The EPA requires reporting of bleach and lye use in the environment. To fulfill this reporting obligation, APHIS and/or State officials are requested to contact the Cervid Health Team to report the amounts of bleach and lye that were used.

Appendix V: Sample Collection

Herd owners are responsible for notifying State representative when animals require sampling and for refrigerating the head for sampling.

Instructions for Veterinarians and Certified CWD Sample Collectors

1) Safety Precautions

The collector should take the following safety precautions to minimize exposure to pathogens:

- A. Wear personal protective equipment (PPE) at all times. (See Section 2 below.)
- B. Cover cuts, abrasions, and wounds with waterproof dressing if not covered by PPE.
- C. Wear gloves while handling specimens and formalin. Optionally, use face and respiratory protection, including a well-fitted respiratory mask and face shield or goggles to protect from infective droplets or tissue particles.
- D. Use 10 percent neutral buffered formalin in a well-ventilated area.
- E. Take steps to avoid creating aerosols, splashes, and dusts.
- F. Wash hands and exposed skin following collection procedures.
- G. Wash and disinfect protective clothing and equipment thoroughly after use. Use equal parts bleach and water to make 1 gallon of disinfectant solution; this solution needs have a wet contact time of 1 hour to be effective. This may require multiple applications. It is best if disposable items are used and then discarded after use.
- H. If rabies is suspected, do **not** proceed with any tissue collection. Instead, contact the approved laboratory for instructions on submission of the entire head to the laboratory for rabies testing. After rabies testing is completed, the laboratory will proceed with CWD sampling on rabies-negative brains.

2) Personal Protective Equipment

Personal protective equipment (PPE) is designed to minimize exposure to pathogens while collecting samples.

The Occupational Safety and Health Administration defines PPE as “specialized clothing or equipment worn by employees for protection against health and safety

hazards.” PPE is designed to protect many parts of the body (i.e., eyes, head, face, hands, feet, and ears).

PPE is selected based on the environment, physical hazards, and ability to complete the task, and is a balance between protection and comfort and should protect an individual from the physical hazards of the collection environment while allowing the individual to comfortably collect specimens. The following PPE is recommended for the collection of CWD specimens, particularly during post-mortem collections:

A. Skin Protection

Protect your skin from contact with fluids during specimen collection. Wear waterproof coveralls, preferably disposable, or coveralls with a waterproof apron and forearm protectors.

B. Eye and Face Protection

Protect your eyes and face from any aerosols, splashes, or dusts that may be created while collecting specimens. Eye protection includes safety glasses, safety goggles, or a face shield.

C. Hand Protection/Gloves

1. Wear metal or mesh gloves. Always wear the cut-resistant glove (Hantover, Koch, or Packer) on the hand that is not holding the knife. Find a cut-resistant glove that fits against your skin and then wear a rubber glove on top of it.
2. Wear latex or nitrile examination gloves or thick rubber gloves on the hand holding the knife.

D. Foot Protection

Protect your feet from injuries or exposure, such as spills or splashes, by using rubber boots.

E. Respiratory Protection

Face masks or respirators are recommended if the environment includes aerosols, splashing, or flying debris as may be encountered with certain methods of brain removal or tissue handling. Zoonotic diseases such as rabies and listeria may be present in the carcass during CWD collection.

3) Paperwork to be Included with Diagnostic Tissue Submission

Accurately complete the specimen collection form (VS Form 10-4 or electronic 10-4, or equivalent submission form). **Note:** Complete VS Form 10-4 with the approval of the

State official or accredited veterinarian who will in turn obtain the approval of the Assistant Director. A link to VS Form 10-4 can be found in Appendix I.

Suspect and presumptive-positive animals should be submitted on separate VS Form 10-4s from routine surveillance samples and shipped promptly to allow NVSL to prioritize testing these cases.

- A. Indicate the reason for submission: Routine herd surveillance, exposed animal, suspect herd/animal.
- B. Indicate whether the animal was exhibiting clinical signs. If the animal exhibited clinical signs, list the signs in the Additional Data Section of the VS Form 10-4 or equivalent form.

4) Document the Following:

- A. Herd identification, species, breed, and sex of animal.
- B. Information from all ID devices, tattoos, and any brands on the animal.
- C. Age of animal based on owner records.

5) Make Four Copies of the Completed VS Form 10-4 or Equivalent Form:

- A. One for your files (submitter's copy),
- B. One for the animal owner or collection site,
- C. One for the VS District Office, and
- D. One to be submitted with the specimen.

