

GOLDEN RETRIEVER  
LIFETIME STUDY

Golden Retriever Lifetime Study

# Biopsy Kit: Collection & Shipping Instructions

# Tips for Participating Veterinarians

Thank you for your participation in this groundbreaking study. We created the following list of tips based on feedback we've received from our Study veterinarians over the last few years. We understand the extra time and effort it takes to participate in the Study and we want to streamline the process as much as possible – you're important to us.

- If a biopsy results in a diagnosis of cancer, please complete a Malignancy Related Questionnaire for your patient as soon as possible. Navigate to [grls.morrisanimalfoundation.org](http://grls.morrisanimalfoundation.org) and use your Study log in username and password. Then click the "Start a new Malignancy Related Questionnaire" button. Timely questionnaire completion helps our Study team stay up-to-date on Study operations.
- We've found that writing your login username and password in each Study patient's chart can make the login process quicker.

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If you have questions or need assistance, the customer care team is a great resource. They're available Monday/Tuesday/Thursday 7 a.m. – 4 p.m. MT, and Wednesday/Friday 7 a.m. – 12 p.m. MT, at **855.4GR.DOGS (855.447.3647)** or **[grdogs@caninelifetimehealth.org](mailto:grdogs@caninelifetimehealth.org)**.

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We appreciate the time and care our Study veterinarians take to help make our study a success – we couldn't do it without you!

# Table of Contents

<b>1 BIOPSY SAMPLE COLLECTION OVERVIEW</b> .....	4
1 Biopsy Sample Collection Requirements .....	5
1a Biopsy Sample Fixation .....	6
1b Clinical Pathology Sample Collection .....	7
1c Sample Packaging & Shipping.....	10
1d Sample Reporting .....	15
<b>2 FLOW CYTOMETRY SAMPLE COLLECTION OVERVIEW</b> .....	16
2a Sample Collection Process .....	17
2b Clinical Pathology Sample Collection .....	18
2c Sample Packaging and Shipping.....	19
2d Sample Reporting .....	20
<b>FIXATION AND SHIPPING OF SMALL OR LARGE SPECIMENS</b> .....	21
<b>TISSUE CODING</b> .....	22

# 1

# Biopsy Sample Collection Overview

**Except for flow cytometry samples for suspected lymphoma (see page 16).**

## **Collection Process:**

- 1 Place a representative sample of the tumor into formalin at a standard ratio of 1 part sample to 10 parts neutral buffered formalin. Store at room temperature.
- 2 Place a tumor sample less than 5 mm<sup>3</sup> (size of a pencil eraser) in one vial of RNA/later. Circle 'DISEASED' on the label. Store at room temperature.
- 3 Place a sample of healthy tissue adjacent to the tumor less than 5 mm<sup>3</sup> (size of a pencil eraser) in a separate vial of RNA/later. Circle 'HEALTHY' on the label. Store at room temperature.

## **Please refer to the following sections for instructions on:**

- 1a Biopsy Sample Fixation
- 1b Clinical Pathology Sample Collection
- 1c Sample Packaging and Shipping
- 1d Sample Reporting

## **Refer to Appendices for guidance on:**

- Appendix 1 – Fixation and Shipping of Small or Large Specimens
- Appendix 2 – Tissue Coding

The biopsy kit contents should be kept together in the labeled biopsy kit box or envelope and stored at room temperature.

Please contact the Study team at 855.4GR.DOGS (855.447.3647) for advice or assistance with any sample submission or to request replacement or additional supplies.

