


BRIEF COMMUNICATION

Sample stability and heparin interference in ionized calcium and ionized magnesium measurements in horses using the Stat Profile Prime Plus co-oximetry electrolyte analyzer

Júlia Sanmartí¹ | José Angel Robles-Guirado² | Eduard Jose-Cunilleras¹ | Anna Bassols² 

¹Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain

²Departament de Bioquímica i Biologia Molecular, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain

Correspondence

Anna Bassols, Departament de Bioquímica i Biologia Molecular, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08913 Bellaterra, Barcelona, Spain.
Email: anna.bassols@uab.cat

Abstract

Background: The determination of iCa and iMg is important in veterinary medicine, but their immediate determination in whole blood is not always possible. Their stability in other sample types and the existence of interferences must be evaluated before its use.

Objectives: We aimed to analyze the effects of storage time on the stability of iCa, iMg, and other analytes in whole blood, plasma, and serum samples in horses and assess the interference of heparin in these measurements.

Methods: Whole blood, heparin-plasma, and serum samples from 10 horses were stored at 4°C and analyzed 1, 2, 3, 4, 5, 6, 7, 8, 24, 48, and 168 hours after sample collection using the Stat Profile Prime Plus Vet equipment (Nova Biomedical, Waltham, MA, USA). Results were analyzed by ANOVA or mixed-effect models.

Results: The concentration of iCa, iMg, total calcium (tCa), total magnesium (tMg), and the ratios iCa/tCa and iMg/tMg did not differ up to 168 hours when compared to the initial time. Total Ca, iMg, and tMg were not significantly different among sample types, but iCa concentrations were slightly but significantly lower in plasma. Freezing at -20°C did not affect iCa, iMg, tCa, and tMg. The pH increased in serum and plasma after 8 hours, and a mild negative correlation existed between plasma iCa concentration and pH. A negative correlation was observed also between the ratios iCa/tCa or iMg/tMg and pH in plasma and serum. A significant decrease in iCa and iMg was detected when comparing homemade syringes at high heparin concentration (~200–300 U heparin/mL) and commercial lithium-heparin tubes (20–30 U/mL).

Conclusions: Samples stored at 4°C can be used to determine iCa and iMg concentrations up to 7 days after collection. Other metabolites are stable for up to 8 hours; heparin interference should be taken into account if using homemade heparin syringes.

KEYWORDS

equine, ionized calcium, ionized magnesium, point-of-care, sample stability, total calcium, total magnesium

Júlia Sanmartí and José Angel Robles-Guirado contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Veterinary Clinical Pathology* published by Wiley Periodicals LLC on behalf of American Society for Veterinary Clinical Pathology.

1 | INTRODUCTION

Calcium (Ca) and magnesium (Mg) are the main divalent cations in body fluids, and their measurement is important in understanding certain body homeostasis imbalances. Both exist in plasma in three different forms: protein-bound, complexed with anions (eg, carbonate, lactate, and phosphates), and free (ionized) divalent cations (iCa and iMg). In horses, the proportion of these calcium fractions is 40%–45%, 5%–10%, and 50%–58% of total calcium, respectively,¹ while the magnesium fractions are 30%, 10%, and 60% of total magnesium.² The free forms of calcium and magnesium are the physiologically active components, responsible for triggering signaling transduction pathways,³ and thus their concentrations are more clinically relevant than their total concentrations, which are affected by total plasma protein concentration. Higher iCa and iMg concentrations are found in acidosis with lower levels in alkalosis, whereas total calcium and magnesium concentrations remain unchanged.^{2,4}