6) Paperwork to be Included with Blood Samples for Codon 96 Genetic Analysis With Ante-mortem Testing of Herds that Contain or Contained CWD-Exposed Animals

Blood samples collected with ante-mortem diagnostic assays must be sent to an approved genotyping laboratory (see Title 9, *Code of Federal Regulations* (9 CFR) section 54.11 – Approval of laboratories to run official scrapie tests and official genotype tests (9 CFR 54.11). Contact the laboratory in advance for submission forms and proper tissue collection and shipping protocols.

7) Sample Quality

All samples should be collected and submitted to the lab irrespective of the state of autolysis. Approved labs should evaluate the condition of the autolyzed samples to

determine if the samples are of sufficient quality to be reliably tested or if the samples should be sent directly to NVSL.

Laboratory diagnosticians will determine the suitability of the samples for CWD testing with guidance from NVSL as necessary. Any concerns for sample quality and suitability, and subsequent interpretation of test results, will be discussed on a case-by-case basis with the Approved State CWD HCP Official and APHIS.

8) Sample Labeling

- A. Properly label all specimen collection containers. The information on the label provides detailed information to the laboratory regarding the specimens. The sample number or sample bar code on the container must be the same as on the completed VS Form 10-4 (or equivalent form).
- B. Clearly label both the top and the sides of the sample container. Identify the sample by using a permanent marker, or affixing a bar code label (if available), or other printed label.
- C. Verify that the sample number that appears on the top and side of the sample container is the same as VS Form 10-4.
- D. The side label should include the following:
 1. Date of collection.
 2. Producer name.
 3. Species.
 4. Type of specimen.
 5. Official animal ID number.
 6. Sample ID number (number assigned to this sample on the VS Form 10-4 or equivalent form).

Correctly package specimens to meet Federal transportation guidelines. For Category B (UN3373) packaging and shipping details, contact the receiving laboratory, or NVSL.

Ensure that the package containing any fresh tissues for CWD testing will be shipped with ice packs for overnight delivery to the laboratory during normal business hours.

9) Tissue Specimens and Preservation

Proper preservation and handling of specimens is critical to ensure accurate CWD test results. Specimens are submitted either formalin-fixed or fresh depending on the type of diagnostic test being used. It is recommended that samples be submitted for testing within 7 days of collection.

- A. **Formalin-fixed specimens** are used for immunohistochemistry (IHC) testing and histopathology. Submerge the specimen in 10 percent neutral buffered formalin (follow the guideline of 10 parts buffered formalin per 1 part specimen). Use a single container for each animal. ***Do not freeze the formalin-fixed specimens.***
- B. **Fresh tissue specimens** are used for Western blot, the ELISA assay, and for DNA/genetic analysis. *Fresh tissue specimens must be kept chilled.* Ensure the sample container correctly lists all specimens included. Use a single container for each animal.
- C. **Blood samples** in EDTA tubes are required for codon 96 genotyping with approved antemortem diagnostic testing as described in a herd plan. *Blood samples must be kept chilled.* Ensure each tube is clearly marked with the animal ID number.

Ship the chilled tissues overnight on ice packs. If dry ice is used, follow all additional shipping regulations associated with using dry ice.

Additional samples may be requested by the State representative or APHIS officials, including samples requested for research.

10) Post Mortem Tissue Specimens

The obex and retropharyngeal lymph node should be collected regardless of sample condition (e.g. autolyzed, frozen, etc.) and submitted to the approved laboratory to comply with the routine herd surveillance requirement. APHIS recommends that an eartag with a fresh piece of ear tissue attached be included with each sample that is submitted for CWD testing.

Required tissues and preservation methods for post mortem diagnostics can be found in the table below.

Fixed: 10% neutral buffered formalin (for histopathology, IHC testing)	Fresh: Chilled or Frozen (for DNA, Western blot, ELISA testing)
Tissues to be Submitted	Tissues to be Submitted
MRPLN. Half of each of the left and right lymph node	MRPLN Half of the left and right nodes
Obex Obex with 1-2 cm brain stem (including the apex of the “V” in the obex)	Obex Obex with 1-2 cm brain stem
Tonsils (optional)	Tonsils (optional)
N/A	Skin Sample Collect the official ID with a quarter-sized (aprox 1” x 1”) piece of tissue (ear, hide, etc.) attached to each device. This will allow DNA verification and/or genotyping if necessary.