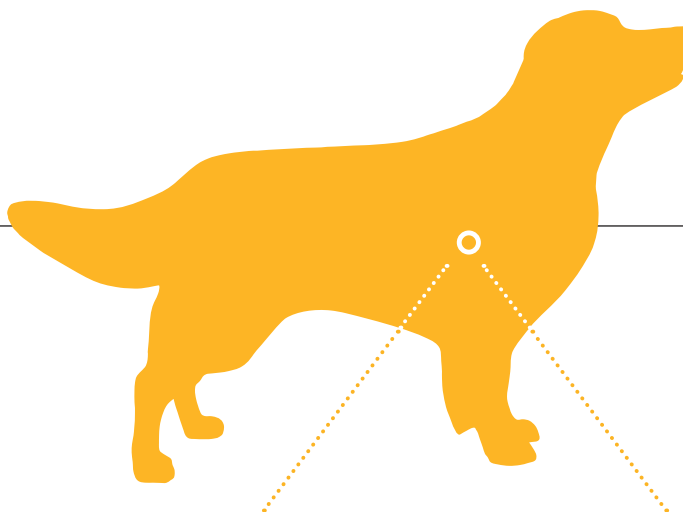
**Read all instructions before sample collection.**

# Biopsy Sample Collection Requirements

1

- 1 ☐ Excise tissue
- 2 ☐ Separate tissue into appropriate sections
- 3 ☐ Mark tissue as necessary
- 4 ☐ Place tissue into respective jars
- 5 ☐ Label jars appropriately
- 6 ☐ Document necessary information
- 7 ☐ Package shipment(s)
- 8 ☐ Wait for histopathology results to post to dog's online account\*
- 9 ☐ Complete online Malignancy-Related Questionnaire (if malignancy diagnosed)
- 10 ☐ Discuss the finding with your client (The Foundation does not provide interpretation)

\* You will not receive any results from the RNA<sup>later</sup> samples.



## Zoetis Reference Laboratories

Majority of the tumor in the formalin for immediate biopsy analysis.



## Azenta Life Sciences

Portion of the tissue (labeled diseased) and adjacent tissue (labeled healthy) in RNA<sup>later</sup> for long-term storage.



# 1a Biopsy Sample Fixation

## Three Biopsy Specimens Total are Requested

- 1 Place a representative sample of the tumor in formalin at a standard ratio of 1 part sample to 10 parts neutral buffered formalin. Store at room temperature.
- 2 Place a tumor sample less than 5 mm<sup>3</sup> (size of a pencil eraser) in one vial of RNA/*later*. Circle 'DISEASED' on the label. Store at room temperature.
- 3 Place a sample of adjacent healthy tissue less than 5 mm<sup>3</sup> (size of a pencil eraser) in a separate vial of RNA/*later*. Circle 'HEALTHY' on the label. Store at room temperature.

## Formalin

Tumor specimens should be placed into formalin immediately after surgical excision unless tissue marking with dye or suture is necessary (see Appendix 1). Samples should be placed in enough formalin to achieve a final ratio of 1 part sample to 10 parts formalin. Formalin jars should be stored at room temperature and shipped the same day of collection.

## RNA/*later*

The tumor sample to be placed into the provided tube of RNA/*later* should be a piece of tissue no larger than 5 mm on each of the three sides (5 mm<sup>3</sup> or size of a pencil eraser). After surgical excision, immediately immerse the sample into the tube of RNA/*later* and replace the cap securely. Circle 'DISEASED' on the label.

The sample of adjacent healthy tissue to be placed into a separate tube of RNA/*later* also should be a piece of tissue no larger than 5 mm on each of the three sides (5 mm<sup>3</sup> or size of a pencil eraser). After surgical excision of the healthy tissue, immediately immerse the sample into a separate tube of RNA/*later* and replace the cap securely. Circle 'HEALTHY' on the label.

When surgical margins are important, select a non-marginal area of the tumor for the sample intended for RNA/*later*. RNA/*later* vials should be stored at room temperature and shipped the same day of collection.

If the entire tumor sample is <1 cm on each side, place half into formalin and half into RNA/*later*.

**If surgical margins are important, then margins and proper sampling for histopathological diagnosis (Zoetis Reference Laboratory shipment) take priority.** Please be sure to collect the sample of adjacent healthy tissue and place in a separate vial of RNA/*later* and circle 'HEALTHY' on the label.

