RNA Sample Protocol

Study: The Genetic Inheritance of Megaesophagus in Friesian Horses

Please notify us before collecting and shipping any samples.
Point of Contact: Angie DePuydt, (920) 944-2073 or angie@fenwayfoundation.com

REQUIRED DOCUMENTATION. Once complete, email the veterinarian report characterizing case history and necropsy/histology report to angie@fenwayfoundation.com

BLOOD SAMPLE. Before euthanasia, draw a blood sample using two (2) 10-12 ml blood tubes with EDTA (purple top). Store the blood samples in a refrigerator before shipping.

TISSUE SAMPLE LOCATIONS & SIZE. To preserve RNA, tissue collection should take place only after the necropsy lab has been prepared and immediately following euthanasia. Tissue samples should be approximately 1 cm in size and encompass all tissue layers.

1. **Center of any dilated portions of the esophagus**. Label as DIL 1, DIL 2, etc.
2. **Center of any hypertrophied portions of the esophagus**. Label as HYP 1, HYP 2, etc.
3. **Cervical esophagus** – 10 cm below the beginning of the esophagus. Label as CER
4. **Thoracic inlet**. Label as TIN
5. **Lower thoracic esophagus** – 10 cm above the lower esophageal sphincter. Label as LTE

RNA SAMPLE PROTOCOL. If possible, collect three different types of samples (see below) from each sample location using the following protocols and preserve them immediately after they are collected using the following protocols:

- **Snap-frozen Protocol**. Place tissue in an empty 2 mL cryovial and immediately plunge into liquid nitrogen. Keep frozen, either in liquid nitrogen or at -80 C. This is the most versatile preservation method and our top-priority sample. **Due to the shipping requirements, this sample is for USA-based cases only and should not be taken outside the USA.** Note: if you do not have the ability to snap-freeze samples, take only RNAlater and histology samples.

- **RNAlater Protocol**: Tissue should be cut into 1 cm pieces, each placed into a 2 mL cryovial containing at least 1 mL of RNAlater stabilization Solution. **This sample should be stored at 4˚C for 24 hours before being frozen before shipment**. Note: If you do not have RNAlater Stabilization Solution on hand and you have advance notice of the necropsy, please contact us to inquire if we can ship you some. Alternatively, take only snap-frozen and histology samples.

- **Histology Sample Protocol**. Tissue should be handled with care to minimize artifacts from forceps or scalpels. Cut a full-thickness section and place it directly in either 10% neutral buffered formalin or fresh 4% formaldehyde in a 2 mL cryovial or another leak-proof container. Store it at 4˚C. If stored long-term before fixation, formalin should be replaced with methanol after 24 hours. **This sample CANNOT be frozen**.

SHIPPING PROTOCOL:

- Place the snap-frozen samples, RNAlater samples, and blood samples in an insulated foam container on dry ice. If there are no snap-frozen samples, a standard ice pack is sufficient.
- Place the leak-proof containers of the formalin samples in a separate box with padding to protect them from damage.
- Place the box of histology (formalin) samples and the foam container with the snap-frozen samples, RNAlater samples, and blood samples in a larger box for shipping.
- Use packing material in between the boxes to protect each box from damage
- Ensure the samples are labeled as indicated above, along with the horse's name and owner's name
- Ship samples via overnight service. DO NOT ship samples with a weekend arrival date. No one is at the lab to receive the samples over the weekend.

SHIPPING ADDRESS (ALL USA SAMPLES)
Dr. Kathryn T. Graves
Animal Genetic Testing & Research Laboratory
108 Gluck Equine Research Center
The University of Kentucky
Lexington, KY 40546-0099 USA
PH: 859-218-1193

SHIPPING ADDRESS (ALL NETHERLANDS SAMPLES)
Ing. B. W. Dibbits
Wageningen University and Research
Animal Breeding and Genomics
Droevendaalsesteeg 1
6708 PB, Wageningen, The Netherlands
PH: +31317482445

Research Consent Form

Genetic Testing at Gluck
108 Gluck Equine Research Center
Lexington KY, 40546-0099
(859) 218-1165

STUDY: Genetic Basis of Megaesophagus, Aortic Aneurysm, Gastroparesis/Gastric Rupture or Connective Tissue Diseases in Friesian Horses

Horse's Registered Name:

_____

Owner Name: _____

Address: _____

Phone: _____ **Email:** _____

I certify that I am the owner/agent of the above horse and that I voluntarily submit material from this animal to be used for the purpose of research at the University of Kentucky. I understand that I shall have no claim to monetary compensation should the use of this sample result in commercial testing applications. Confidentiality will be strictly maintained for all submitted samples.

X_____ **Date:** _____