11) Ante-mortem Tissue Specimens - White-tailed Deer ONLY

Ante-mortem sampling is done as part of a herd plan for CWD-exposed animals only. Required tissues and preservation methods for ante-mortem diagnostics can be found in the table below. All ante-mortem tissue and blood samples collected as part of herd plans in CWD-positive or exposed herds must be performed or directly monitored by a State animal health official (SAHO) or Veterinary Services (VS) representative to verify the identity of the animal, the tissues taken for biopsy, and the chain of custody of the biopsy and blood samples.

Whole blood collection by a State or Federal veterinarian or a licensed accredited veterinarian is required for determining the genetic polymorphism at codon 96 in white-tailed deer. This polymorphism has a significant impact on CWD propagation and consequently detection, and is used to determine repeat sampling times. Blood samples are to be sent to an approved genotyping laboratory and the results reported to the Cervid Health Team.

Fixed: 10% neutral buffered formalin (for histopathology, IHC testing)	Fresh: Chilled or Frozen (Avoid repeated freeze/thaw; for genotyping)
Tissues to be Submitted	Tissues to be Submitted
MRPLN Biopsy 2cm X 1cm X 1cm (at least 40 follicles required)	Blood 3-5 mL of whole blood in EDTA tube
Rectal Biopsy 1 cm x 1.5 cm	

12) Collection Procedures for Post-Mortem MRPLN

The post-mortem collection of the MRPLNs can be completed using several methods. However, these collection procedures describe the preferred methods to prevent inadvertent damage to the tissues during collection.

A. The following equipment will help ensure proper specimen collection:

1. Sharp boning knives.
2. Disposable scalpel blades or disposable scalpels (a large scalpel blade is acceptable).
3. Brown-Adson or rat-tooth forceps.
4. Disposable cutting surfaces such as cardboard, plastic, or Styrofoam.
5. Small hand nippers can be used on the hyoid bones or you may cut through at the soft cartilage of the joint using a knife.
6. Sharp stainless steel scissors.

B. MRPLN removal

1. The MRPLNs are medial to the stylohyoid bones on the dorsolateral surface of the pharyngeal muscles and dorsal to the carotid artery.
2. With the head positioned upside down, locate the esophagus and trachea in relation to the foramen magnum (FM).

3. Lift the trachea and dissect muscles forward of the FM (rostrally). Locate the left and right medial retropharyngeal lymph nodes (MRPLN) halfway between each corner of the jaw bone and the FM, caudal to the nasopharynx, and deep to the salivary gland. Lymph node consistency is much firmer and rounder than the surrounding tissue.
4. Remove each left and right medial RPLN and longitudinally incise each LN to confirm lymphoid tissue.

For IHC testing: Place the medial RPLNs in the same formalin jar with the obex.

For ELISA testing: Place the fresh medial RPLNs in labeled whirl-pak bags (do NOT use formalin).

13) Collection Procedures for Ante-Mortem MRPLN

A licensed, accredited, veterinarian must perform the sample collection as described in the herd plan. The accredited veterinarian must be monitored by a SAHO or VS representative to verify the identity of the animal, the tissues taken for biopsy, and the chain of custody of the biopsy and blood samples.

A. Tissue Collection

1. Anesthesia will be administered by a licensed accredited veterinarian or by personnel under the direct supervision of a licensed accredited veterinarian.
2. All biopsy collections will be performed using aseptic procedures at the surgical site, including surgical gloves, masks, sterile instruments, and other aseptic techniques.
3. Surgical instruments must be sterilized according to prion-specific disinfection or be disposed of after each use.
4. Biopsy – a single side or bilateral biopsy – may be performed.
5. With the head positioned upside down, identify the medial retropharyngeal lymph node located between the larynx and the floor of the skull. If the lymph node is cut through the center an outer layer (the cortex) and an inner layer (the medulla) will be visible. The lymph node is about 1-2 cm diameter x 2-3 cm long.
6. The whole lymph node or a section of the lymph node is surgically removed. Typically a biopsy of approximately 2 cm x 1 cm x 1 cm will be large enough

to meet or exceed the required 150 square millimeter of total surface area and 40 total follicles when the biopsy is sectioned and examined microscopically.

7. The incision is closed with absorbable sutures in a 2-3 layer closure.
8. Place the biopsy in a jar of 10 percent neutral buffered formalin (10:1 ratio of formalin to tissue sample).
9. Submit MRPLN biopsies collected from CWD-positive or -exposed herds directly to NVSL.

