

Effects of Freezing on Measurement of Plasma Adrenocorticotrophic Hormone Concentrations in Horses¹

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Introduction

Measurement of adrenocorticotrophic hormone (ACTH) has become the most common diagnostic method utilized for the diagnosis of pituitary pars intermedia dysfunction (PPID) in horses. Adrenocorticotrophic hormone has reportedly been understood to be fragile in whole blood samples, and most affected by heat and time spent on cells. Consequently, the recommendation has been to refrigerate whole blood samples prior to centrifugation and separate plasma within 8 hours of collection. If plasma samples cannot be shipped to the respective laboratory the day of collection, plasma should be frozen until shipment. It is imperative for the veterinarian to understand if freezing plasma has any negative effects on the stability of resting ACTH concentration.

Study Objective

The objective of the study was to determine the stability of ACTH in plasma after freezing for different lengths of time prior to determination of resting ACTH concentration.

Materials and Methods

Plasma samples were obtained from 12 horses and resting ACTH levels were measured at Day 0 (baseline) and over time (variable by storage method). Plasma samples were stored in either -80°C, -20°C or samples placed between ice packs and stored at -20°C. Prior to determination of resting ACTH concentration, plasma samples were stored at -80°C for 3, 7, 30, 60 and 90 days, or stored at -20°C for 3, 7, 30 and 60 days, or stored between ice packs at -20°C for 3 and 7 days. Plasma samples were shipped to the Animal Health Diagnostic Center, Cornell University, Ithaca, NY for measurement of resting ACTH concentration.

Statistical Analysis

Within each storage method, ACTH levels over time were compared to baseline (non-frozen Day 0 plasma) using a paired t-test (i.e., each horse serving as its own control and $p < 0.05$ was considered statistically significant). Data were summarized with mean, standard error (SE) and percent change from baseline (%CFB).

Results

Mean ACTH level on Day 0 for plasma stored at -80°C was 392.2 pg/mL. By Day 90 the % CFB was -6.9% although not reaching statistical significance ($p=0.1042$), a trend toward significant degradation was observed. Mean ACTH level in plasma stored at -20°C declined 5.3% from baseline by Day 60 to 371.4 pg/mL, but was not statistically significant ($p=0.0590$). Mean ACTH level on Day 0 for plasma stored between ice packs at -20°C declined 1.1% by Day 7, but was not statistically significant ($p=0.4860$). None of the storage methods resulted in sample degradation that differed significantly from baseline. Trends toward degradation were observed on Day 90 in -80°C stored samples and on Day 60 at -20°C stored samples.

Table 1. ACTH levels (pg/mL) from samples stored at -80°F over time

	Day 0	Day 3	Day 7	Day 30	Day 60	Day 90
Mean	392.2	393.7	388.0	399.7	387.0	365.0
SE	106.19	103.21	103.03	105.8	104.54	100.86
%CFB	-	0.4%	-1.1%	1.9%	-1.3%	-6.9%
p-value	-	0.9003	0.5418	0.1335	0.2281	0.1042

SE= standard error, %CFB = percent change from baseline

Table 3. ACTH levels (pg/mL) from samples stored at -20°C between ice packs over time

	Day 0	Day 3	Day 7
Mean	392.2	391.0	387.9
SE	106.19	105.30	105.14
%CFB	-	0.3%	-1.1%
p-value	-	0.8080	0.4860

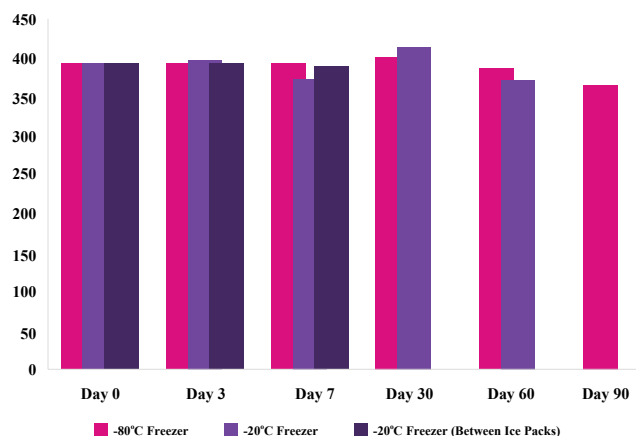
SE= standard error, %CFB = percent change from baseline

Table 2. ACTH levels (pg/mL) from samples stored at -20°C over time

	Day 0	Day 3	Day 7	Day 30	Day 60
Mean	392.2	395.8	373.2	414.4	371.4
SE	106.19	106.04	101.62	113.68	97.60
%CFB	-	0.9%	-4.8%	5.7%	-5.3%
p-value	-	0.6852	0.1667	0.1128	0.0590

SE= standard error, %CFB = percent change from baseline

ACTH Levels From Samples Stored Over Time



Discussion

None of the storage methods resulted in sample degradation that differed significantly from baseline. Trends toward degradation were observed on Day 90 in -80°C stored samples and on Day 60 at -20°C stored samples. No significant degradation was observed in ACTH from plasma samples stored between ice packs at -20°C for 7 days. Freezing plasma for short periods of time at -20°C or at -80°C for at least 90 days resulted in no statistically significant degradation of plasma ACTH compared to non-frozen plasma baseline samples. Additional studies should be conducted to further evaluate the effects of freezing on plasma ACTH concentrations in larger populations of horses.

Take Home Message

None of the storage methods resulted in sample degradation that differed significantly from baseline. Based on the results of this study, ACTH appears stable for at least 60 days when properly stored at -20°C. If freezer temperature could potentially fluctuate, it would be advisable to store plasma between ice-packs when storing plasma at -20°C.

Acknowledgments

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References

- Haffner J, Neal D, Grubbs S. Effects of freezing on measurement of plasma adrenocorticotrophic hormone concentrations in horses. 2018. Accepted for ACVIM Forum, Seattle, WA.