MORRIS ANIMAL FOUNDATION

GOLDEN RETRIEVER LIFETIME STUDY

Golden Retriever Lifetime Study

Necropsy Kit: Collection & Shipping Instructions

Necropsy Goals

- Determine the cause of death.
- Assess the dog's health status at the time of death via blood, urine and fecal testing.
- Stage malignancy by sampling visibly or commonly affected organs.
- Harvest samples for a tissue bank, providing a valuable resource for future research.

1 Clinical Pathology Sample Collection

Premortem or Postmortem

Blood Collection

Collect 13 ml of blood into the lavender-top EDTA tubes provided by the study or from your clinic.

- Label a 3 ml tube with a provided dog name, ID# (094-xxxxxx), and date (MM/DD/YYYY) sticker and fill in the date
- Label a 10 ml tube with the provided barcoded whole blood sticker (EDTA 094xxxxxxC1099).

Collect 40 ml of blood into red-top tubes (NOT serum separator tubes) provided by the study or from your clinic.

Serum Separation and Collection

Allow serum tubes (red tops) to clot for at least 45 minutes at room temperature, and then centrifuge per your clinic's normal protocol (we recommend 1100–1300 RCF for 10 minutes).

After centrifugation, remove the Vacutainer cap from each serum tube (red top) and using a pipette (DO NOT POUR) transfer the serum into 2 separate red-top tubes.

- Label a 3 ml tube with a provided dog name, ID# (094-xxxxxx), and date (MM/DD/YYYY) sticker and fill in the date.
- Label a 10 ml tube with the provided barcoded serum sticker (SERUM 094XXXXXXC2099).

If additional serum is needed to achieve the requested volume, try re-centrifuging the blood remaining in the tubes. Once the appropriate amount of serum has been collected, discard the clotted red-top tubes.

Please read all instructions before proceeding.

Clinical Pathology Sample Collection (Cont.) 1

Premortem or Postmortem

Urine Collection

Collect at least 10 ml of urine via cystocentesis or free catch and place into the green screw-top tubes provided by the study or two red-top tubes from your clinic.

- Label one tube with a dog name, ID# (094-xxxxxx), and date (MM/DD/YYYY) sticker and fill in the date.
- Label the remaining tube with the barcoded urine sticker (URINE 094XXXXXXC3099).

Fecal Collection

Collect two marble-size samples of feces and transfer each into a separate plastic fecal tube provided by the study or from your clinic.

- Label the white screw-top tube with a dog name, ID# (094-xxxxxx), and date (MM/DD/YYYY) sticker and fill in the date.
- Label the brown screw-top or remaining tube with the barcoded fecal sticker (FECES 094XXXXXXC6099).

1 Clinical Pathology Sample Collection (Cont.)

Premortem or Postmortem

Toenail Trimmings Collection

Collect 5–10 toenail trimmings from the dog and place into the blue-top tube provided by the study or a red-top tube from your clinic.

- Label with the barcoded nails sticker (NAILS 094XXXXXXC5099).

Hair Collection

Cut a lock of hair ≈1/4” in diameter (about the diameter of a wooden pencil) from an inconspicuous location, and a minimum of 2” long as close to the root as possible and place into the orange tube provided by the study or a red-top tube from your clinic.

- Label with the barcoded hair sticker (HAIR 094XXXXXXC4099).

Fluids

Collect pleural effusion, pericardial effusion, ascites, and/or synovial fluid if available and relevant to this dog’s death/disease into the appropriate lavender- and/or red-top tubes.

- Label each tube with a dog name, ID# (094-xxxxxx), and date (MM/DD/YYYY) sticker, and fill in the date and note the fluid type.

Histopathology Sample Collection 2

These instructions are for a full necropsy. For a cosmetic necropsy or conditions under which ideal sample collection is not possible, use your best judgment, but please try to collect as many diseased and core tissues as possible.

For suspected malignant tumors or lesions of interest

- Place a representative tissue sample into a formalin jar, label with the date, "Diseased" tissue type, and the appropriate tissue code.
  - In the case of multiple metastases, select 2–5 representative lesions to harvest.
- Place a 5 mm cube of diseased tissue into a tube of RNAlater, label with the date and appropriate tissue code, and circle "DISEASED."
- Place a 1 cm cube of normal tissue at least 2 cm away from the tumor/lesion into a separate formalin jar, label with the date, "Healthy" tissue type, and the appropriate tissue code.
- Place a 5 mm cube of normal tissue at least 2 cm away from the tumor/lesion into a separate tube of RNAlater, label with the date and appropriate tissue code and circle "HEALTHY."