# Clinical Pathology Sample Collection 1b

Please collect clinical pathology samples if it has been more than 30 days since the last Annual Study examination.

## Blood Collection

To collect blood, please use the Vacutainer blood collection system included in this kit and:

- The butterfly catheter attached to the Vacutainer needle holder when collecting from the cephalic or lateral saphenous vein

OR

- The straight needle attached to the Vacutainer needle holder when collecting from the jugular vein

Using the Vacutainer holder and either the butterfly or straight needle provided, draw blood in the following order:

- 1 Fill the 3 mL EDTA\* tube (purple top; no barcode) for Zoetis Reference Laboratories clinical testing– verify the information on the label and print the date on the label.
- 2 Fill the 10 mL EDTA\* biorepository tube (purple top; barcode: 094XXXXXXB10yy, EDTA.)
- 3 Fill the four 10 mL serum tubes (red tops; no barcodes.)

\* To prevent clotting, immediately invert the EDTA tubes several times to mix the blood with the anticoagulant.



You will need to collect at least 43 mL of blood.

If necessary, use a needle and syringe (not supplied) to collect additional blood to top off any underfilled tubes.

Read all instructions before collecting sample.

# 1b Clinical Pathology Sample Collection (Cont.)



## Serum Isolation

Allow the four serum tubes (red tops) to clot for at least 45 minutes and then centrifuge per your clinic's normal protocol (we recommend 1,100–1,300 RCF for 10 minutes).

After centrifugation, remove the Vacutainer cap from each of the four serum tubes (red tops) and using the 3 mL transfer pipette provided (DO NOT POUR), transfer the serum as follows:

- Place 3 mL of serum into the 3 mL serum tube for Zoetis Reference Laboratories clinical testing. The black line on the left side of the tube label indicates the appropriate fill volume. (white top; no barcode.)
  - Verify the information on the label and print the date on the label.
- Place 10 mL of serum into the 10 mL biorepository serum transport vial (label includes bar code: 94XXXXXXByy, SERUM.)

If additional serum is needed to achieve the required volumes, try re-centrifuging the blood remaining in the tubes.



## Urine Collection

Collect a minimum of 10 mL of urine via free catch or cystocentesis.

A transfer pipette is provided for your convenience to:

- Place a minimum of 5 mL of urine into the Zoetis Reference Laboratories urine tube (no barcode.)
  - Verify the information on the label and print the date on the label.
- Place a minimum of 5 mL of urine into the biorepository urine tube (label contains bar code: 094XXXXXXB30yy, URINE.)



# Clinical Pathology Sample Collection (Cont.)

1b

## Fecal Collection

For owner-provided sample, fill tubes about 1/2 way.  
DO NOT OVER-FILL. If the owner did not bring the dog's fecal sample to the visit, please obtain a sample from the patient during the visit.

Transfer a marble-sized sample (approximately 1 g) of feces into each of the following:

- 30 mL fecal collection tube, (no barcode)
  - Verify the information on the label and print the date on the label.
- Biorepository fecal collection tube, (label includes bar code: 094XXXXXXB60yy, FECAL.)



## Toenail Trimmings Collection

Collect 5–10 toenail trimmings from the dog.

- Place the toenail trimmings into the biorepository 2 mL plastic cryogenic tube, (screw top; bar code: 094XXXXXXB50yy, NAIL.)



## Hair Collection

Cut a lock of hair  $\approx 1/4$ " in diameter (about the diameter of a wooden pencil) and a minimum of 2" long as close to the root as possible.\*

- Place the lock of hair into the 3.5 mL biorepository tube, (screw top; bar code: 094XXXXXXB40yy, HAIR.)

Proceed to shipping instructions on the next page.

\* Please ask the owner for the preferred collection site.



