

Information regarding the collecting of samples for DNA Research and Diagnostics for ruling out other Diseases/Disorders

The attached forms are being provided to give further information regarding the submittal of samples to be used for DNA Research as well as for diagnostics for the purpose of ruling out possible other diseases/disorders. The following is a synopsis of those needed samples:

DIAGNOSTICS (Needed To Rule Out Other Possible Disease/Disorder):

The following blood workups and tests should be performed on your dog if you suspect he/she has had a CECS episode. These diagnostics should be performed within 48 hours of the dog's suspect episode and are important to rule out other possible diseases/disorders that your dog may have. This will not confirm that your dog does or does not have CECS/Spike's Disease.

- Routine blood workups: CBC and Chemistries Panel are warranted after any episode that resembles a seizure the first time and should be done within 48 hours after that episode.
- Because there may be an increased incidence of Hepatic Microvascular abnormalities in Border Terriers, a Bile Acid Stimulation Test (BAST) is also recommended, although your veterinarian may decide to do this at a later date. If the BAST is abnormally high, your veterinarian may want to do additional liver function tests, ultrasound, and/or liver biopsy.
- The results of these diagnostics may be sent along with any DNA information you plan to submit to the University of Missouri.

THE FOLLOWING IS INFORMATION REGARDING DNA RESEARCH AND INSTRUCTIONS ON HOW YOU CAN BECOME INVOLVED:

DNA Research information:

CECS/Spike's Disease is a relatively newly discovered disorder. It most likely has been around for many years, but wasn't brought to light until a few years ago.

DNA research has just begun on this disorder and it is a very important factor in discovering the cause. Eventually it will help to discover an effective way to treat this condition. More importantly, DNA research can result in finding a DNA marker that will enable breeders to create their own intelligent breeding program that will enable them to exclude affected dogs. As well, it will allow breeders to test young puppies to determine if they are affected, carriers or clear.

The following information explains what you can do, as a Border Terrier fancier, to help in finding this very important DNA marker in our breed.

HOW CAN I HELP?

If you have a Border Terrier suspected of having CECS/Spike's Disease, you can supply important DNA information for the project. Participation by the owners of affected dogs and their relatives is essential to the success of this project.

DNA RESEARCH NEEDS:

Because CECS/Spike's Disease is still a mystery in many ways, important information must be gathered to help find both the cause of this disorder as well as a way to accurately diagnose it (by finding the DNA marker for this disorder and to eventually find an accurate way to medicate for it). Most importantly, finding a DNA marker for this disorder will allow breeders to do intelligent breedings to avoid producing CECS dogs. The following material should be submitted to the University of Missouri to aid in their DNA research:

- **Blood sample** – The ideal sample for DNA extraction is 5-10cc's of whole blood, in purple-topped (EDTA) tubes. For very small dogs or puppies, 3ccs should be sufficient. The blood sample needs only to be put in the tubes and rocked gently a few times to distribute the anticoagulant – do not spin, extract serum or anything further. Refrigerate if the sample is being held for any time before shipping. *There is no cost for this beyond your local veterinarian's draw and shipping.*

There are further instructions regarding shipping on the attached "Sample Handling" sheet.

- **DOG'S PEDIGREE** (preferably a 4-5 generation pedigree) - If you only know your dog's sire and dam and their AKC registration numbers (found on their AKC registration slip, you can go to the American Kennel Club site and order an online pedigree for \$10. If you do not have a way to gather a full pedigree, then you can send your dog's sire and dam's name and registration numbers to the University of Missouri and they will take the time to do this. You may also contact Kris Blake for help on gathering your dog's pedigree information.

*If you do not have your dog's pedigree, please contact Kris Blake for suggestions as to how you can obtain that information. Kris can be e-mailed at: KrisBSaga@comcast.net or reached by phone at: (763) 208-3961

- **INDIVIDUAL DOG'S INFORMATION FORM** - There is an attached short form created by the University of Missouri that must be filled out and accompany the blood sample and pedigree.
- If you have performed the CBC, Chemistries Panel and BAST (Bile Acid Stimulation Test), it would be most helpful to send the results of these tests to the University of Missouri as well at this time.

