**Note*: It is best to describe things in everyday language instead of trying to use technical jargon, since inappropriate use of technical terminology results in incorrect diagnostic interpretation!

All participants must be familiar with <u>USFWS Wildlife Health Office - Donning and Doffing PPE Protocol</u> before proceeding; keep biosafety and biosecurity in mind throughout necropsy procedure.

If you have any reason to suspect anthrax, DO NOT PROCEED without using an anthrax field testing kit and/or contacting the Wildlife Health Office.

- Collect 1 freeze (chunk at least 1 inch) + 1 fix (formalin jar) of every lesion, including crossing from "normal" into "abnormal" areas
- If more than 1 type of lesion in a tissue (such as one nodule filled with pus in addition to another area that is tough, thick and white), collect a set of tissues for each type of lesion
- Tissue in formalin jar = $1/16^{\text{th}} 1/8^{\text{th}}$ inch thick slice and handle tissue very gently with forceps
- Tissue to formalin ratio = 1:10; if you run out of jars, OK to overpack if you immediately pour off, separate and refill into separate jars with fresh formalin right after necropsy
- Photos of everything you collect
- 1. Collect blood using needle and syringe, or expose jugular vein and puncture to drain into blood tube
- 2. External exam both sides: eyes, nose, mouth, ears, anal, hooves, ectoparasites, dermatitis photo and sample as needed
- 3. Collect hair with follicles, bloodcard or small earnotch for genetics
- 4. Position carcass left side down
- 5. Flip right front leg over back/neck away from rest of body by cutting through hide and natural fascia layers
- 6. Flip right hind leg up and over rear of body by cutting deeply through muscle, well away from GI, to and through ball and socket hip joint
- 7. Check synovial fluid like thin, runny snot; check ball and socket for "crazing" cracks or worn spots
- Peel right hide up from just above midline to near spine; look for punctures and areas of bruising
 Continue hide cut up neck
 - -Cut through trachea and esophagus a few vertebrae below skull if planning to collect CWD samples, then cut and pull trachea/esophagus down to point of entry at chest
 - -Or cut hide up to the underside of jaw if no CWD sampling to examine oral cavity more carefully, Split jaw, cut around and pull tongue/trachea/esophagus down to point of entry at chest
- 9. Start at highest point on abdomen just behind rear ribs, carefully make a window in abdominal wall until the omentum covering GI is visible; OK to puncture rumen if need to purposely deflate for easier handling
- 10. Draw 3 lines to clear tissue away from ribcage, remove ribcage using rib cutters; cut diaphragm close to ribs -If ribcage is attached to underlying organs and/or difficult to lift, photograph and proceed slowly
- 11. Examine ribcage photograph
- 12. Examine everything in place: size, position, matched lesions to ribcage photo total, thoracic cavity and abdominal cavity
- 13. OK to photo and sample lungs before remove pluck if needed; then remove pluck to examine thoroughly -Cut pluck away from spine, then away from diaphragm, then away from sternum and remove
 - -Palpate lungs, scrape all surfaces clean photos
 - -Esophagus, trachea slit open, examine, photo and sample as needed
 - -Shred one side lungs and bread slice the other worms, photos and samples
 - -Heart: examine pericardial sac fat; carefully open and sample pericardial fluid without contamination -Examine coronary fat and pulmonary artery – photos & sample as needed
 - -Open heart right side, check glossy, free valves AV and semilunar; check pulmonary artery lining
 - -Three surfaces: outside (epicardium), meaty muscle (myocardium), inside (endocardium)
 - -Three structures: papillary muscle bulging muscles attached to strings
 - trebecular carnae grooves and divits in muscle

cordae tendonae – heart strings

-Heart left side, repeat exam, up into aorta

- -Check thickness = 3:1 of left ventricle freewall to right ventricle freewall
- 14. Kidney exam evaluate renal fat, capsule; butterfly and scrape kidney photos and samples
- 15. Remove liver: scrape, palpate and bread slice; fan and examine; density punch test photos and samples 16. GI: Examine omental fat
 - -4 stomachs: outside, inside and content RROA (rumen, reticulum, omasum, abomasum)
 - -Small intestine, large intestine, colon -- photo & sample (include ICC Jxn + mesenteric LNs for JD)
- 17. Roll out (or up if needed) rumen for spleen sample and photos
- 18. Remove and examine bladder sample and photos if needed
- 19. Examine repro and record reproductive status
- 20. If cervid: collect medial retropharyngeal lymph nodes and obex for CWD, referring to USFWS Wildlife Health Office protocols: <u>CWD Sample Collection Obex</u> and <u>CWD Sample Collection RLNs</u>

Standard samples (with photos) for all necropsies:

Blood: ONLY if carcass is freshly dead within 3-8 hours (depending on environmental conditions)
Genetics: hair with follicles, bloodcard or small earnotch (photos not necessary)
Lung (1 freeze, 1 formalin)
Pericardial fluid, if collected cleanly (freeze)
Kidney (1 freeze, 1 formalin)
Liver (2 freeze, 1 formalin)
Retropharyngeal LNs and obex (deer and elk only)

Diarrhea-specific or ulcers or extra confirmation for unknown cause of death, collect these additional samples: Colon (1 freeze, 1 formalin) Small intestine (1 freeze, 1 formalin) Ileocecalcolic junction (freeze only) Ileocecalcolic junction LN (½ freeze, ½ formalin) Colic LN, if found (½ freeze, ½ formalin) Mesenteric LN (1 part freeze, 1 formalin) Bladder (1 freeze, 1 formalin) Spleen (1 freeze, 1 formalin) Fecal – collect from colon in the pelvic canal (1 refrigerate, 1 freeze)

Necropsy photography

• For each item:

-First photo of entire organ (include trachea in pluck photos for proper orientation) -Second photo up close of lesion

• Take a second set of photos from a different angle to reveal subtle tissue texture

Difficult lighting:

Outside bright sun: take one shot with direct sunlight, take another in a shadow Indoor lighting: take one shot with flash, take another without flash

Don't use the zoom lens!

Keep lens retracted, and position camera 12 -18 inches from lesion for close-up; zooming results in blurry pictures with the tiniest movement

Don't use the macro (flower) setting!

Focus is more difficult to achieve with macro setting, such that many photos during a necropsy end up blurry using macro. Better for me to zoom in on my computer screen for added detail.

Have the necropsy person stabilize their arm, hand, or tissue as much as possible to keep photos sharp.

If camera has autofocus window, take the time to refocus and move the camera with tiny adjustments until the focus window is surrounding the area you are targeting. Variations in tissue depth often result in the wrong tissue being in focus without careful adjustments.

If doing more than one necropsy at a time, take a photo of ID tag or datasheet with ID number before necropsy Photos to ensure photos are organized with the correct animal.

Once photos are downloaded, you can use Microsoft Office photo editor to compress them all at once:

Upper left, select filmstrip view

Shift click to select all photos

File, export

Browse to a new location (so you don't overwrite your original high resolution pictures)

Resize to document large – this size works well for most photos, compressing to 180 – 350 kb

Sample handling

Store formalin jars at room temperature; ensure they don't freeze during shipping Centrifuge blood and draw or pour off serum; or let tube sit at room temperature for 6 hours and draw or pour

off serum; or contact Wildlife Health Office and ship immediately Minimize freeze-thaw cycles of tissues and serum

Refer to USFWS Sample Packaging and Shipping Protocol, or contact Wildlife Health Office for guidance.