## 1c Sample Packaging & Shipping



### **Placing A Service Call For FedEx Shipment(s)**

Place a service call to FedEx directly at 800.463.3339. Let them know you have packages for pickup and tell them that the shipping cost is being billed to Morris Animal Foundation with a "Billable Stamp". If your clinic is inadvertently charged any fees, please contact the Study team.

Please contact the Study team at 855.4GR.DOGS (855.447.3647) for advice or assistance with any sample submission or to request replacement or additional supplies.

# Sample Packaging & Shipping (Cont.)

1c

## Zoetis Reference Laboratories

### 1 Clinical Pathology

- Verify all four tubes (no bar codes on labels) are properly filled and labeled, including the date. Place the fecal, 3 mL serum, 3 mL EDTA and urine tubes in the biohazard zip-closure bag.
- The Zoetis Reference Laboratories Manifest form will already be pre-filled with Morris Animal Foundation's information as well as the Study dog's information.
- The Manifest will come prepopulated with the test "GRLS Study Bundle."  
**Failure to include the manifest may result in charges to your clinic. No additional tests may be run with this order.**
- *If you fail to use the provided manifest, the Study will not receive the test results.*
- Place the preprinted manifest into the biohazard zip-closure bag (which should already contain the necessary samples, along with 2 paper towels (provided) or an absorbent sheet).
- Place the zip-closure bag inside the shipping box.



### 2 Histopathology

- Ensure all formalin container lids are tightly sealed.
- Tape lids to bottles.
- Handwrite the date and tissue code (see Appendix 2) on the label of all formalin jars.
- Place the labeled formalin jar(s)\*, together with 1 absorbing sheet per formalin jar, into the 5"x8" zip-closure bag and seal the bag.
- Complete the green Zoetis Reference Laboratories pathology form. Include your clinic information as well as the clinical history for the Study Dog. **Failure to include the clinical history may delay results.**
- Place the sealed bag containing the labeled formalin jar(s) plus the preprinted Zoetis References Laboratories Manifest (code: Histopathology Simple) and the green pathology form, along with any other documents or photos into the same shipping box as the clinical pathology samples (provided). Seal the box.

**\*If using supplies you have on hand at the clinic, please label the jars with the required information.**

Acct: 4761  
Dog ID: 094-XXXXXX  
Owner: Last Name, Dog Name

Date:  
Tissue Type:

# 1c Sample Packaging & Shipping (Cont.)

## 3 Packaging

- Place the sealed box containing formalin jars, clinical pathology samples and documents into the FedEx Clinical Pak that is pre-labelled with a shipping stamp to Zoetis Reference Laboratories. Close the Clinical Pak by removing the clear strip to expose the adhesive seal. Call FedEx at 800.463.3339 to arrange for a pickup.

# Sample Packaging & Shipping (Cont.)

1c

## Biorepository

### 1 Clinical Pathology

- Fill out the Shipment Inventory Form—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:
  - 5 mL of urine—10 mL tube with "Urine Only" label
  - 10 mL of EDTA blood—purple-top tube
  - 10 mL of serum— tube with "Serum Only" label
- Close the Styrofoam shipping container and place it inside a zip-closure Lab-Loc Biohazard bag. Also place the following five items inside the zip-closure Lab-Loc Biohazard bag:
  - Fecal sample tube—brown-top tube
  - Nails cryogenic tube—blue-top tube
  - Hair cryogenic tube—orange-top tube
  - Absorbent paper towel
  - Shipping inventory form



### 2 RNAlater

There should usually be two vials of RNA<sup>later</sup> shipped together - one labeled 'DISEASED' and one labeled 'HEALTHY.'

