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!

• As soon as possible after the annual Study examination and sample collection, please complete the veterinary questionnaire for your patient. (On the morrisanimalfoundation.org homepage, click on the GRLS icon in the upper right corner, and scroll down to log in). 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.

• Remember to report relevant findings on the annual questionnaire in the case of a benign mass and on a Malignancy-Related Questionnaire if a mass is malignant.

If you have any other ideas, please feel free to share them with the Study team!

If you have questions or need assistance, the customer care team is a great resource. They’re available 9 a.m. — 6 p.m. ET at 855.4GR.DOGS (855.447.3647), or grdogs@caninelifetimehealth.org.

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

Except for fine needle aspirates 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 mm³ (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 mm³ (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 – Identification of Surgical Margins
Appendix 3 – Tissue Coding

The biopsy kit contents should be kept together in the labeled biopsy kit box or envelope and stored at room temperature. (Please check expiration dates on all supplies prior to biopsy collection).

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 □ 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 applicable)
10 □ Discuss the finding with your client

* You will not receive any results from the RNAlater samples.

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

**Fisher BioServices**
Portion of the tissue (labeled diseased) and adjacent tissue (labeled healthy) in RNAlater for long-term storage.
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 mm³ (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 mm³ (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 Appendices 1 and 2). 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 (unless collected Friday through Sunday).

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 mm³ 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 mm³ 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 (unless collected Friday through Sunday).

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 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

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 ZNLabs 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: 094XXXXXXB10, 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 53 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.
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 ZNLabs 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: 94XXXXXXB20, 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 ZNLabs 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: 094XXXXXXB30, URINE.)
**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: 094XXXXXXXB60, FECAL.)

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**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: 094XXXXXXXB50, NAIL.)

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**Hair Collection**
Cut a lock of hair ≈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: 094XXXXXXXB40, HAIR.)
Proceed to shipping instructions on the next page.
* Please ask the owner for the preferred collection site.
**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.

**Samples can ONLY be shipped Monday through Thursday.**

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.
ZNLabs

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.
• In the top header of the ZNLabs requisition form, provide the following information: your clinic name, address, email, phone and fax number.
• Complete the information (doctor, date, client name, pet name, species, breed and age) at the top of the form.
• All laboratory tests are ordered with code 4761; this is a no-charge study code, so 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. No additional tests may be run with this order.
• If you fail to use the provided requisition form, the Study will not receive the test results.
• Place the completed copy of the ZNLabs requisition form 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 3) 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.
• Place the sealed bag containing the labeled formalin jar(s) plus the completed ZNLabs histopathology requisition form along with any other documents or photos into the same shipping box as the clinical pathology samples (provided). Seal the box.

3 Packaging see figure 1

*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:
Tissue Code: ___ ___
Sample Packaging & Shipping (Cont.)

- Place the sealed box containing formalin jars, clinical pathology samples and documents into the FedEx Clinical Pak that is pre-labelled with a billing stamp to ZNLabs. 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.

Figure 1: ZNLabs Shipment

ZN Labs Samples: Histopath and clinical pathology report will be shared via the Study Veterinarian portal at no charge.
Biorepository

1 Clinical Pathology

- 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:*
  - 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 RNAlater shipped together - one labeled ‘DISEASED’ and one labeled ‘HEALTHY.’

- Fill out the Tissue Submission Form for Tissues in RNAlater (Fisher BioServices)—Golden Retriever Lifetime Study.
- Ensure you have handwritten the date and tissue code (see Appendix 3) on the label of all RNAlater tubes and circled ‘DISEASED’ or ‘HEALTHY’ on the appropriate vials.
- Place both labeled RNAlater 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 RNAlater into a biohazard zip-closure bag and seal the bag.

* NOTE: All tubes for the biorepository shipment are barcoded.
3 Packaging  see figure 2

- Place the biohazard zip-closure bag containing the biopsy samples and the zip-lock bag containing the serum, whole blood, urine, feces, hair and nail samples into the FedEx Clinical Pak and seal the envelope by removing the clear seal to expose the adhesive strip.
- The FedEx Clinical Pak contains a pre-applied shipping label. Call FedEx at 800.463.3339 to arrange for a pickup.

Figure 2: Fisher Bioservices Shipment

Fisher BioServices Samples: Healthy and diseased tissue and whole blood, serum, urine, feces, hair and toenails for long-term storage.
You will receive the test results from the samples you ship to ZNLabs within five to seven business days on your patient’s record through your online Study account at morrisanimalfoundation.org. The results are posted under the tab 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 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 or call toll-free at 855.4GR.DOGS (855.447.3647). We are here to help!
Lymph Node Aspirate Collection Overview

In cases where a diagnosis of lymphoma is made from a fine needle aspirate (FNA), 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 FNA Collection process  
2b Sample Packaging and Shipping  
2c Sample Reporting

Please contact the Study team at 855.4GR.DOGS (855.447.3647) for advice or assistance with any sample submission.
FNA Collection Process

Once cytologic evaluation confirms a diagnosis of lymphoma, 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 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.
Clinical Pathology Sample Collection

If Annual Study Visit clinical pathology samples have not been collected in the 4 weeks preceding FNA or biopsy please refer to page 7 for instructions.
Sample Packaging and Shipping

• Fill out the CSU Clinical Immunology Submission Form (the form can be downloaded at http://csu-cvmbs.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). This will route the flow cytometry billing to the Study.

• 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.

• On a FedEx Express US Airbill, complete the required “From” section. Retain the top copy for your records. Place the shipment bill inside a clear FedEx mailer pouch and seal the pouch. Affix the pouch to the outside of the Styrofoam shipping box. Make sure the sample is shipped Priority Overnight.

• Call FedEx at 800.463.3339 to arrange for a pickup.

• The Study team will reimburse your office for FedEx shipping costs. Alternatively, please call 855.447.3657 for a FedEx billing number. You may also use your preferred shipping company and call for shipping cost reimbursement so long as the sample is on ice.
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.
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

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<th>Description</th>
<th>Additional Indications</th>
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<tr>
<td>99</td>
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</table>
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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.

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