

NOTICE:

Our procedures have changed
Effective March 03, 2021

Please read the instructions on pages 2-4 carefully.

For more information contact:

rlwlab@hawaii.edu

or

Call 808-932-7148

Why we are changing our methods...

We are trying to increase our detection capabilities by applying new methods from the latest research on cell-free DNA which has been growing as a diagnostic tool for human and veterinary parasitic infections over the last 5 years. Detection of cell-free DNA in the blood might be the best chance of early detection.

Based on recently published scientific literature we are changing our methods for DNA detection infections to new blood collection vials, new blood DNA extraction methods, and testing all samples with our previous qPCR methods (CDC ITS 1 assay) as well as a new qPCR methods (NIH 3990 assay) that is 100-1000x more sensitive.

We would like to thank you for participating in our research studies involving testing for rat lungworm disease in local animals. Over the last 3 years, we have tested 110 animals with symptoms consistent with RLWD and seen roughly 20% positivity by qPCR. We hope these new methods will increase our detection capabilities.

As a reminder, testing is for research purposes only; a negative qPCR result does not necessarily mean the animal does not have rat lungworm disease. There are many variables outside of our control that might affect genetic testing, such as parasite load, immune response, and stage of infection. Fees charged in this study cover the cost of materials only, the cost of personnel time is paid for by funding from the Hawaii State Legislature for rat lungworm research.

Veterinary Sample Submission Instructions

Our team is working to develop a blood-based diagnostic test for the presence of *Angiostrongylus cantonensis*, the parasite that causes Rat Lungworm Disease (RLWD). Testing is for research purposes only and veterinarians should rely on standard methods for clinical diagnosis. Real-time PCR (qPCR) is used to identify the presence of *A. cantonensis* DNA in tissue samples. This is the same technique used on human CSF to clinically diagnose RLW disease. We are also optimizing an antibody test and appreciate donations of EDTA blood or CSF.

To have a sample analyzed, follow the directions below and complete the sample submission form. **The shipment of biological substances is highly regulated. Be sure to follow the instructions exactly to avoid federal and/or state fines.** Feel free to contact our lab if you have any questions. Thank you for your interest in rat lungworm disease.

Non-human animal tissue protocol

1. **Tissues** of non-human animals must be collected by and submitted to our lab by a **licensed veterinarian**. Samples received from the general public will be destroyed without testing.
2. **Whole blood :**
 - a. **ACUTE INFECTIONS:**
 - **Ideally:**
 - Collect 10 mL of blood directly into a **STRECK cell-free DNA BCT tube** (provided by Jarvi lab).
 - Follow instructions for the specialized tubes (see page 4).
 - Store sample at **room temperature**. See below for shipping instructions.
 - Sample must be received by the lab within 14 days from collection.
 - If the animal is large and healthy enough to draw a 2nd vial of blood, please also submit an EDTA blood sample for antibody testing (refrigerate and ship on ice).
 - **For small or weak animals:**
 - Submit ≥3-4 mL of EDTA blood.
 - Refrigerate fresh samples until delivered or picked up by lab personnel (≤4 hrs after draw).
 - Isolate plasma for storage if sample cannot be delivered or picked up ≥4hrs after draw.
 - Centrifuge for 5 minutes at 3,000 g and transfer plasma to a new sterile tube.
 - Freeze both tubes at -20°C (keep frozen in transit)
 - Sample should be submitted to the lab as soon as possible, for best results.
3. **CSF:** A minimum of 1 mL of CSF should be collected and shipped in a sterile screw-cap tube. Freeze and ship on ice.
4. **Tissues:** Best tissues for testing: brain (cerebrum, cerebellum, brain stem), spinal cord, lungs, and heart.
 - a. ~**100 mg** is ideal for tissue collection.
 - b. Samples should be collected into separate, sterile screw cap vials.
 - c. To minimize contamination between samples use sterile disposable instruments or instruments soaked in 10% bleach for 20 minutes, then rinsed in water and dried. Use separate instruments for each sample.
 - d. Freeze samples or preserve in DNA lysis buffer (provided by the Jarvi lab). Ship on ice.
5. **Fill out our sample submission form** (see page 3).
6. **Contact us by phone at (808) 932-7148** for pick-up in the Hilo area, or to obtain a copy of the BSP2 permit that must accompany your shipment.
7. **Pack** all tubes in a Ziploc bag, with absorbent material, such as paper towels to absorb leaks and place into a 2nd Ziplock bag. STRECK tubes are glass, pack carefully, and ship at room temperature. All other samples should be packed in a Styrofoam box with ice, ice packs, or dry ice (< 5 lbs).
8. **Your package MUST have these labels. Attach labels** to a vertical side of the box (not the top or bottom).
 - Shipper and receiver name, address and telephone number.
 - BSP 2 permit
 - UN 3373, Biological Substance, Category B
 - UN 1845 Dry Ice (**only if dry ice is used**). Containers must allow the release of carbon dioxide gas (i.e. unsealed styrofoam cooler within cardboard box). Write in the weight of dry ice on the label.
9. **Ship by overnight mail to Dr. Jarvi, Pharmacy Research, 200 W. Kawili St, Hilo, HI 96720 (808)932-7148**

