

MODIFIED ENVIROVET  
FLAMINGO NECROPSY PROTOCOL  
FOR DIE-OFF EVENTS

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## FLAMINGO NECROPSY PROTOCOL

1. **On site at time of capture:** Collect blood sample from LIVE birds into 8 ml heparinized (green top) tube, 2 ml into EDTA tube and remainder in red top tubes (4 ml minimum). If handling the birds, wear protective clothing and wash up afterwards.
2. Euthanize sick and/or dying birds with barbiturate overdose via the occipital sinus.
3. Take pictures of the lesser flamingos using the Flamingo Research\_Carotenoids\_Digital Photography Protocol.
4. Collect feathers for carotenoid analyses.
5. Place in cooler filled with ice and transport to lab for necropsy. Make sure that the feathers are wetted to cool the bird.
6. Don gloves, protective clothing, and mask.
7. Wet down bird/feathers with alcohol or disinfectant, part feathers along ventral midline.
8. Open coelomic cavity with sterile blade and sterile forceps—**careful not to contaminate internal organs prior to bacteriology (spleen and liver) sampling!**
9. Using aseptic technique, collect a 1cm by 2cm sample of liver and place directly in whirl pack labeled “Assay: Bacteriology.”
10. Next (still using aseptic technique), excise spleen. Cut it in half while holding over open Whirl-Pak® so one half drops in. Place the other half into formalin (avoid touching formalin or container with instruments).
11. Incise skin over keel, reflect. Incise lateral extent of pectorals or reflect pectorals from midline laterally.
12. Using heavier scissors, cut both sides of thorax where keel meets ribs and remove keel, breaking corocoid as reflect toward head.
13. Use sterile scalpel blade to incise edge of one lung lateral/ventral edge, use back of scalpel handle to elevate lung, excise and again, cut in half over Whirl-Pak® labeled “Assay: Bacteriology,” put the other half into formalin.
14. Isolate and tie-off a 4 cm to 8 cm loop of jejunum, place in Whirl-Pak® labeled “Assay: Bacteriology.”
15. Put these 4 Whirl-Pak®s (labeled with the flamingo ID, tissue, date,) in dry ice cooler to freeze.
16. Observe organs grossly *in situ* and record any abnormalities.
17. If bird was found dead, or died during transport or insufficient blood (less than 2 ml) was collected, tie off great vessels of heart, cut distal to ties, remove heart, place in Whirl-Pak® labeled “Assay: botulinum toxin.”
18. Systematically examine all systems, note any abnormalities, collect all listed samples for histopathology (Check off list as collected and placed in formalin).
19. Collect several representatives of any parasites in alcohol-filled vials and label with bird ID#, date and location.
20. Lift the root of the mesentery and cut the mesenteries as close to the intestines as possible. Continue to lift and cut until liver is removed preserving as much of the mesenteric and portal vessels as possible.
21. Collect two pieces of liver from different lobes for histology (no thicker than 0.25 cm) and then separate the remaining liver into three Whirl-Pak®s as follows: at least 3 g for cyanotoxins, about half of the remaining for metals; about 0.1 g for carotenoids, and the rest of the liver with the mesenteries and vessels still attached for parasitology. Freeze all.
22. Collect adipose tissue, place on shiny side of foil, fold foil over so no tissue is exposed, place in Whirl-Pak® and freeze.
23. Collect kidneys—put one in a Whirl-Pak® to freeze and fix the other in formalin.
24. Remove brain—slice along mid-sagittal plane. Put one half in a Whirl-Pak® to freeze and fix the other half in formalin.
25. Collect one eye ball—fix in formalin and the other eye for carotenoids-freeze.
26. Collect skin, muscle, sciatic nerve, tibiatarus—fix in formalin.
27. Make sure your name and all information are recorded on gross pathology report form. Have the recorder sign the form.
28. Verify that all samples have been collected. Have the necropsy coordinator sign off on sample collection and gross pathology report before leaving lab.

## FLAMINGO TISSUE COLLECTION CHECKLIST

### FRESH FROZEN TISSUE COLLECTION

**CAROTENOIDS:** using protocols, place in separate labeled (flamingo ID, tissue, date) Whirl-Pak®s and freeze on dry ice immediately.

- Plasma.
- Liver.
- Tarsal Skin.
- Eye.
- Body Fat.
- Intestinal Contents in separate vials indicating the regions collected from.

**BACTERIOLOGY:** use aseptic technique, place in separate labeled (flamingo ID, tissue, date, ) Whirl-Pak®s and freeze on dry ice immediately.

- Liver.
- Spleen.
- Intestine.
- Lung.

**TOXICOLOGY:** collect as per protocol into Whirl-Pak®, freeze.

- Liver–cyanotoxins, 5 g.  
Liver–collect 2 pieces for  histopath into formalin, half of remaining in Whirl-Pak®  for metals.
- Kidney–one frozen whole.
- Adipose –on foil, then Whirl-Pak®.
- Brain–cut sharply sagittally and **freeze half in whirl pak** , other **half into formalin**
- Heart–if bird dies before sufficient blood obtained, tie off great vessels, freeze entire heart to retain heart blood. *For Euthanized birds:* entire heart is fixed after opened, DO not collect for botulism or will invalidate assay.

