

## **Sampling Protocol For Dogs With Hereditary Ataxia That Are Euthanized**

### **Aims**

We are trying to get three types of samples from dogs diagnosed with hereditary ataxia that are euthanized. To be a candidate for participation in the study the dog must either have been diagnosed with hereditary ataxia by MRI of the brain, or show signs consistent with hereditary ataxia and be directly related to a dog confirmed to have this disease. Dr Olby can be contacted on 919 513 8286 (office), or 919 513 6692 (clinic) or by e mail ([Natasha\\_olby@ncsu.edu](mailto:Natasha_olby@ncsu.edu)) to discuss the dogs clinical signs and its candidacy for the trial.

The samples we are collecting include:

1. Blood to extract DNA.
2. Tissue fixed in formalin for the purposes of establishing a diagnosis.
3. Frozen brain tissue to extract RNA.

Tissue for extraction of RNA requires immediate removal of the brain in a sterile fashion, and immediate freezing of the samples taken in liquid nitrogen. This is rarely possible unless performed at North Carolina State University so we have given details of all protocols below, but are most concerned about getting tissue for confirmation of diagnosis and blood for DNA extraction.

### **Protocol**

#### **1. *Initial contacts***

If you are planning to euthanize your dog, please contact Dr. Olby by e mail ([Natasha\\_olby@ncsu.edu](mailto:Natasha_olby@ncsu.edu)) or by telephone (919 513 8286 or 919 513 6692 (clinic)) as soon as possible as she will be able to talk directly to your veterinarian about these protocols. If there is a College of Veterinary Medicine close by, she will be able to contact someone there and see if the full protocol can be carried out. Although it is difficult, the more time you can give Dr. Olby, the better she will be able to organize additional support. If Dr. Olby is not available (the receptionist in the neurology clinic will know when she is out of town), please call or e mail her research technician Pragna Mehta at 919 513 7235 (e mail: [pragna\\_mehta@ncsu.edu](mailto:pragna_mehta@ncsu.edu)).

#### **2. *Blood sample and pedigree***

We need 6-10mls of blood taken into an EDTA tube (or tubes). If you have already sent blood, this is not a priority although we like to take duplicate samples if possible. If we do not have your dog's pedigree we would like a copy of that as well.

#### **3. *Tissue to establish a diagnosis***

We need your veterinarian to remove your dog's brain after euthanasia causing as little damage as possible and place it in a container of 10% formalin with enough formalin in it to entirely cover the brain. The container should then be tightly sealed and placed in at least two layers of plastic bags, then placed in a Styrofoam, or well padded cardboard box with the blood samples and sent to us overnight using a shipping service that allows the package to be traced such as Fed Ex. Many veterinarians are not comfortable with, and do not have the equipment necessary for removal of a dog's brain, in which case we ask that they decapitate the dog and send the head to us packed in ice as soon as possible.

#### **4. *Tissue for RNA extraction***

In order for this tissue to be obtained, the veterinarian must have access to liquid nitrogen and be able to remove the brain in a sterile fashion immediately after death. Once the brain has been removed, a portion of one of the cerebellar hemispheres should be removed, placed in aluminum foil or a cryotube and frozen in liquid nitrogen. We need a 1cm square block of tissue. This frozen tissue has to be shipped on dry ice to prevent thawing. The remainder of the brain (and cerebellum)

should be placed in 10% formalin and shipped to us as listed above: it has to be shipped separately to the frozen sample to avoid freezing of formalin fixed tissue.

### **5. Shipping**

Please avoid shipping on a Friday as this would mean the samples would sit in the mail over the weekend. The blood samples will be fine if simply kept in a 4 degree centigrade refrigerator until is shipped on Monday, and formalin fixed tissue is stable at room temperature. The shipping address is:

Pragna Mehta at: Room 332 CVM Research Building, College of Veterinary Medicine, NCSU, 4700 Hillsborough St, Raleigh, NC 27606 (telephone 919 513 7235). Please notify us on the day the sample is shipped if possible by phone or by e-mail ([pragna\\_mehta@ncsu.edu](mailto:pragna_mehta@ncsu.edu)).

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