**Jarvi Lab, Genetics of Infectious Disease
Laboratory Sample Submission Form**

Customer Information

Veterinarian Name: _____
Veterinary Clinic/ organization: _____
Vet Phone: _____ Vet Email: _____

Sample Information

Name of animal (if domestic) or descriptor (if wild): _____
Vet identifier for animal (ex/ ID # or owner name): _____
Number of Samples: STRECK blood ____ EDTA Blood ____ plasma ____ CSF ____ Tissue ____
Type(s) of solid tissue: _____
Tissues were drawn or collected: Date: _____ Time: _____
Species: _____ Breed: _____ Age: _____ Sex: M F
Was the animal alive or deceased at the time of collection? Body Weight: _____
How long has the animal exhibited RLWD symptoms? _____
Clinical symptoms include: _____

ZIP code where the animal lives: _____
Suspected route or source of infection: _____

The veterinarian and/or veterinary clinic is willing to collaborate with Dr. Jarvi to publish results of these samples. Additional information on clinical symptoms, treatments, and outcomes may be needed.

Yes Maybe No

***The results of this analysis are exclusive to the samples provided
and cannot be extrapolated to other samples or populations.
TESTING IS FOR RESEARCH PURPOSES ONLY***

Internal Use Only

Received by: _____ Date Received: _____ Time: _____
Processed by: _____ Invoice/Report No.: _____
Animal ID #: _____ DNA Log #: _____ qPCR run #: _____



Dr. Susan Jarvi

The Daniel K. Inouye College of Pharmacy
University of Hawaii at Hilo
Physical Address: 722 South Aohoku Dr., Hilo, HI 96720
Shipping Address: 200 W. Kawili St., Hilo, HI 96720
808-932-7148

Veterinary Sample Submission Instructions

Additional STRECK tube Instructions

1. **Contact the Jarvi lab for the STRECK cell-free DNA BCT tubes.**
2. **PRECAUTIONS:**
 1. Tubes are for Jarvi Lab research purposes only.
 2. DO NOT freeze the tubes (empty or full)
 3. DO NOT refrigerate the tubes (empty or full)
 4. DO NOT centrifuge the tubes
 5. DO NOT use after the expiration date.
 6. DO NOT use for collection of materials to be injected into patients.
 7. DO NOT dilute or add additional components to the tube.
 8. Over or under filling will lead to an incorrect blood-to-additive ratio and may affect results.
 9. Tubes are GLASS, handle carefully.
3. **Instructions for use:**
 - a. Collect specimen by venipuncture and **prevent backflow**.
 - b. Fill tube completely with blood (10 mL)
 - c. Remove tube from adapter and immediately mix by gentle inversion (180°) 8-10 times.
 - d. Store and ship STRECK tube at room temperature (6-37°C, 43-99°F).
 - e. Deliver to the Jarvi Lab within 14 days from collection.

❖ If collecting multiple tubes of blood for different analyses, see the STRECK recommended draw order below. *NOTE***: the Jarvi lab only needs the STRECK cell-free DNA BCT tube or the EDTA tube if the animal is large and healthy enough.

