Investigating Equine Muscular Storage Diseases. Recommended protocol for New Zealand Equids.

STEP 1: Collect a detailed history and complete a thorough physical examination to characterise the lesions.

STEP 2: A complete biochemical profile should be run.

a. CK and AST can provide evidence of exertional rhabdomyolysis and elevated SAA (serum amyloid A) may be seen with inflammation.
   - The absence of, or only mildly elevated muscle enzymes or SAA does not exclude storage diseases or other myopathies.

b. Assessing serum selenium and Vitamin E concentrations should also be considered.
   - While deficiencies do not cause storage diseases, their presence can exacerbate frequency or severity of the clinical signs.
   - Whole blood rather than serum selenium should be assessed. Serum Vitamin E concentrations are rarely low in NZ horses but could also be determined.

STEP 3: Urinalysis should be considered if there is evidence of rhabdomyolysis at the time of examination. Urinalysis can determine if myoglobinuria is present and, in conjunction with biochemical analysis, if renal dysfunction has developed.

STEP 4: Exercise response tests: These are helpful in diagnosing chronic exertional rhabdomyolysis in horses that do not present with clinical signs.

STEP 5: +/- Ultrasound: to identify muscle trauma and fibrosis (presuming there is physical disruption of the muscle).

STEP 6: Genetic testing (see below).

STEP 7: Muscle biopsy (see below).

Genetic testing available in NZ (performed at Massey University)

PSSM1 (Polysaccharide storage myopathy type 1), GBED (glycogen branching enzyme deficiency), HYPP (hyperkalaemic periodic paralysis) and MH (malignant hyperthermia) genetic tests are available in NZ and myopathies that have genetic tests available should be ruled out, as indicated by breed. These are performed on hair samples.

Directions from the Massey website for hair collection:

- Please pull mane or tail hair to preserve the root bulbs, do not cut.
- About 30-40 hair bulbs is sufficient.
- Please do not tie or knot the hair.

Disease	Breed(s) & their crosses	Mode of inheritance
Hyperkalaemic periodic paralysis (HYPP)	QH, Paint, APP	Autosomal dominant
Glycogen branching enzyme deficiency (GBED)	QH, Paint	Autosomal recessive
Malignant hyperthermia (MH)	QH, Paint	Autosomal dominant
PSSM 1 (Polysaccharide storage myopathy type 1)	QH, Paints, Morgan, Warmbloods, TB, Arabians, etc	Autosomal dominant

Abbreviations: QH = Quarter horse; TB = Thoroughbred; APP = Appaloosa

For more information: https://www.massey.ac.nz/massey/learning/colleges/college-of-sciences/clinics-and-services/equine-parentage-animal-genetics/horse-tests/horse-tests.cfm

Muscle biopsies

CAVEAT: *Muscle biopsies are indicated for horses with existing clinical signs only.* Biopsies have poor sensitivity and specificity in clinically normal and young horses and should not be used to try to predict if a horse will develop chronic exertional myopathies or immune-mediated diseases. Similarly, young horses may not show classic histologic features of storage myopathies, as these accumulate over time and false negative results can occur.

- ✓ Care must be taken to biopsy the correct muscle group and ensure an adequate biopsy size.

- ✓ MINIMUM BIOPSY SIZE: 10x10x10mm (Tissue will shrink by 20% during the fixation and processing steps and smaller biopsies may compromise the ability to obtain a diagnosis).

  - For specific instructions on biopsy collection refer to the Michigan State University: Valberg Neuromuscular lab website: https://cvm.msu.edu/research/faculty-research/comparative-medical-genetics/valberg-laboratory/for-veterinarians/obtaining-and-submitting-a-biopsy#forms-and-instructions

Biopsy sites based on site of muscle atrophy and disease concern:

Distribution of Atrophy/Disease concern	Muscle to biopsy
Generalized atrophy & suspected *Equine* motor neuron disease (*EMND*)	Sacrocaudalis muscle
Topline atrophy & suspected immune-mediated myopathies	TWO biopsies from (1) Semimembranosus (2) Epaxial muscles (Multiple Trucut biopsies okay)
Focal muscle atrophy	Affected muscle
Exertional Myopathies and suspected PSSM	Semimembranosus muscle

- ❖ Michigan State University (MSU) does not hold import permits to allow receipt of frozen or formalin-fixed tissue from NZ horses. Tissues can be processed and read at SVS Labs or processed and referred to MSU as formalin-fixed paraffin-embedded sections mounted onto glass slides.

References:

Vet Pathol 46:1281-1291 (2009)
J Vet Diagn Invest 20:572-579 (2008)
Vet Pathol 55:68-75 (2018)
J Equine Vet Sci. 25(2):52-61 (2005)