Effects of sample handling on plasma adrenocorticotrophic hormone (ACTH) stability in horses with normal and elevated ACTH concentrations.

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Presented at ANZCVS Science Week, Equine Chapter 5-7th July 2018.

Introduction
Basal ACTH concentration is recommended to diagnose pituitary pars intermedia dysfunction (PPID); however, previous studies have questioned accuracy when sample handling is less than ideal.

Objective: to describe the effects on ACTH concentration and subsequent diagnosis of PPID after exposure of samples to various temperatures, separation techniques and storage conditions.

Materials and Methods
Animals: 16 mature horses, 4 with ACTH concentrations considered negative for PPID (<35 pg/mL, group N), 6 with equivocal ACTH concentrations (35-100 pg/mL, group E) and 6 with ACTH concentrations positive for PPID (>100 pg/mL, group P).

Blood was collected in EDTA tubes and stored for 2, 4, 8, 12, 24 or 48 hours at -20°C, 4°C, 20°C, 30°C; and for 10, 20, 30, 60 and 120 minutes at 40°C or 70°C, as whole blood or after plasma separation by centrifugation or gravity. The effects of freeze-thaw cycles, storage in a silica-containing tube and hemolysis were also investigated. ACTH concentrations were analysed using a chemiluminescent assay and effects of different factors determined by linear mixed effects models.

Results
In group N, processing had a clinically limited effect; however, in groups E and P, temperature, time, separation methods, freeze-thaw cycles and hemolysis induced significant (P<0.05) changes in ACTH concentrations resulting in false negative and false positive results.

Relevance to Equine Practice
Further studies are required, but it appears that ACTH should be measured from non-haemolysed samples kept at 4°C, centrifuged within 2 hours and analyzed within 8 hours of collection to prevent misdiagnosis of PPID.