See Appendix 2 for tissue codes. See histopathology labeling examples on page 10.

In addition to any tumors or lesions, or if no lesions are found, collect samples as described below. This list also is provided in the Necropsy Checklist for patient-side use.

Please collect the seven core tissues listed below and as many of the secondary tissues as possible. Samples may be combined in as few formalin jars as possible, maintaining the ratio of one part sample to 10 parts formalin. Any samples too small to identify on gross examination after fixation, or that have particular importance, should be identified by putting them in a cassette labeled with pencil or by placing them in a separate formalin jar.

2 Histopathology Sample Collection (Cont.)

Core Tissues

- **Liver:** 1 cm cube of normal liver.
- **Right Kidney and Adrenal Gland:** right adrenal gland and 1 cm wedge from the right kidney to include cortex, medulla and pelvis and any lesions.
- **Left Kidney and Adrenal Gland:** left adrenal gland and 1 cm wedge from the left kidney to include cortex, medulla and pelvis and any lesions.
- **Spleen:** 1 cm cube of normal spleen.
- **Lymph Node(s):** submandibular, axillary, tracheobronchial, mesenteric, and/or prescapular.
- **Heart:** Provide a gross description of the heart in your report. Rinse as much blood as possible from the heart samples with water. Submit a full thickness 1 cm wide sample from the right ventricular free wall (ideally through the papillary muscle), the left ventricular free wall (ideally through the papillary muscle) and the interventricular septum.
- **Thyroid Gland and Parathyroid Glands:** entire thyroid gland and the parathyroid glands.

Prior to shipment, record the tissues submitted on the Tissue Inventory Checklist (appendix 3).

Histopathology Sample Collection (Cont.) 2

Secondary Tissues

- **Haired Skin Sample:** 1 cm x 1 cm from ventral abdomen, full thickness, with subcutaneous fat.
- **Eyes:** collect both entire eye globes, inject 0.25 mL formalin into the vitreous humor of each eye, and immerse both globes into one formalin jar. Please indicate the left globe by placing a suture through the optic nerve.
- **Lung:** 1 cm cube of normal lung.
- **Esophagus:** 2 cm x 2 cm portion of normal esophagus.
- **Stomach:** 2 cm x 2 cm portion of normal stomach.
- **Duodenum and Pancreas Together:** 4 cm length of normal tissue. Rinse intestinal contents with water prior to immersion into a formalin jar.
- **Jejunum:** 4 cm length of normal tissue. Rinse intestinal contents with water prior to immersion into a formalin jar.
- **Ileocecocolic Junction:** 4 cm in each of the three directions. Rinse intestinal contents with water prior to immersion into a formalin jar.
- **Urinary Bladder:** 2 cm x 2 cm piece.
- **Skeletal Muscle:** 2 cm x 2 cm crosssection of semimembranosus/semitendinosus muscle from a hind leg to contain sciatic nerve.
- **Bone:** 2 cm sample of rib or other bone, record the specific bone submitted.
- **Bone Marrow:** core biopsy in formalin and/or smears from an aspirate.
- **Synovial Fluid:** If joint disease is present, collect into a purple- and/or red-top tube. Label with 'Synovial Fluid,' dog name and ID# (094-XXXXXX), and date (MM/DD/YYYY) for analysis by ANTECH.
- **Nervous System:** brain, spinal cord or both.

2 Histopathology Sample Collection (Cont.)

Histopathology Labeling Examples

Formalin jars containing healthy tissue should be labeled with a completed dog name, ID#, and date sticker.

Acct: 22725 **Chart:** 094-012345

Smith, Fido

DATE: 12/1/2015

Formalin jars containing a tumor, lesion of interest, or normal tissue adjacent to such lesions should have a completed dog name, ID#, and date sticker with a completed "Diseased [tissue type]" or "Healthy [tissue type]" sticker. Tissue codes are in Appendix 2.

Acct: 22725 **Chart:** 094-012345

Smith, Fido

DATE: 12/1/2015

TISSUE TYPE: Diseased liver

TISSUE CODE: 85

RNAlater jars should each contain a single tissue sample and be labeled with the dog name, ID#, date, and tissue code and marked "Diseased" or "Healthy." Tissue codes are in Appendix 2.