- Fill out the Tissue Submission Form for Tissues in RNA<sup>later</sup>—Golden Retriever Lifetime Study.
- **Ensure you have handwritten the date and tissue code (see Appendix 2) on the label of all RNA<sup>later</sup> tubes and circled 'DISEASED' or 'HEALTHY' on the appropriate vials.**
- Place both labeled RNA<sup>later</sup> tubes, together with one absorbing sheet, into the padded envelope and seal the envelope.
- Place the sealed padded envelope and Submission Form for Tissues in RNA<sup>later</sup> into a biohazard zip-closure bag and seal the bag.



\* NOTE: All tubes for the biorepository shipment are barcoded.

# 1c Sample Packaging & Shipping (Cont.)

## 3 Packaging

- Place the biohazard zip-closure bag containing the RNAlater samples and the zip-lock bag containing the serum, whole blood, urine, feces, hair and nail samples into the FedEx Clinical Pak. Also include a frozen ice pack from your supply. Use the ice packs provided in the kit to replace the ice pack being sent out. Seal the envelope by removing the clear seal to expose the adhesive strip.
- The FedEx Clinical Pak contains a pre-applied shipping label to Azenta Life Sciences. Call FedEx at 800.463.3339 to arrange for a pickup.

# Sample Reporting 1d

You will receive the test results from the samples you ship to Zoetis Reference Laboratories within five to seven business days on your patient's record through your online Study account at [grls.morrisanimalfoundation.org](http://grls.morrisanimalfoundation.org). The results are posted under the dropdown labeled 'Lab Results.' The results will come as two separate reports: one report for the biopsy and a second report for the clinical pathology.

If malignant histopathology results are received, log on at [grls.morrisanimalfoundation.org](http://grls.morrisanimalfoundation.org) to complete a Malignancy Related Questionnaire (MRQ) form for your patient. You can access the form by selecting the appropriate patient from your portal page. If you have any questions, please do not hesitate to email the Study team at [grdogs@caninelifetimehealth.org](mailto:grdogs@caninelifetimehealth.org) or call toll-free at 855.4GR.DOGS (855.447.3647). We are here to help!

Results will upload to your patient's online study account.

## 2

# Flow Cytometry Sample Collection Overview

In cases where a diagnosis of lymphoma is made from a fine needle aspirate (FNA) or blood smear an additional FNA cell sample should be collected for flow cytometry. Samples will be processed at no extra charge by the Clinical Immunology Laboratory at Colorado State University.

**Please refer to the following sections for instructions on:**

**2a** Sample Collection process

**2b** Sample Packaging and Shipping

**2c** Sample Reporting

Please contact the Study team at 855.4GR.DOGS (855.447.3647) for assistance with any sample submission.



# Sample Collection Process 2a

Once cytologic evaluation confirms a diagnosis of lymphoma/leukemia, additional cells should be collected for flow cytometry prior to chemotherapy.

- Make a cell aspirate solution substitute solution by mixing 0.9 ml of sterile saline with 0.1 ml of serum. Serum can be derived from the patient or from a different, unrelated dog.
- Collect a fine needle aspirate of affected lymph node or solid tumor using suction with a 20 gauge needle.
- Add material to the cell aspirate solution.
- Cap the tube tightly and keep refrigerated (4 degrees Celsius) until shipping. **DO NOT FREEZE the cells.**
- Refrigerated samples should be shipped within 48 hours of collection.

If the dog has lymphocytosis or appears to have bone marrow involvement, an EDTA tube containing at least 500ul of blood/bone marrow can be submitted. **Blood samples need to be accompanied by a current (within 2 days) CBC.**

If you have specific questions regarding flow cytometry, you can contact the Colorado State University Clinical Immunology Laboratory at 970-491-1170.

## **2b Clinical Pathology Sample Collection**

If Annual Study Visit clinical pathology samples have not been collected in the 4 weeks preceeding lymphoma/leukemia testing, please refer to page 7 for instructions.

