Friesian Histopathology Sampling Protocol

Megaesophagus, Foregut & Connective Tissue Disease

1. Research Objectives

This protocol is intended to guide standardized tissue sampling for histopathologic evaluation in Friesian horse necropsies. Histopathology findings and associated reports will be shared with the Fenway Foundation for Friesian Horses to support ongoing research into Friesian megaesophagus, foregut dysfunction, vascular disease, and related connective tissue phenotypes. Routine standardized evaluation of both grossly normal and abnormal tissues may help improve understanding of:

- Classic megaesophagus phenotypes
- Distal focal narrowing and hypertrophic phenotypes
- Mixed esophageal and gastric phenotypes
- Aortic rupture and vascular connective tissue phenotypes
- Potential subclinical or early-stage lesions in clinically unaffected horses

2. General Sampling Guidelines

- Collect full-thickness tissue sections whenever feasible.
- Histopathologic evaluation of all standardized landmark samples is requested regardless of whether gross abnormalities are identified.
- In addition to standardized anatomic landmark samples, collect targeted samples from any visible lesion.
- Whenever feasible, tissue orientation should be maintained consistently to facilitate comparison of mucosal, submucosal, muscular, and neural structures between cases.
- When a lesion is present, collect:
    - One sample from the center of the lesion
    - One sample from the transition zone between abnormal and more normal-appearing tissue, if identifiable
- Standardized labeling of sampled regions is requested to improve consistency across cases and facilitate comparison between horses.

3. Histopathology Submission & Processing

- Tissue samples should be collected and processed according to the laboratory's standard histopathology procedures.
- Histopathologic evaluation of all standardized landmark samples is requested, including regions that appear grossly normal at necropsy.
- Additional lesion-targeted sections are requested whenever gross abnormalities are identified.
- For aortic samples, evaluation of the tunica media is especially important, including assessment for medial degeneration or necrosis, mucoid material accumulation, elastic lamina fragmentation or disorganization, smooth muscle hypertrophy, fibrosis or abnormal collagen morphology, mineralization, inflammation, dissection, pseudoaneurysm formation, and vasa vasorum changes.

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- Special stains for elastic fibers and connective tissue components may be valuable in selected vascular cases when deemed appropriate by the pathologist.
- Full-thickness sections are preferred whenever feasible.
- Standardized labeling of sampled regions is requested to improve consistency across cases and facilitate comparison between horses.
- Histopathology reports, including descriptions of sampled locations and identified lesions, should be shared with the Fenway Foundation for Friesian Horses for research purposes.
- Representative photos may be valuable for future comparative review when available.

4. Standardized Labeling Framework

- **Standard Landmark Samples**: CER, TIN, MTE, LTE, GEJ, SQ-MP, GL-B, ANT, PYL, DUO, AOR-LA, AOR-CR, AOR-CA, PA-LA.
- **Lesion-Targeted Samples**: DIL-C/T, HYP-C/T, DFN-C/T, AOR-RUP, AOR-DIS, AOR-PSA, or other descriptive lesion codes as appropriate

5. Esophagus: Standard Landmark Samples

The following esophageal samples are recommended for routine histopathologic evaluation in every Friesian necropsy case, regardless of gross findings.

Location	Description	Label
Cervical esophagus	Approximately 10 cm distal to the beginning of the esophagus	CER
Thoracic inlet	Thoracic inlet region	TIN
Mid-thoracic esophagus	Midway between thoracic inlet and lower thoracic sample	MTE
Lower thoracic esophagus	Approximately 10 cm cranial to the lower esophageal sphincter	LTE
Gastroesophageal junction/cardia region	Collect when feasible, particularly in horses with distal esophageal Abnormalities, narrowing, or suspected mixed foregut phenotypes	GEJ

6. Esophagus: Lesion-Targeted Samples

For each gross lesion identified, additional histopathologic sections are requested:

- One sample from the center of the lesion
- One sample from the transition zone, if identifiable

Suggested lesion labels

Lesion Type	Center	Transition
Dilated segment	DIL-C 1	DIL-T 1
Hypertrophied segment	HYP-C 1	HYP-T 1
Distal focal narrowing	DFN-C 1	DFN-T 1

Additional lesions may be labeled similarly using descriptive lesion codes as appropriate.

Gross abnormalities that should trigger additional sampling include:

- Esophageal dilation
- Hypertrophy or wall thickening
- Distal focal narrowing
- Diverticular change
- Structural irregularities
- Any other visible abnormality

7. Stomach: Standard Samples

The following gastric samples are recommended for routine histopathologic evaluation in every Friesian necropsy case, regardless of gross findings.

Location	Description	Label
Squamous mucosa adjacent to margo plicatus	Squamous region bordering glandular mucosa	SQ-MP
Glandular body/fundic region	Gastric body/fundus	GL-B
Pyloric antrum	Antral region proximal to pylorus	ANT
Pylorus	Pyloric outflow region	PYL
Proximal duodenum	Immediately distal to pylorus, if feasible	DUO

8. Stomach & Duodenum: Lesion-Targeted Samples

For each gross lesion identified, additional histopathologic sections are requested from:

- One sample from the center of the lesion
- one sample from the transition zone, if identifiable

Examples include:

- Focal thickening
- Ulceration
- Scarring
- Mucosal irregularity
- Pyloric narrowing
- Discoloration or other visible abnormalities

9. Aorta: Standard Samples

The following aortic samples are recommended for routine histopathologic evaluation in every Friesian necropsy case, regardless of gross findings. Circumferential full-thickness sections are preferred whenever feasible to facilitate evaluation of regional medial abnormalities.

Location	Description	Label
Aortic arch near ligamentum arteriosum	Thoracic aorta at/adjacent to the ligamentum arteriosum attachment, the region most commonly associated with Friesian aortic rupture	AOR-LA
Adjacent thoracic aorta, cranial to ligamentum arteriosum	Grossly normal-appearing aorta cranial to the ligamentum arteriosum region, when feasible	AOR-CR
Adjacent thoracic aorta, caudal to ligamentum arteriosum	Grossly normal-appearing aorta caudal to the ligamentum arteriosum region, when feasible	AOR-CA
Pulmonary trunk/artery adjacent to ligamentum arteriosum	Especially important if aorto-pulmonary fistulation, pulmonary artery rupture, or perivascular hemorrhage is suspected	PA-LA

10. Aorta & Pulmonary Artery: Lesion-Targeted Samples

For each gross vascular lesion identified, additional histopathologic sections are requested from:

- one sample from the center or edge of the lesion, including the rupture margin when present
- one sample from the transition zone between abnormal and more normal-appearing vessel wall, if identifiable
- associated perivascular hemorrhage, pseudoaneurysm wall, or dissection tract when present

Lesion Type	Center/Lesion	Transition
Aortic rupture	AOR-RUP-C 1	AOR-RUP-T 1
Periaortic hemorrhage/hematoma	AOR-HEM 1	—
Aortic dissection	AOR-DIS 1	—
Pseudoaneurysm	AOR-PSA 1	—
Pulmonary artery rupture/fistulation	PA-RUP 1	PA-T 1

11. Fenway/KFPS Contact Information

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