14) Collection Procedures for Post-Mortem Obex (Via Foramen Magnum)

A. The following equipment will help ensure proper specimen collection:

1. Sharp boning knives.
2. Disposable scalpel blades or disposable scalpels (a large scalpel blade is acceptable).
3. Brown-Adson or rat-tooth forceps.
4. Meat-cutting bone saw, hacksaw, or electric saw when brain removal is required.
5. Disposable cutting surfaces such as cardboard, plastic, or Styrofoam.
6. Small hand nippers can be used or you may cut through at the soft cartilage of the joint using a knife.
7. Sharp stainless steel scissors.
8. Brain stem/obex spoon, grapefruit knife, or other brain stem scoop.

B. Obex removal

1. Incise the head of the animal at the atlanto-occipital joint (between skull and first vertebra). Cut behind the back of the ears and extend the cut around and through the front of the larynx. Sever the brain stem as far to the posterior as possible during the removal process.
2. Position the head upside down (ventral side up). Locate the occipital condyles and foramen magnum (FM). Locate the brain stem inside the FM. Trim the dura mater around the brainstem and cut the attached cranial nerve trunks.

3. Gently lift the brain stem with forceps and insert the spoon into the **dorsal** aspect of the FM between the brainstem and **dorsal** calvarium.
4. Advance the spoon 2-3 inches rostrally until it contacts bone to sever the cerebellum.
5. Reposition the spoon in the **ventral** aspect of the FM between the brainstem and the **ventral** calvarium. Advance the spoon until it contacts bone and transversely sever the brain stem.
6. Remove the brain stem using the spoon and forceps. Examine to ensure the proper obex sample (bifurcation or “V”) is preserved.
7. Further trim the brain stem section by making a transverse cut 3/4 inch in front of the “V” shape bifurcation and an equal distance behind the bifurcation for good fixation.

For IHC testing: Place the trimmed obex and brainstem pieces in a jar of 10 percent neutral buffered formalin (10:1 ratio of formalin to tissue sample).

For ELISA testing: Place the fresh obex sample and trimmed pieces in a conical tube (do NOT use formalin). Samples should be placed individually in a labeled plastic bag and kept chilled or frozen.

Including official animal identification with a quarter-sized (aprox 1” x 1”) piece of tissue (ear, hide, etc.) attached to each device provides verification of sample identity and material for DNA analysis, if needed. The owner may observe the sampling and labeling procedures to assure his or her sample is properly identified.

15) Whole Head Submission

Refrigerated heads may be shipped to an APHIS-approved CWD laboratory. Prior notification and approval is required from the laboratory before shipping whole heads. Owners must ensure that fresh samples or heads can be refrigerated over weekends and holidays prior to shipping. Heads should be double bagged and shipped with ice packs overnight. Be sure to properly label shipment as biological specimens as per shipper requirements.

Whole heads submitted to a laboratory by the owner must include:

- A. The owner’s name, address, and phone number.
- B. All animal IDs (official and herd).
- C. Age of animal.

D. Sex of animal.

E. Description of any observed clinical signs.

16) Collection Procedures for Ante-Mortem Rectal Biopsy

Collection of rectal biopsies is to be conducted only by trained State, Federal, or accredited veterinarians following the recommendations given below to avoid cross infection of animals, and to ensure sample quality. The accredited veterinarian must be monitored by a SAHO or VS representative to verify the identity of the animal, the tissues taken for biopsy, and the chain of custody of the biopsy and blood samples.

CWD can be transmitted between animals through the use of contaminated instruments. Gloves and instruments must be changed between each animal. All instruments described below should be disposable. After use, instruments should be soaked in 1:1 bleach and water solution for 1 hour, then thrown away.