094-012345

Smith, Fido

DATE: 12/1/2015

TISSUE TYPE CODE: 85

DISEASED **HEALTHY**

094012345N7099

3 Sample Packaging & Shipping

Placing a Service Call for FedEx Shipment(s)
Place a service call to FedEx directly at 800.463.3339 Monday through Thursday. Let them know you have packages for pickup and tell them that the shipping cost is being billed to ANTECH Diagnostics and Fisher BioServices with a “Billable Stamp.” ANTECH and Fisher BioServices also will be covering the costs for the biohazard shipping fees. If your clinic is inadvertently charged any fees, please contact the study team.

Please notify us by calling 855.447.3647 when you perform a necropsy and are ready to ship your samples.

Sample Packaging & Shipping (Cont.) 3

ANTECH Diagnostics Shipment

Part I: Clinical Pathology Samples

- Verify all tubes are properly filled, labeled and dated. Place the fecal (white top), 3 ml serum (red top), 3 ml EDTA (purple top) and one of the urine (green top) tubes in the ANTECH zip-closure bag. Also, if you collected any other fluids for analysis such as ascites or pericardial fluid, add these tubes to the bag.

Tubes for ANTECH do not have barcodes.

- In the top header of the ANTECH Diagnostics laboratory requisition form, provide the following information: date, doctor, clinic name, address, phone number, fax number and email.
- Verify the additional information (client name, pet name, species, breed and age, Chart #/Dog's Study ID) on the form is correct.
- All laboratory tests are ordered with code 87007; this is a no-charge study code. You must use this requisition form and verify that the study code is preprinted on the form. Failure to do so may result in charges to your clinic. Please call the Study Veterinarian at 303.708.3405 to discuss fluid analysis or other testing as appropriate for each case.
- Retain the bottom copy of the form for your records.
- Place the completed front copy of the ANTECH Diagnostics requisition form into the ANTECH zip-closure bag containing the necessary samples and seal the bag.
- Place the zip-closure bag inside the ANTECH Diagnostics shipping box if available.

*Tubes for Antech DO NOT have barcodes.

3 Sample Packaging & Shipping (Cont.)

Part II: Formalin containers

- Make sure all formalin jars are tightly sealed.
- Tape the lids to the jars.
- Make sure the labels on all formalin jars have the date, the dog's name, the Study ID# and enclosed tissue type(s) if appropriate. Complete Appendix 3 - Tissue Inventory Checklist to document all tissues submitted and make a copy for the Study Veterinarian.
- Place all formalin containers into a zip-closure bag or bags. Include one absorbing sheet per two jars of formalin. Seal the bag(s).
- Complete the enclosed ANTECH Diagnostics histopathology requisition form and make a copy for your records.
- Place the completed ANTECH Diagnostics histopathology requisition form, Appendix 3 – Tissue Inventory Checklist, a copy of your gross necropsy report, and any other documents or photos into a zip-closure bag.
- Place the sealed bag(s) of formalin jars and the sealed documents bag into a large zip-closure bag and seal.

You MUST include a copy of your gross necropsy findings with the tissue samples.

Sample Packaging & Shipping (Cont.) 3

Part III: Packaging (see Fig 1)

- Place the sealed double bag(s) of formalin jars and documents, and the clinical pathology samples, into the FedEx ClinPak, Necropsy Kit box, or any other appropriate size box.
- Close and seal the ClinPak or box. If using a box, ensure that a UN3373 Biological Substances sticker is placed on the outside.
- Complete your return address on the right side of the provided FedEx Expanded Billable Stamp labeled for ANTECH Diagnostics, affix it to the shipping container.
- Retain the left portion of the label for your records and tracking.
- Call FedEx at 800.463.3339 to arrange for a pick up.

Figure 1

3 Sample Packaging & Shipping (Cont.)

Biorepository Shipment

Part I: Clinical Pathology Samples

- Fill out the Shipment Inventory Form (Fisher BioServices) – Golden Retriever Lifetime Study and include the patient information, collection date, collection times, sample shipment inventory and any optional comments.
- Place the following three samples into the Styrofoam shipping container if available:
  - Urine
  - EDTA blood (purple-top tube)
  - Serum (red-top tube)
- Close the Styrofoam shipping container and place it inside the zip-closure Lab-Loc Biohazard bag.
  (If the Styrofoam shipping container is not available, place all tubes directly into the Lab-Loc Biohazard bag.)
- Also place the following five items inside the zip-closure Lab-Loc Biohazard bag:
  - Fecal sample
  - Nails
  - Hair
  - Absorbent paper towel
  - Shipping inventory form
    (All tubes in the biorepository shipment should have a barcode.)