Electrolyte imbalances are common in critically ill humans and animals, and hypocalcemia constitutes a frequent finding in sepsis or endotoxemia.^{5,6} In horses, electrolyte disturbances play an important role in diseases such as colic, renal failure, or sepsis. Specifically, severe enterocolitis is frequently accompanied by hypocalcemia, which can manifest deleterious consequences and may result in death.⁶ Possible causes of hypocalcemia in ill horses include renal loss of calcium, sequestration of calcium in the gastrointestinal tract due to inflammation, and impairment in calcium mobilization.¹ Likewise, hypomagnesemia is possibly related to poor magnesium absorption in the inflamed bowel or excessive urinary losses.⁷ Serum iMg concentration is also decreased in horses with enterocolitis and hypocalcemia.⁶ The role of iMg in critically ill patients has received less attention than that of iCa, but both electrolytes are closely associated. The effects of iMg on mineral metabolism and its interaction with calcium homeostasis, and vice versa, may be important in chronically ill patients, and it has been suggested that iMg may serve as an iCa antagonist and prevent iCa entry into cells during sepsis or endotoxemia.² Calcium homeostasis is further influenced by plasma magnesium since secretion of the parathyroid hormone is inhibited by high concentrations of magnesium.^{8,9}

Monitoring and restoration of calcium and magnesium homeostasis is an important aspect of managing severely ill horses. Thus, measuring the free forms of calcium and magnesium is important in clinics. The clinical meaning of iCa is widely acknowledged in human medicine, whereas the role of iMg is recognized especially in critically ill patients.^{10,11} In veterinary medicine, these studies are less common and mostly analyze total calcium and magnesium, although some reports claim the usefulness of measuring iMg.¹² In addition, it is important to note that veterinary samples are not always immediately processed, so the knowledge about the stability of these analytes in collected samples is of utmost importance.

The ionized forms of calcium and magnesium are usually determined using ion-selective electrodes (ISE) analyzers that selectively

measure the activities of specific ions. Although several companies have developed point-of-care equipment that allows the measurement of iCa and other analytes in clinical settings, there is only one that allows the determination of iMg in whole blood, plasma, and serum that is marketed for veterinary use (Stat Profile Prime Plus Vet, Nova Biomedical, Waltham, MA, USA). The measurement of ions (calcium, magnesium, sodium, potassium, chloride) using this equipment is based on ISE.

Considering this, the objectives of our study were to evaluate the effect of storage time on the measurement of iCa, iMg, and other analytes after sample collection, and the potential heparin interference in heparinized syringe samples from equine samples, analyzed by the Stat Profile Prime Plus Vet analyzer (Nova Biomedical).

2 | MATERIALS AND METHODS

In the first experiment, blood was obtained from 10 horses using a previously placed indwelling intravenous catheter. Horses were client-owned animals in the veterinary teaching referral hospital for medical reasons, and the study did not require ethical permission since it was categorized as a nonexperimental clinical veterinary practice by the institutional Ethics Committee on Animal and Human Experimentation (CEEAH, UAB). The horses were hospitalized with various medical conditions: five colic, one chronic weight loss, one distal limb laceration, one septic arthritis, one recurrent uveitis, and one breeding mare accompanying a septic foal. There were five geldings and five mares, and five cross-bred, two Andalusians, and one Arabian, Friesian, and pony each. The horses ranged in age 9.3 ± 5.2 years (average \pm standard deviation [SD]).

The sample size was calculated assuming a significance level (alpha or *P*-value) set at <0.05 , the statistical power at $>80\%$, a standard deviation of 0.05, and an expected effect size of 0.1. The power analysis gave an adequate sample size of $n = 10$.

Blood was aliquoted in 1 mL evacuated tubes containing lithium-heparin (Aquisel blood tubes, Barcelona, Spain) for analyte measurements in whole blood. For analyte measurements in plasma, 2 mL lithium-heparin tubes were used (Aquisel blood tubes) and after collection, blood samples were centrifuged at 1500 *g* for 5 min at room temperature, and plasma was transferred to Eppendorf tubes. To obtain serum, blood was evacuated in 5 mL tubes without additives (Becton Dickinson, Madrid, Spain) and was left to coagulate for 30 minutes. Once the clot was formed, samples were centrifuged at 1500 *g* for 10 min at room temperature, and serum was collected in Eppendorf tubes. All samples were stored in closed tubes at 4°C until use, minimizing oxygen exposure as much as possible. Just before measurements were made, tubes were inverted several times (blood) or were vortexed (plasma and serum) to obtain a homogeneous solution. Sample collection (time 0 of the measures) was performed at 9 am, and the overall procedure ended at 9:30–9:40 am. Determinations were performed every hour during the first 8 hours and after 24, 48,

and 168 hours of storage. Additionally, three aliquots of plasma obtained at 0 hours were stored frozen at -20°C for ~168 h and analyzed like the refrigerated samples. Care was taken regarding the time of collection since the circadian rhythm has been acknowledged in humans for both calcium⁷ and magnesium.³ Samples with hemolysis were discarded since red blood cells contain approximately three times the concentration of Mg in serum.²