**PARASITOLOGY (Alcohol fixed and frozen):**

- 4 separate 3 to 5 cm samples of small intestine into 70% isopropyl alcohol in double-bagged Whirl-Pak®s.
- Liver + mesenteric and portal veins in Whirl-Pak® and frozen.

### FIXED TISSUE COLLECTION FOR HISTOPATHOLOGY

Sections of tissues should be no more than 0.25 cm in width and should be placed in 10% neutral buffered formalin at a ratio of one part tissue to ten parts formalin.

**Sample normal appearing as well as any lesions or abnormalities within these or other organs:**

- Thymus.
- Thyroids and parathyroids.
- Trachea including syrinx.
- Lungs–one entire, and the retained half of the other.
- Air sacs.
- Heart–collect entire heart, open ventricles prior for proper fixation (unless bird is found dead–see toxicology).
- Tongue–cross section.
- Esophagus–3 cm in length, opened longitudinally.
- Crop–3 cm in length, opened longitudinally.
- Proventriculus–3 cm long sections.
- Ventriculus–3 cm long sections.
- Small intestine–multiple sections, each 3 cm in length. Inject formalin into lumen or flush through with formalin.
- Ceca and large intestine–multiple sections, each 3 cm in length.
- Liver–(should have been collected at same time as toxicology was done).
- Pancreas.

- Spleen–(the remaining spleen after some is collected for bacteriology).
- Adrenals.
- Testis or Ovary.
- Oviduct–with longitudinal cut into lumen.
- Kidney–fix one kidney only. Freeze the other (see toxicology).
- Brain–half in formalin and half frozen (slice mid-sagittally).
- Skin with feather.
- Skeletal muscle–longitudinal section of thigh and pectoral muscle.
- Bone/bone marrow–cut off small piece of proximal tibiatarus and fix it whole, don't attempt to remove marrow.
- Peripheral (Sciatic) nerve.

**FLAMINGO NECROPSY DATA COLLECTION**

PRINT LEGIBLY BELOW

Bird Identification No:		Ring No:	
Date of collection:		Date of necropsy:	
Time of collection:		Time at start of necropsy:	
Date and time of death:		Time at end of necropsy:	

Gross Necropsy Performed By: \_\_\_\_\_  
 Assistants: \_\_\_\_\_  
 Recorder: \_\_\_\_\_  
 Digital Photography/Videography Recorder: \_\_\_\_\_

Age (Adult/Immature/Juvenile):	Sex :
Mass (g):	Flattened wing length (mm):
Culmen length (mm):	Skull (mm):
Total tarsus length (mm):	Head-and-bill length (mm):

**GROSS PATHOLOGY**

- Describe any lesions or abnormalities seen grossly  
 Remember: **DDD TAP** =  
**D**egree -- slightly, minimal, moderately, mild, marked, severe  
**D**istribution -- focal, multifocal, diffuse, locally extensive, cranial, caudal, circumferential, ¼, ½, 90%, etc....  
**D**uration -- acute, subacute, chronic  
**T**issue  
**A**djective -- describe surface, consistency, shape, color, size, smell  
**P**rocess -- inflammation, fibrosis, granuloma, abscess....
- Obtain samples for bacteriology before contaminating carcass with intestinal contents, etc.
- Cut samples of tissue for histopathology that are no more than 0.25 cm in thickness, take extra samples of any lesions.

**General Condition: (Nutritional status/physical condition)**

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**Integumentary System: (Skin/feathers, note lice or mites and collect)**

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**Musculoskeletal System: (Skeletal muscle, bone, joints)**

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**Coelomic Cavity: (Fat stores, abnormal fluid, exudates, fibrin, etc.)**

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**Hemolymphatic System: (Spleen, thymus, bursa of Fabricius)**

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**Respiratory System: (Nares, sinuses, choanae, syrinx, trachea, lungs, air sacs)**

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**Cardiovascular System: (Heart, pericardium, great vessels)**

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**Digestive System: (Beak, oral cavity, tongue, esophagus, proventriculus, ventriculus, intestines, cloaca)**

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**Glandular Organs: (Liver, pancreas)**

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**Urinary System: (Kidneys, ureters)**

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**Reproductive System: (Testis/ovary, oviduct)**

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**Endocrine System: (Adrenals, thyroid, parathyroids, pituitary)**

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**Nervous and Sensory Systems: (Brain, spinal cord, peripheral nerves, eyes, ears)**

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**Gross Diagnoses: (List each lesion separately. Include organ, type of lesion, severity, etc.)**

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**Most Significant Finding(s)/Lesion(s): (Based on gross examination)**

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**Photography/Videography Performed? (List organs/lesions photographed/videographed; who has the film/tapes)**

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**Miscellaneous comments/remarks**

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Verified completed: \_\_\_\_\_ (date and signature of recorder)

Verified completed: \_\_\_\_\_ (date and signature of necropsy coordinator)