Part II: RNAlater tubes

- Place RNAlater tubes into the small padded envelope(s). Include one absorbing sheet per envelope and seal the envelope(s).
- Place the sealed padded envelope(s) and completed Submission Form(s) for Tissues in RNAlater into a Fisher Lab-loc biohazard zip-closure bag and seal the bag.

All tubes in the biorepository shipment should have a barcode.

Sample Packaging & Shipping (Cont.) 3

Part III: Packaging (see Fig 2)

- Place the plastic bag containing the clinical pathology samples and the Fisher Lab-Loc biohazard bag(s) containing the RNAlater samples into one of the provided FedEx Clinical Paks and seal the envelope by removing the clear seal to expose the adhesive strip.
- On the provided FedEx air shipment bill labeled “FedEx Express US Airbill,” complete the required “From” section. Retain the top copy for your records. Place the shipment bill inside the provided pouch and seal the pouch. Peel the backing from the pouch and affix it to the indicated location on the FedEx Clinical Pak.
  (see Fig 2)
- Call FedEx at 800.463.3339 to arrange for a pickup.

Figure 2

3 Sample Packaging & Shipping (Cont.)

Part IV: Unused RNAlater tubes (see Fig 3)

- Place any unused RNAlater tubes (ideally in the original shipment carton) into a zip-lock bag with one absorbing sheet and seal the bag.
- Place this sealed bag into the FedEx padded mailer addressed to UBC.
- Call FedEx at 800.463.3339 to arrange for a pickup.

Figure 3

Sample Reporting 4

Test results from ANTECH Diagnostics will be available to the patient's registered study veterinarian at morrisanimalfoundation.org within 3-5 business days. From the homepage, click on the GRLS icon in the upper left corner, and scroll down to log in. Alternatively, if you have bookmarked the study log in page, your bookmark will still work. The results are posted under the "Lab Results" tab. The results will come as two separate reports - one report for the histopathology and a second for the clinical pathology results.

Once the test results are received, the patient's registered study veterinarian will log on at morrisanimalfoundation.org to complete the Death and Necropsy Report for this patient. (You can access this report by selecting the appropriate patient from your "My Patients" page.) Use the completed Necropsy Checklist for Veterinarians to enter the necropsy findings. If you have any questions, please do not hesitate to contact the study team at grdogs@caninelifetimehealth.org or 855.4GR.DOGS (855.447.3647).

5 Appendix 1: Presumed Cause of Death

Cancer/Neoplasia		Behavioral	Cardiovascular/Respiratory
Adrenal Tumor	Melanoma	Aggression	Arrhythmia
Basal Cell Tumor	Multiple Myeloma	Anxiety	Cardiomyopathy
Bile Duct Tumor	Nasal Tumor	Cognitive Dysfunction (Senility)	Congestive Heart Failure
Bladder Tumor	Osteosarcoma		Heartworm Infection
Brain/Spinal Cord Tumor	Pancreatic Tumor		Pneumonia
Eye Tumor	Perianal Adenocarcinoma		Pulmonary Hypertension
Heart Tumor	Prostate Tumor		Pulmonic Stenosis
Hemangiosarcoma	Soft Tissue Sarcoma		Subaortic Stenosis
Histiocytic Sarcoma	Squamous Cell Carcinoma		Valvular Disease
Kidney Tumor	Stomach/Intestinal Tumor		
Leukemia	Testicular Tumor		
Liver Tumor	Thyroid Tumor		
Lung Tumor			
Lymphoma			
Mammary Tumor			
Mast Cell Tumor			

Dermatologic	Ear-Nose-Throat	Endocrine
Atopy	Epistaxis	Addison's Disease (Hypoadrenocorticism)
Dermatitis	Hearing Problem	Cushing's Disease (Hyperadrenocorticism)
Sarcoptic Mange	Otitis Externa/Media/Interna	Diabetes Insipidus
	Upper Respiratory Infection	Diabetes Mellitus
		Hypercalcemia
		Hyperparathyroidism
		Hypoparathyroidism
		Hypothyroidism
		Pancreatic Insufficiency