In the second experiment, the potential interference by heparin was evaluated by analyzing iCa and iMg in whole blood samples with decreasing dilutions of sodium heparin. To simulate a widely used procedure in equine clinics, homemade heparin syringes were used to collect blood for subsequent determination of blood gases and electrolytes. The preparation consists of the partial filling of a 1 or 2 mL syringe with commercial Na heparin (5000 IU/mL) (Laboratorios Farmacéuticos Rovi, Madrid, Spain) followed by complete injection back into the vial, leaving a residual volume within the needle hub. The residual heparin within the tip of the syringe and needle hub is considered enough to avoid blood coagulation. In preliminary studies, using a precision scale and weighing before and after prefilling, the volume of residual fluid in a 2 mL syringe was estimated as 127 μL . The final heparin concentration once the syringe is filled with 2 mL of blood was calculated as 297.6 IU/mL. Serial dilutions (1:10, 1:20, and 1:30) of commercial Na heparin (5000 IU/mL) in 0.9% NaCl were used to similarly prefill syringes to obtain final concentrations of 29.7, 14.9, and 9.9 IU/mL, respectively, once filled with 2 mL of blood. In addition, for comparison purposes, blood samples were collected in parallel with Li-heparin vacutainer tubes.

Sample analysis was carried out using the Stat Profile Prime Plus Vet Analyzer (Nova Biomedical), following the instructions of the manufacturer, which is based on ISE technology, as stated above. tCa and tMg were measured by colorimetric methods in the Beckman Coulter AU400 analyzer (Hamburg, Germany) with the Arsenazo III method (OSR 60117) and the xylidyl blue method (OSR 6189), respectively.

Statistical analysis was performed using GraphPad Prism 7 software. For all determinations, Rout tests using $Q = 1\%$ were performed to identify possible outliers. Data distribution was evaluated using Shapiro-Wilk tests. To evaluate differences over time, comparing each type of sample, iCa, iMg, tMg, tCa, pH, Na, K, and Cl were analyzed using Two-Way ANOVA tests. Factors "time," "type of sample," and their interaction "time-type of sample" were evaluated. Multiple comparisons were carried out using Dunnett tests to identify simple and main effects. Heparin interference experiments were analyzed using Brown-Forsythe and Welch ANOVA tests and Dunnett T3 tests for multiple comparisons. The same analysis was carried out to evaluate possible differences over time in blood analysis measurements for tBil, Hct, and O_2HB . Kruskal-Wallis tests, using Dunn's tests for multiple comparisons, were performed to evaluate pCO_2 , pO_2 , SO_2 , tHb, and COHb variations. Pearson correlations were performed to evaluate possible associations between pH and iCa, tCa, iMg, tMg, and iCa/tCa, or iMg/tMg using a 95% percent of confidence. Descriptive data are presented as the mean \pm SD.

P -values <0.05 were considered significant, presented as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

3 | RESULTS

3.1 | Effect of storage time on ionized and total Ca and Mg in different sample types

Whole blood, plasma, and serum iCa and iMg concentrations did not change significantly over 168 hours (7 days) when kept refrigerated at 4°C (Figure 1A,C). Similar results were obtained for tCa and tMg in plasma and serum over 7 days (Figure 1B,D). The ratios iCa/tCa and iMg/tMg (Figure 1E,F) did not change either, except for serum iCa/tCa, which decreased significantly after 168 hours compared with the time of collection ($t = 0$; $P < 0.05$). In addition, the storage of plasma samples for 7 days (168 hours) at -20°C did not result in significant differences in iCa (1.66 ± 0.07 and 1.61 ± 0.05 mmol/L at 0 and 7 days) or iMg concentrations (0.52 ± 0.06 and 0.49 ± 0.06 mmol/L at 0 hours and 7 days, respectively; mean \pm SD). Variability between samples from different animals was higher in serum than in plasma or blood.