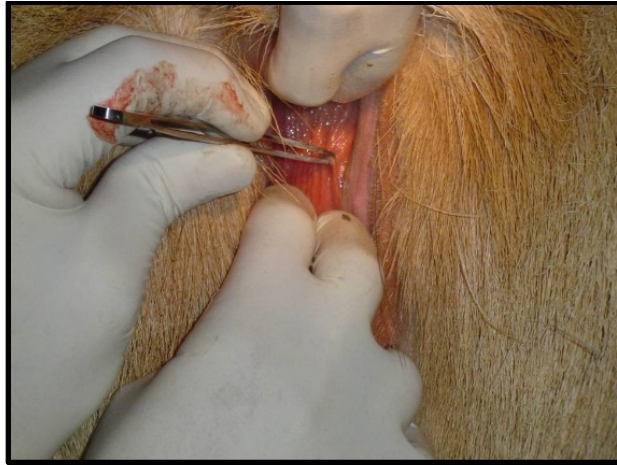
A. The following equipment will help ensure proper sample collection:

1. Nitrile gloves.
2. Disposable toothed Adson forceps.
3. Disposable curved Metzenbaum scissors.
4. Disposable rectal speculum (an extra pair of hands also works).
5. Obstetrical lubricant containing 2 percent lidocaine or 0.5 percent proparacaine.
6. Individually labeled tissue cassettes with foam inserts, labeled with pencil, *not* marker or pen.
7. Specimen collection containers with 10 percent buffered formalin.
8. Head lamp.

B. Collection of biopsy sample:

1. Animals need to be immobilized safely in a chute or chemically.
2. The rectal speculum is put in place, or the rectum held open.
3. The obstetrical lubricant with lidocaine is inserted approximately 10 cm into the rectum.

4. Five or more seconds after application of lubricant, pull the rectal epithelium away from the submucosa with forceps approximately 1 cm anterior to the mucocutaneous junction on the lateral wall (fig. 1A, B). Try to avoid sampling at 12 (tail) or 6 (feet) o'clock. Quickly snip an 1.5 cm X 1 cm biopsy.
5. Place the biopsy mucosal side down on the one of the foam inserts in the tissue cassette, carefully spread the sample out, place the other foam insert on top, close the cassette, and drop the cassette into the labeled formalin sample container (fig.1C).
6. Rectal biopsy samples collected from CWD-positive or -exposed herds must be sent to NVSL.



A.



B.

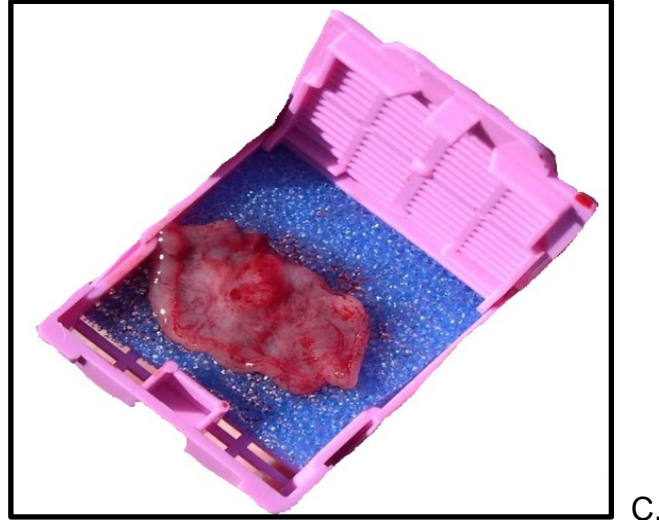


Figure 1. Grasping of rectoanal mucosa-associated lymphoid tissue (A.) Collection of rectal biopsy (B.) Placement of rectal biopsy in tissue cassette (C.) Photos courtesy of Dr. Thomas Gidlewski.

17) Collection Procedures for Blood Sample with Ante-Mortem Testing of Herds that Contain or Contained CWD-Exposed Animals

Whole EDTA blood collection is required for determining genetic polymorphisms at codon 96 in white-tailed deer together with ante-mortem diagnostic assays. Collection is only to be performed by a State or Federal veterinarian or a licensed, accredited veterinarian under the supervision of a State or Federal veterinarian. Polymorphism at codon 96 has a significant impact on CWD propagation, and consequently detection, and is used to determine intervals for sampling times in herds.

A. Collection of blood sample:

1. Animals need to be immobilized safely in a chute or chemically.
2. 3-5 ml of blood is collected into a commercial EDTA blood tube (purple top tube), then immediately inverted several times to ensure mixing of EDTA and blood.
3. Blood samples should be immediately placed in a cooler with ice or ice packs.
4. Blood samples should be sent overnight with ice or ice packs, with the associated sample submission form, to an approved genotyping laboratory.

Appendix VI: Diagram for Response to a CWD-Positive Case

The following diagram may be used to assist in response to a CWD-positive animal. All CWD-exposed cervids should be traced forward and back to include the 5 years since the exposure to the CWD-positive animal occurred.