Appendix 1: Presumed Cause of Death (Cont.) 5

Eye	Gastrointestinal	Hematologic
Cataract(s)	Bloat with Torsion (GDV)	Hemophilia
Corneal Ulcer	Bloat without Torsion	Immune-mediated Hemolytic Anemia
Glaucoma	Chronic Colitis	Immune-mediated Thrombocytopenia
Keratoconjunctivitis Sicca (KCS)	Food Allergy/Sensitivity	Pancytopenia
Pigmentary Uveitis	Gastritis/Gastroenteritis	Von Willebrand Disease
Progressive Retinal Atrophy/Degeneration	Gastrointestinal Foreign Body	
Trauma/Injury	Inflammatory Bowel Disease	
Uveitis (Other Than Pigmentary)	Megaesophagus	
	Pancreatitis	

Infectious	Musculoskeletal	Nervous
Babesia	Bone Fracture(s)	Cervical Spondylomyelopathy
Ehrlichia	Cruciate Ligament Rupture	Degenerative Myelopathy
Fungal infection (specify)	Elbow Dysplasia	Epilepsy
Influenza	Growth Deformity	Laryngeal Paralysis
Leishmania	Hip Dysplasia	Limb Paralysis
Leptospirosis	Immune-mediated Polyarthropathy	Lumbosacral Stenosis
Lyme disease	Intervetebral Disc Disease	Meningitis
Rocky Mountain Spotted Fever	Osteoarthritis	Myasthenia Gravis
	Osteochondrosis Dessecans (OCD)	Steroid-responsive Meningitis-arteritis
	Panosteitis	
	Patellar Luxation	
	Rheumatoid Arthritis	
	Spondylosis	
	Trauma/Injury	

Reproductive	Toxicosis	Trauma
Dystocia	Anticoagulant Rodenticide	Bite Wounds
Mastitis	Chocolate	Hit By Car
Prostate Abscess	Ethylene Glycol (Antifreeze)	
Prostatitis		
Pyometra		

Urinary	Other	Unknown
Acute Renal Failure	Specify	
Chronic Renal Failure		
Cystitis		
Bladder Stones		
Crystalluria		
Ectopic Ureter		
Glomerulonephritis		
Incontinence		
Kidney Infection/Pyelonephritis		
Kidney Stones		

6 Appendix 2: Tissue Coding

Code	Description	Additional Indications
70	Other tissue, source	Tissue: Diseased | Healthy
71	Adrenal Gland	Left | Right | Both
72	Bone	None
73	Bone Marrow	None
74	Brain	None
75	Colon	None
76	Duodenum	None
77	Esophagus	None
78	Eye	Left | Right | Both
79	Gonads	Left | Right | Both
80	Heart	None
81	Ileocecocolic Junction	None
82	Ileum	None
83	Jejunum	None
84	Kidney	Left | Right | Both
85	Liver	None
86	Lung	Specify Lobe:
87	Lymph Node	Left | Right : Axillary | Mesenteric | Prescapular | Mandibular | Popliteal | Other:
88	Oral Cavity	None
89	Pancreas	None
90	Parathyroid Gland	None
91	Prostate	None
92	Rectum	None
93	Skeletal Muscle	None
94	Skin	None
95	Spinal Cord	None
96	Spleen	None
97	Stomach	None
98	Thyroid	None
99	Urinary Bladder	None

Necropsy Kit
Appendix 3: Tissue Inventory Checklist

Please check all tissues submitted in formalin. Submit one copy of this sheet with the formalin containers and send another copy to Morris Animal Foundation Study Veterinarian.

- ☐ **Liver**
- ☐ **Right Kidney/Adrenal**
- ☐ **Left Kidney/Adrenal**
- ☐ **Spleen**
- ☐ **Lymph Node(s)**
  Specify: _____
- ☐ **Heart**
- ☐ **Thyroid and Parathyroid Glands**
- ☐ **Haired Skin**
- ☐ **Eyes**
- ☐ **Lung**
- ☐ **Esophagus**
- ☐ **Stomach**
- ☐ **Duodenum and Pancreas**
- ☐ **Jejunum**
- ☐ **Ileocecocolic Junction**
- ☐ **Urinary Bladder**
- ☐ **Skeletal Muscle**
- ☐ **Bone**
  Specify: _____
- ☐ **Bone Marrow**
- ☐ **Synovial Fluid**
- ☐ **Nervous System**
  Specify: _____
  _____
- ☐ **Other Lesions or Tissues of Interest:**
  Specify: _____
  _____
  _____
  _____