Additionally, the concentrations of iCa, iMg, tCa, and tMg in the three different sample types were compared (Table 1). The concentrations of tCa, iMg, tMg, and the ratios iCa/tCa and iMg/tMg were similar in all samples, showing low variability after sample manipulation. The concentration of iCa was lower in plasma than in serum ($P < 0.01$).

Although precautions were taken to keep the samples in anaerobic conditions, pH was higher in serum and plasma than in blood (Table 1). An increase in pH after 8 hours was observed in serum and plasma ($P < 0.05$), and a decrease in whole blood at 168 hours ($P < 0.05$) (Figure 2). Figure 2 shows no apparent relationship between pH and iCa or iMg throughout the study since both ions remained stable in serum and plasma. Nevertheless, the correlation analysis showed a negative correlation between iCa vs pH in plasma (Figure 3A, $R = -0.3554$, $P < 0.001$), whereas it did not reach significance in serum (Figure 3B). There was no correlation between iMg vs pH in serum or plasma in this pH range (Figure 3C,D). However, a clear negative correlation with pH was observed with the ratios iCa/tCa and iMg/tMg in both plasma and serum ($r = -[0.363-0.674]$, $P < 0.001$) (Figure 3E-H).

3.2 | Heparin interference on the determination of iCa and iMg

Heparin interference was evaluated in blood and plasma, comparing homemade Na-heparin syringes at different dilutions versus Li-heparin vacutainer tubes. In blood, interference by heparin was detected when comparing commercial Li-heparin tubes (20–30 IU Li heparin/mL) with homemade syringes with high concentration (~200–300 IU Na heparin/mL), whereas no effect was observed