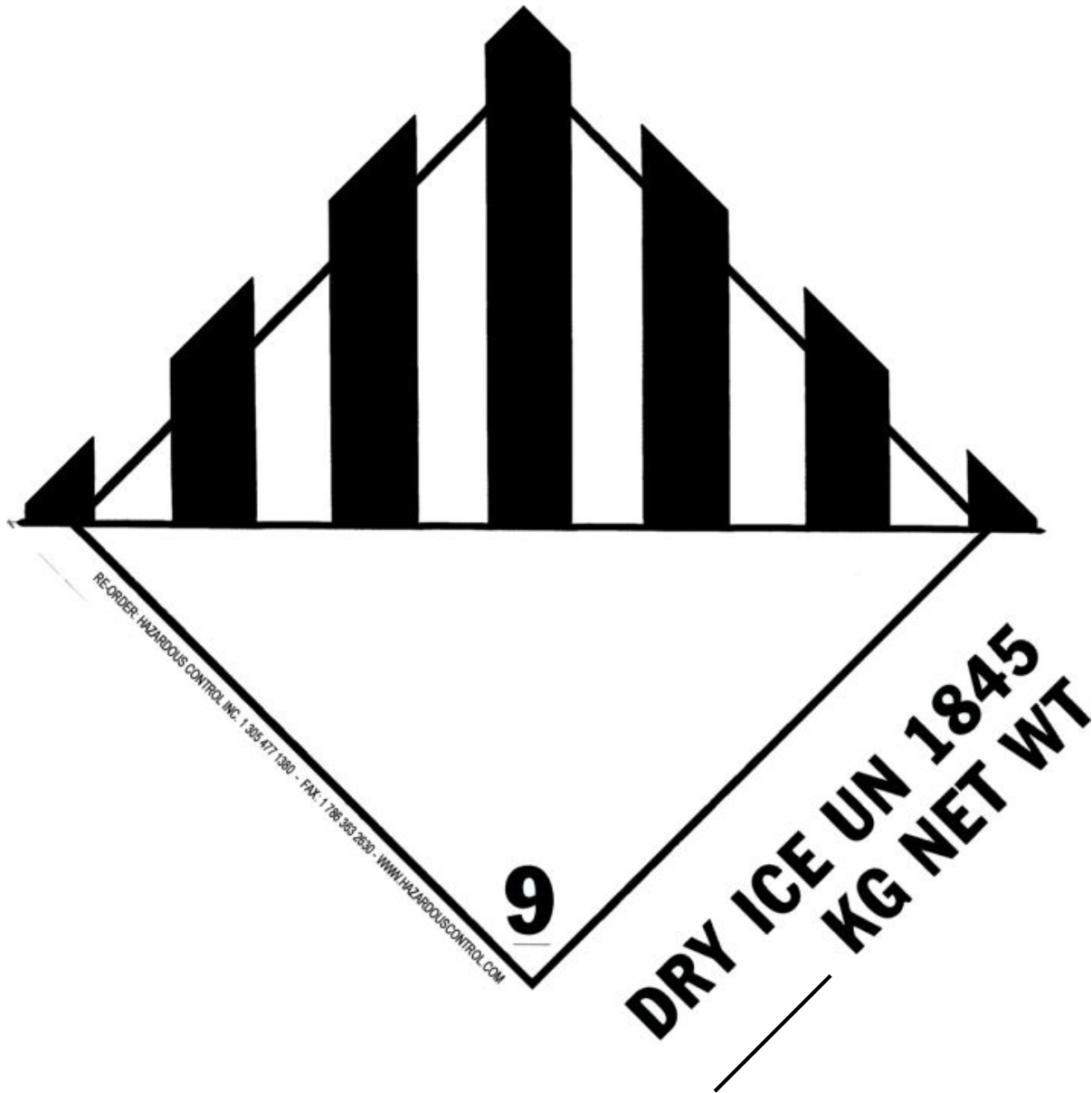
 - a. Sodium citrate
 - b. Serum
 - c. Clot activator
 - d. Heparin
 - e. EDTA tube **
 - f. **STRECK cell-free DNA BCT tube ****
 - g. Fluoride Oxalate (glycolytic inhibitor)
4. **Additional information (optional):**
 - a. <https://www.streck.com/products/stabilization/cell-free-dna-bct/>
 - b. [https://www.streck.com/wp-content/uploads/sync/Stabilization/Cell-Free_DNA_BCT_RUO_CE/01_Instructions_\(IFU\)/01_Cell-Free_DNA_BCT_RUO_IFU.pdf](https://www.streck.com/wp-content/uploads/sync/Stabilization/Cell-Free_DNA_BCT_RUO_CE/01_Instructions_(IFU)/01_Cell-Free_DNA_BCT_RUO_IFU.pdf)



Biological Substance, Category B

Do NOT resize or rotate these labels. Attach labels to a vertical side of the box (not the top or bottom). Each diamond must be 4" x 4" large, "square-on-point", ≥ 3 inches away from other markings that may reduce its effectiveness.

Shipping Labels for Rat Lungworm Samples



Do NOT resize or rotate these labels. Attach labels to a vertical side of the box (not the top or bottom). Each diamond must be 4" x 4" large, "square-on-point" with the #9 at the bottom, ≥3 inches away from other markings that may reduce its effectiveness. NOTE: USPS will not transport dry ice, use UPS or FedEx.