5 About Morris Animal Foundation

Morris Animal Foundation is a nonprofit organization that invests in science to advance animal health. The foundation is a global leader in funding scientific studies for companion animals, horses and wildlife. Since its founding in 1948, Morris Animal Foundation has invested in studies that have led to significant breakthroughs in diagnostics, treatments, preventions and cures to benefit animals worldwide. Learn more at morrisanimalfoundation.org.

Thank You to Our Partners

FOUNDING PARTNER

The Mark & Bette Morris Family Foundation

PLATINUM PARTNERS

GOLD SPONSORS

Golden Retriever Foundation
Hadley and Marion Stuart Foundation
Zoetis

GOLDEN CHAMPIONS

Mars Veterinary

If you have any questions, please contact us at 855.4GR.DOGS (855.447.3647)

MORRIS ANIMAL FOUNDATION
GOLDEN RETRIEVER LIFETIME STUDY

Golden Retriever Lifetime Study

Necropsy Checklist for Veterinarians

Complete this checklist as you perform the necropsy.

This information will be used to enter the necropsy findings into the online Death and Necropsy Report. If you are not this dog's registered study veterinarian, please forward a copy of the completed form to the study veterinarian for online entry.

For detailed instructions on how to perform a necropsy, see the link "Necropsy Techniques" under the heading Tumor Sampling and Necropsy Resources on your study homepage.

Please contact the Golden Retriever Lifetime Study Veterinarian at 303.708.3405 for necropsy consultation and questions.

Dog Name: _____

Study ID: 094-_____ **Date of Death:** ___/___/___

Current Sex Status:

- ☐ **Intact Female** ☐ **Intact Male**
- ☐ **Neutered Female:** (Circle) OHE/OVH OE/OVE
- ☐ **Neutered Male:** (Circle) Testicles Surgically Removed Chemically Altered Vasectomized
- ☐ **Unknown**

- ☐ **Natural Death**
- ☐ **Euthanasia**

Drug Name: _____

Concentration: _____ **mg/ml**

Amount: _____ **ml**

Reason for Euthanasia: _____

Presumed Cause of Death or Euthanasia (See appendix 1): _____

_____

Date of Necropsy: ___/___/___

Approximate number of whole hours between time of death and time of necropsy: ____

Necropsy performed by: ☐ **Registered study veterinarian** ☐ **Other veterinarian**

Veterinarian Name: _____

Clinic Name: _____

Email: _____

Address: _____

Telephone number: _____ **Fax number:** _____

MORRIS ANIMAL FOUNDATION
GOLDEN RETRIEVER LIFETIME STUDY

Golden Retriever Lifetime Study

Necropsy Checklist for Veterinarians

Clinical Pathology Samples

- ☐ Before death, collect 13 ml of blood into lavender-top EDTA tubes plus 40 ml into red-top serum tubes.
- ☐ Collect 10 ml of urine via cystocentesis, and divide into the two green screw-top tubes provided by the study or 2 red top tubes available in your clinic.
- ☐ Collect 2 gm of feces and divide into two separate plastic tubes.
- ☐ Collect a lock of hair ¼" in diameter into the orange-top tube provided by the study or a red-top tube from your clinic
- ☐ Collect 5-10 nail trimmings into the blue-top tube provided by the study or a red-top tube from your clinic.
- ☐ Collect pleural effusion, pericardial effusion, ascites, and/or synovial fluid into appropriate lavender- and/or red-top tubes if available and relevant to this dog's death/disease.

Gross Examination

- ☐ **Scan and record the microchip number:** _____
- ☐ **Record the most recent body weight:** _____ ☐ **lb** ☐ **kg**
- ☐ **Record Purina Body Condition Score:**
  (Circle) **1 2 3 4 5 6 7 8 9**
- ☐ **Record general notes on a separate page.***

*** For the online questionnaire you will be asked to note NORMAL, ABNORMAL, or NOT EVALUATED for each organ/body cavity.**

Histopathology Samples

These instructions are for a full necropsy. For a cosmetic necropsy or conditions under which ideal sample collection is not possible, use your best judgment, but please try to collect as many of the core tissues as possible.