# Sample Packaging and Shipping

2c

- Fill out the CSU Clinical Immunology Submission Form (the form can be downloaded at <http://csu-cvmbbs.colostate.edu/Documents/cilab-submission-form.pdf>.)
- Under Test Requested, indicate "flow."
- Under the History section, write the patient's Study ID number (the number beginning 94-0xxxxx) and write "Golden Retriever Lifetime Study." **If you are inadvertently charged any fees, please send an itemized invoice to [grdogs@caninelifetimehealth.org](mailto:grdogs@caninelifetimehealth.org) for reimbursement.**
- Place the tube containing the cell aspirate solution into a Zip-lock bag along with a liquid absorbing sheet ( e.g. paper towel).
- Place the completed form, cell aspirate, and ice packs into a biohazard shipping container.
- Make sure the sample is shipped Priority Overnight. You may either reach out to the Study team for a pre-paid shipping label or ask for shipping reimbursement from the Study.
- Call FedEx at 800.463.3339 to arrange for a pickup.
- The Study team will reimburse your office for shipping costs. Alternatively, please call 855.447.3657 for a pre-paid label. **You may also use your preferred shipping company and call for shipping cost reimbursement so long as the sample is on ice.**

## 2d Sample Reporting

Completion of the CSU Clinical Immunology Submission Form as specified on page 19 will ensure that the test results are transmitted to the Study veterinarian, for discussion with the dog's owner. A copy of the test results will also be transmitted to the Golden Retriever Lifetime Study team.

**Reporting from CSU takes about 3 business days.**

# Appendix 1

## **Fixation and Shipping of Small or Large Specimens**

Specimen shipping can be complicated when dealing with very small or very large tissues that cannot be routinely processed.


- Samples too large to fix as a whole (e.g., spleen) should be sectioned into portions and submitted in separate, appropriately labeled formalin jars. An annotated digital photograph or sketch of the original specimen to depict sectioning and orientation along with area(s) of gross lesions should accompany the samples. Dye margins before sectioning if appropriate.
- If an osteosarcoma is suspected, please overnight ship the entire limb. DO NOT FREEZE. Overnight ship prerefrigerated fresh tissue with ice packs to help minimize autolysis. \*For non-bony limb tumors, remove as much of the noncancerous tissue as possible prior to shipping, and then immerse the tumor into an appropriate-size container of formalin.
- Very small samples, such as endoscopic or punch biopsies, should be placed into the provided tissue cassette, the Dog Study ID# labeled with a #2 pencil, and then put into a formalin container for shipping. Do not use gauze sponges or cardboard because tissue may become compromised upon retrieval. Very small samples may, at times, yield insufficient artifact-free section for diagnosis; therefore, submission of multiple specimens from a single lesion is preferable.
- For luminal organs (e.g., intestine, uterus, large vessels), gently flush the intact lumen with formalin. For long sections of luminal tissues, make a partial longitudinal incision, leaving areas of interest (e.g., resection sites, mass) intact, or submit three labeled sections (e.g., proximal, middle and distal).
- Thin, flat samples (e.g., urinary bladder, stomach, diaphragm) should be placed directly into a formalin container. Larger flat samples can be sutured onto a flat piece of cardboard presoaked in formalin or water before immersion into a formalin container.
- Regional or draining lymph nodes associated with the primary tumor, including those that result in limb amputation, warrant microscopic examination. To ensure proper evaluation, consider dissection or biopsy of the regional lymph node during surgery. Submit the lymph node in a separate, appropriately labeled container.

**\* Contact the Study team at 855-4GR-DOGS (855-447-3647) for assistance with this shipment.**

# 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	Ileocecolic 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



Please contact the Study team at 855.447.3647 for advice or assistance with any sample submission, or to request replacement or additional supplies.

# Thank You to Our Partners

## FOUNDING PARTNER

The Mark & Bette Morris Family Foundation

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Please contact the Study team at 855.447.3647 for advice or assistance with any sample submission or to request replacement or additional supplies.

Morris Animal Foundation is a nonprofit organization that bridges science and resources to advance the health of animals. 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](http://morrisanimalfoundation.org).