Note: Gathering info on the affected *and* non-affected siblings, parents and nearest relatives of CECS affected dogs helps the researchers the most and will shorten the time it takes them to find this DNA marker. There are instructions in the forms package that explain how to submit more than one dog's info at one time.

If you need further clarification regarding the details of a collection, or the completion of the forms contact:

Kris Blake
KrisBSaga@comcast.net

Liz Hansen
HansenL@missouri.edu

Please send all DNA Research material to:

Dr. Gary Johnson - (Breed of Dog) DNA Research
320 Connaway Hall
University of Missouri
Columbia, MO 65211

**Canine DNA Research for Individual Dog Information, Sample Handling
and Submission Information on following pages**

CANINE DNA RESEARCH

Individual Dog Information

Breed: _____

Litter ID code: _____

Blood – Tissue – Other: _____

Registered Name: _____

Call name: _____

AKC #: _____ Birth Date: _____

Male Female Intact Neutered

Sample Submission Date: _____

Color: _____

Sample submitted for which research project? _____

Owner Info:

Alternate Contact:

Name: _____

Name: _____

Address: _____

Address: _____

Phone (Day): _____

Phone (Day): _____

Phone (Eve): _____

Phone (Eve): _____

Fax: _____

Fax: _____

E-Mail: _____

E-Mail: _____

Does this dog exhibit any of the following conditions? (Please attach history for any Yes answer.)

Y – N Allergies

Y – N Heart Problems

Y – N Arthritis

Y – N Hernia (Where? _____)

Y – N Autoimmune Disorders

Y – N Reproductive Problems

Y – N Bite or Tooth Abnormalities

Y – N Seizures

Y – N Cancer/Tumors

Y – N Skin/Coat Problems

Y – N Cataracts/Vision Problems

Y – N Skeletal Abnormalities (Hip Dysplasia, etc.)

Y – N Deafness/Hearing Impaired

Y – N Temperament Problems (Shy, aggressive, etc.)

Y – N Digestive difficulties

Other (Please list):

Testing done on this dog:

OFA/PennHip Y – N Age at test: _____ Result: _____ # _____

CERF Y – N Age last tested: _____ Result: _____ # _____

Thyroid Y – N Age last tested: _____ Result: _____

Other (Please list):

Other Comments/Questions/Concerns?

Please circle your response to the following:

I am / am not willing to provide additional blood samples if needed for research.

I will / will not consider donation of a tissue sample (spleen, kidney, or liver) upon the death of this dog, and will discuss this decision with my veterinarian so that a notation is placed in my file.

I submit this sample and pedigree for the purpose of DNA research; I understand that the identity of dogs and owners participating in the research will not be revealed; and I have supplied complete and accurate information, to the best of my knowledge.

Signed: _____ Date: _____

SAMPLE HANDLING
For Canine DNA Research at the University of Missouri

Blood Sample - The ideal sample for DNA extraction is 5-10cc's of whole blood, in purple-topped (EDT A) tubes. For very small dogs or puppies, 3ccs should be sufficient. The blood sample needs only to be put in the tubes and rocked gently a few times to distribute the anticoagulant - do not spin, extract serum, or anything further. Refrigerate if the sample is being held for any time before shipping.

Frozen Semen - If there is frozen semen stored from sires or affected dogs, DNA can be extracted from it. Please send 2 straws. They do not need to be shipped frozen, but do pack them in a crush-proof container.

Tissue Sample - Tissue removed as a result of surgery, or an organ sample upon death of the dog will provide a large amount of DNA for research. Please discuss this with your vet ahead of time if you intend to do this. (If the dog is to be euthanized, have a blood sample pulled first, if possible, and send both samples.) First choice is spleen, second choice kidney, and third choice is liver (a piece about the size of your thumb is all that is needed - not the entire organ). One tissue sample is sufficient. Have the organ removed as soon as possible following death, place into a labeled freezer bag, put that into a second bag, freeze, and ship.

Label sample with the following;
call name - owner's last name
(If samples from several dogs are sent together, number samples and forms)

An **Individual Dog Information form** should be completed, and a **pedigree copy** must be included with the sample to tie it in with the correct family. If the dog is not affected but is a relative of an affected, please indicate the relationship.