For suspected malignant tumors or lesions of interest

- Place a representative tissue sample into a formalin jar, label with the date, "Diseased" tissue type, and the appropriate tissue code.
  - In the case of multiple metastases, select 2-5 representative lesions to harvest.
- Place a 5 mm cube of diseased tissue into a tube of RNAlater, label with the date and appropriate tissue code, and circle "DISEASED."

Necropsy Checklist for Veterinarians

- Place a 1 cm cube of normal tissue at least 2 cm away from the tumor/lesion into a separate formalin jar, label with the date, "Healthy" tissue type, and the appropriate tissue code.
- Place a 5 mm cube of normal tissue at least 2 cm away from the tumor/lesion into a separate tube of RNAlater, label with the date and appropriate tissue code see and circle "HEALTHY."

See Appendix 2 for tissue codes. See histopathology labeling examples on page 10.

In addition to any tumors or lesions, or if no lesions are found, collect samples as described below.

Please collect the seven core tissues listed below and as many of the secondary tissues as possible. Samples may be combined in as few formalin jars as possible, maintaining the ratio of one part sample to 10 parts formalin. Any samples too small to identify on gross examination after fixation, or that have particular importance, should be identified by putting them in a cassette labeled with pencil or by placing them in a separate formalin jar.

Core tissues

- **Liver:** 1 cm cube of normal liver.
- **Right Kidney and Adrenal Gland:** Right adrenal gland and 1 cm wedge from the right kidney to include cortex, medulla and pelvis and any lesions.
- **Left Kidney and Adrenal Gland:** Left adrenal gland and 1 cm wedge from the left kidney to include cortex, medulla and pelvis and any lesions.
- **Spleen:** 1 cm cube of normal spleen.
- **Lymph node(s):** Submandibular, axillary, tracheobronchial, mesenteric and/or prescapular.
- **Heart:** Provide a gross description of the heart in your report. Rinse as much blood as possible from the heart samples with water. Submit a full thickness 1 cm-wide sample from the right ventricular free wall (ideally through the papillary muscle), the left ventricular free wall (ideally through the papillary muscle), and the interventricular septum.
- **Thyroid gland and parathyroid glands:** Entire thyroid gland and the parathyroid glands.

Necropsy Checklist for Veterinarians

Secondary Tissues

- **Haired skin sample:** 1 cm x 1 cm from ventral abdomen, full thickness, with subcutaneous fat.
- **Eye:** Collect both entire eye globes, inject 0.25 mL formalin into the vitreous humor of each eye, and immerse both globes into one formalin jar. Identify the left globe by placing suture through the optic nerve.
- **Lung:** 1 cm cube of normal lung.
- **Esophagus:** 2 cm x 2 cm portion of normal esophagus.
- **Stomach:** 2 cm x 2 cm portion of normal stomach.
- **Duodenum and Pancreas Together:** 4 cm length of normal tissue. Rinse intestinal contents with water prior to immersion into a formalin jar.
- **Jejunum:** 4 cm length of normal tissue. Rinse intestinal contents with water prior to immersion into a formalin jar.
- **Ileocecocolic Junction:** 4 cm in each of the three directions. Rinse intestinal contents with water prior to immersion into a formalin jar.
- **Urinary Bladder:** 2 cm x 2 cm piece.
- **Skeletal Muscle:** 2 cm x 2 cm cross section of semimembranosus/semitendinosus muscle from a hind leg to contain sciatic nerve.
- **Bone:** 2 cm sample of rib or other bone, record the specific bone submitted.
- **Bone Marrow:** Core biopsy in formalin and/or smears from an aspirate.
- **Synovial Fluid:** If joint disease is present, collect into the appropriate lavender- and/or red-top tube. Label with 'Synovial Fluid,' dog name and ID# (094-XXXXXX), and date (MM/DD/YYYY) for analysis by ANTECH.
- **Nervous System:** Brain, spinal cord or both.

Prior to shipment, record the tissues submitted on the Tissue Inventory Checklist (Appendix 3). You MUST include a copy of your gross exam findings with the tissues submitted.

Please gather any photographs or radiographs you took during the necropsy, as well as any pertinent clinical pathology or diagnostic results, and contact the Study Veterinarian at 303.708.3405 for submission instructions. Thank you again for supporting the Golden Retriever Lifetime Study.