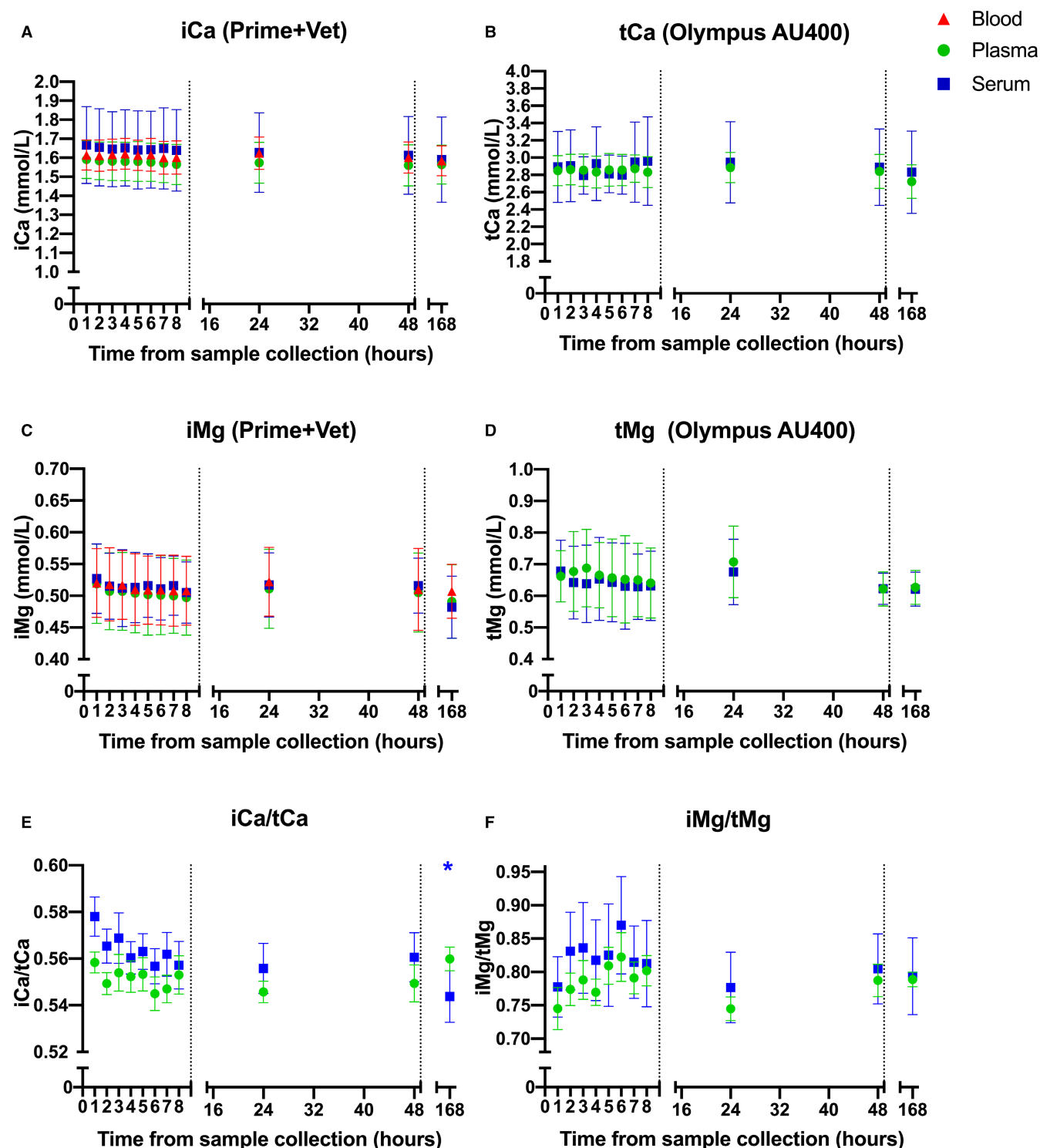


FIGURE 1 Effect of storage at 4°C up to 168 hours on measured concentrations of (A) ionized Ca (iCa), (B) total Ca (tCa), (C) ionized Mg (iMg), (D) total Mg (tMg), (E) ratio iCa/tCa, and (F) ratio iMg/tMg of samples obtained from 10 horses in serum (blue), heparin-plasma (green), and whole blood (red) hours and using the Prime Vet Plus (Nova Biomedical) analyzer (iCa and iMg) and the AU400 analyzer (tCa and tMg). Data are presented as mean and standard deviation. For each type of sample, Rout tests with $Q = 1\%$ were carried out to identify outliers. Data distribution was evaluated using Shapiro–Wilk tests. Two-way ANOVA tests were performed to compare variations within each sample and between sample types. Factor “time” and “type of sample” and their interaction were evaluated. For multiple comparisons, Dunnett tests were performed using a sample at time 1 as the control. Serum iCa/tCa was significantly different at 168 hours of storing versus time 1 (* $P < 0.05$).

TABLE 1 Concentrations of ionized and total calcium and magnesium and their ratios in whole blood, plasma, and serum samples.

Analyte	Type of sample	Mean \pm SD (mmol/L)	Minimum (mmol/L)	Maximum (mmol/L)	Number of samples (n)
iCa	Blood (Prime+Vet)	1.61 \pm 0.08 ^{a,b}	1.40	1.76	110
	Plasma (Prime+Vet)	1.58 \pm 0.10 ^b	1.34	1.74	110
	Serum (Prime+Vet)	1.64 \pm 0.20 ^a	1.35	2.18	109
tCa	Plasma (Olympus)	2.85 \pm 0.17	2.35	3.11	95
	Serum (Olympus)	2.89 \pm 0.39	2.48	4.32	95
iMg	Blood (Prime+Vet)	0.51 \pm 0.05	0.41	0.61	110
	Plasma (Prime+Vet)	0.50 \pm 0.06	0.38	0.60	110
	Serum (Prime+Vet)	0.51 \pm 0.05	0.41	0.60	110
tMg	Plasma (Olympus)	0.67 \pm 0.12	0.49	1.26	95
	Serum (Olympus)	0.64 \pm 0.10	0.45	0.93	95
iCa/tCa	Plasma (Prime+Vet)	0.55 \pm 0.02	0.51	0.60	95
	Serum (Olympus)	0.56 \pm 0.03	0.50	0.63	95
iMg/tMg	Plasma (Prime+Vet)	0.78 \pm 0.07	0.47	0.93	95
	Serum (Olympus)	0.81 \pm 0.11	0.54	0.98	95
pH	Blood (Prime+Vet)	7.39 \pm 0.05 ^d	7.18	7.51	106
	Plasma (Prime+Vet)	7.66 \pm 0.10 ^c	7.50	7.99	104
	Serum (Prime+Vet)	7.67 \pm 0.12 ^c	7.38	7.98	92