Shipping - Ideally the sample should be shipped immediately (with a tissue sample make certain it is completely frozen first). If samples are held for a day or over a weekend, blood must be refrigerated, and tissue samples must be kept frozen. Ship via overnight delivery (US Mail, UPS, or FedEx). **Do not send on a Friday** - there will not be anyone to accept the delivery on a weekend, and the sample could be unusable by Monday. Pack in a small insulated container (most vets have these for shipping samples to labs), with one or more cool packs - it is important that blood samples be kept cool but not frozen, and tissue samples be kept as frozen as possible.

The delivery address is;
Dr. Gary Johnson - (Breed of Dog) DNA Research
320 Connaway Hall
University of Missouri
Columbia, MO 65211

If you need clarification, or have any questions about any of these procedures, please contact Liz Hansen by phone (573-884-3712), email (HansenL@missouri.edu), or regular mail (321 Connaway Hall, University of Missouri, Columbia, MO 65211). Liz is Dr. Johnson's Project & Information Coordinator, and can help with any questions you may have.

Thank you for your cooperation and participation!

SENDING INFORMATION AND SAMPLES
For DNA Research at the University of Missouri

First, THANK YOU for participating in this important research project! The samples you provide will allow researchers to continue discovering the genes controlling traits in your breed, and dogs in general. As the canine genome is mapped, breeders will have an unprecedented opportunity to identify and avoid producing disease, and concentrate on positive advances in their breeding programs.

It is of utmost importance that the information you provide with the samples is as complete and accurate as possible. The presence of disease, unusual, or "undesirable" characteristics should be revealed to the researchers wherever it has been identified. Information on specific, individual dogs will not be revealed - results of the research will identify what markers have been found, but not the names of those who submitted the samples where a characteristic was located, nor which individual dogs show affected or carrier status for any given condition. Information provided will be kept strictly confidential. As the research produces results, participants may request information on the genetic status of their dog(s).

Complete families are critical to locating specific genes and markers. Wherever possible, submit samples from all siblings, both parents, and all available grandparents.

Begin by gathering the pedigree, litter information, and litter list(s) for each family you plan to submit. You will need a correctly formatted (sire on top, dam on the bottom), typed or computer-generated pedigree (3- to 5 generation) of the litter where an affected appeared. If the sample is for a DNA bank, send a pedigree of the individual dog. The pedigree will connect each sample you submit to the family it comes from, so make copies for each individual dog who will be sampled. The breeder of the litter, or other person familiar with the litter should make a "Litter Packet" for each litter - this consists of the *Litter Information* sheet, *Litter List*, and the *Pedigree*. For the Litter 10 code use the kennel name or breeder name, plus the date of birth of the litter, so if Pat Doe had a litter born May 15, 1992, the code would be "Doe 05-15-92". Dr. Johnson's staff has a different system of coding in the lab to anonymously identify samples, but the Litter 10 code is a way to tie your information together and place individuals in the families where they belong as samples are submitted. This 10 code should be on each form sent in. Keep a copy of the packet for your own records, and send a copy to Dr. Johnson. This family information may be sent with the blood samples, or separately.

Next, begin collecting and submitting samples for DNA extraction. See the *Sample Handling* sheet for procedures. The *Individual Dog* submission form should accompany each sample, along with the marked *Pedigree* copy that will tie in with the family information sent. Make copies of the *Sample Handling* and *Individual Dog* forms as needed for all samples to be submitted. If several dogs' samples are being sent together, number the forms and samples to be certain there is no confusion (Sample #1, #2, etc). On a spare copy of the pedigree you may want to mark (for yourself) who is alive and sampled, not sampled, and those no longer living, to keep track of who you need to get samples from. As stated before, entire families will give the best chance at finding specific genes - do your best to include all living family members.

Send samples and information to Dr. Johnson's lab at this address;

Dr. Gary Johnson - (breed of dog) DNA Research
320 Connaway Hall
University of Missouri
Columbia, MO 65211

If you need clarification, or have any questions about any of these procedures, please contact Liz Hansen by phone (573-884-3712), email (HansenL@missouri.edu), or regular mail (321 Connaway Hall, University of Missouri, Columbia, MO 65211). Liz is Dr. Johnson's Coordinator of Veterinary Information, and can help with any questions you may have.

Once again, thank you for participating in this important research - you are contributing to the betterment of future generations.