

## USDA APHIS Veterinary Services

### Chronic Wasting Disease Program – *Post Mortem* Sample Collection Guidance

#### Procedure for Removal of Obex & Medial Retropharyngeal Lymph Nodes (MRPLN)

Chronic Wasting Disease (CWD) has not been shown to infect humans, but other cervid diseases, such as rabies, can be zoonotic diseases. Therefore, personal protective equipment (PPE) should be worn when handling animal tissues. Dedicated or disposable instruments, work area covers, and PPE are recommended for sampling.

*Obex & MRPLN are **required** tissues for APHIS CWD Herd Certification Program (HCP) CWD testing*

*Samples **MUST** be submitted within 7 days of collection*

#### Sampling Procedure for Obex:

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 brainstem as far caudally 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 brainstem inside the FM. Trim the dura mater around the brainstem and cut the attached cranial nerve trunks.
3. Gently lift the brainstem 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. Re-position the spoon in the ventral aspect of FM between the brainstem and the ventral calvarium. Advance the spoon until it contacts bone and transversely sever the brainstem.
6. Remove the brainstem using the spoon and forceps. Examine to ensure the proper obex sample (Bifurcation or “V”) is preserved.
7. Further trim the brainstem 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.

#### SUBMIT

**FIXED:** [formalin] (IHC) place the trimmed obex in 10% buffered formalin jar (10:1 ratio of formalin to tissue sample).

**FRESH:** [chilled or frozen] Place the second piece of brain stem and place in a labeled whirl pack. For ELISA testing – place the second piece of brainstem sample and trimmed pieces in a conical tube.

Please see Sample Submission Procedure section in the HCP Program Standards for further details.

**Recommended PPE**  
Gloves, eye protection, coveralls

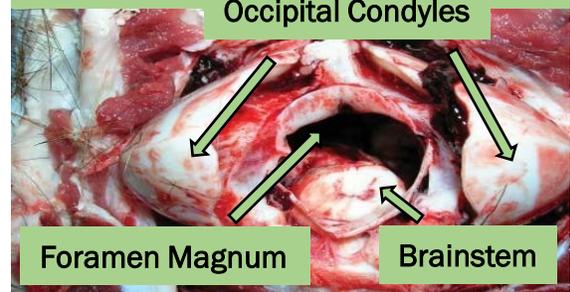
#### Equipment

Extraction spoon	Formalin Jars
Scalpel	Whirl-paks
Forceps	Icepacks
Scissors	Coolers
Knife	Bleach
Permanent Marker	

#### Ventral Side Up



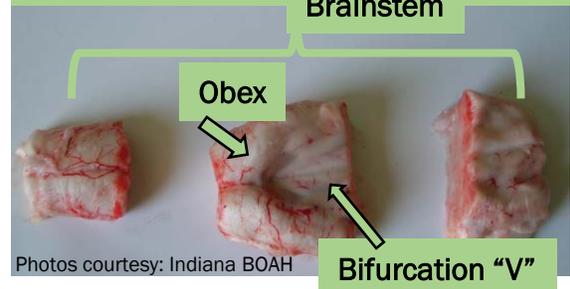
#### Occipital Condyles



#### Foramen Magnum

#### Brainstem

#### Brainstem



Photos courtesy: Indiana BOAH

#### Bifurcation "V"

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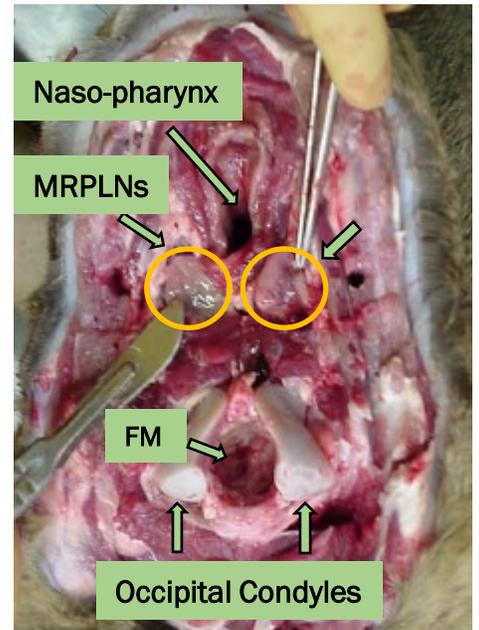
### Sampling Procedure for Medial Retropharyngeal Lymph Nodes (MRPLN)

1. With the head positioned ventral side up, locate the esophagus and trachea above the foramen magnum (FM).
2. Lift the trachea and dissect muscles forward of the foramen magnum (rostrally). Locate the left and right Medial Retropharyngeal Lymph Nodes (MRPLN) half-way between each corner of the jaw bone and the FM, caudal to the naso-pharynx, and deep to the salivary gland. Lymph node (LN) consistency is much firmer and rounder than the surrounding tissue.
3. Remove each left and right MRPLN and longitudinally incise each LN. Place **half of the left and half of the right** node into formalin, and the other halves into a whirl-pak.

#### SUBMIT

**FIXED:** [formalin] (IHC) place **half of the left**, and **half of the right** MRPLN in the same 10% buffered formalin jar as the obex (10:1 ratio of formalin to tissue sample).

**FRESH:** [chilled or frozen] place the other **halves** of the MRPLNs in a labeled whirl pack. For ELISA testing – place MRPLNs in a labeled whirl pack.



### Sampling Procedure for ID and Skin

1. Remove the official ID with a 1" X 1" piece of ear attached for each animal sampled and place in a labeled whirl-pak. If the animal is a trophy animal, submit the official ID and attach a new official ID to another piece of skin. If no official ID is present, the collector must affix one and record the information. Microchips must be in tissue.

#### SUBMIT:

**FRESH:** [chilled or frozen] place the **official ID with attached skin** (ear or hide) attached into a labeled whirl-pak and submit with other fresh tissue. **DO NOT PLACE IN FORMALIN**

### Sample Submission Procedures:

1. Label sample containers / bags with sample number, producer name, official ID number, species, date, etc. Refer to **appendix V** in the current **HCP Program Standards** for a specifics on required paperwork, and sample labeling.)
2. Complete VS 10-4 Form. List any notes or clinical signs in the *Additional Data* section.
3. Sample Shipping: visit [www.fedex.com/us/packaging/guides/Clinical\\_fxcom.pdf](http://www.fedex.com/us/packaging/guides/Clinical_fxcom.pdf) for packaging instructions.

**Fixed:** [formalin] Obex and MRPLN  
Ship samples in formalin jars

**Fresh:** Obex, MRPLN, and tag w/skin  
Keep samples chilled on ice packs  
and use overnight delivery

Packages containing biological specimens must be labeled as a **Category B (UN3373)** for shipping.

4. Ship to a VS approved CWD laboratory