Note: Data are presented in mmol/L by mean \pm standard deviation (SD). Minimum and maximum values, the number of samples and P-values comparing each sample type for each analyte were also defined. Superindexes: a \neq b: $P < 0.01$; c \neq d: $P < 0.01$.

Abbreviations: iCa, ionized Ca; iMg, ionized Mg; tCa, total Ca; tMg, total Mg.

with syringes with low heparin concentrations (9.9 up to 30 IU Na heparin/mL). Concentrations of iCa and iMg decreased by ~20% in samples using undiluted heparin compared with commercial tubes ($P < 0.01$ for iCa and iMg) (Figure 4).

3.3 | Effect of storage time on other electrolytes and biochemical parameters

The Stat Profile Prime Plus Vet equipment also allows the quantification of other parameters in blood, plasma, or serum samples, whose stability over time was also examined.

Monovalent ions (Na, Cl, and K) showed good stability up to 48 hours with low variability, except for K in whole blood, as expected (Figure S1A–C). The concentrations of Na and Cl were significantly higher in serum than in plasma or blood (136.10 \pm 1.96, 132.50 \pm 2.78, and 133.00 \pm 2.05 mmol/L for Na and 106.60 \pm 2.04, 104.30 \pm 2.20, and 102.90 \pm 1.92 mmol/L for Cl, respectively, being $P < 0.001$ for all comparisons described). The concentration of K was stable in serum and plasma over time (4.02 \pm 0.39 for serum and 3.82 \pm 0.12 mmol/L for plasma) and progressively increased in blood samples from 3.82 \pm 0.19, 4.23 \pm 0.33, 4.87 \pm 0.58, 5.39 \pm 0.79, and 7.81 \pm 1.50 mmol/L by 0, 8, 24, 48 and 168 hours, respectively (time 0 vs 24, 48, and 168 hours; $P < 0.001$).

Additionally, the stability of glucose and lactate in the three sample types was evaluated (Figure S2A,B, respectively). In whole blood, a decrease in glucose and an increase in lactate was observed, which

was significant at 168 h ($P < 0.05$). Serum and plasma glucose and lactate did not change significantly.

Finally, blood-gas analysis measurement in samples collected with vacutainer tubes was performed for exploratory purposes. High stability was observed for up to 48 hours in partial pressure carbon dioxide (pCO₂), partial pressure of oxygen (pO₂), total hemoglobin (tHb), oxyhemoglobin (O₂Hb), carboxyhemoglobin (COHb), oxygen saturation (sO₂), total bilirubin (tBil), and hematocrit (Hct) (Figure S3).

4 | DISCUSSION

Determining the ionized forms iCa and iMg, in addition to tCa and tMg, is increasingly important since these analytes are physiologically important and do not always correlate with the total concentration. Both iMg and iCa are likely physiologically interdependent, and in equine patients, both hypocalcemia and hypomagnesemia have been associated with decreased survival in critical illness.^{5,6,13,14} In veterinary medicine, the determination of iCa and iMg in whole blood is not always possible and, in addition, veterinary samples are not always immediately processed, so the knowledge about the stability of these analytes and a comparison of these analytes between different sample types are important issues. The results of the present study have shown that iCa, iMg, and the total forms (tCa, tMg) and the ratios iCa/tCa and iMg/tMg are stable up to 168 hours. Furthermore, concentrations are similar among sample

types, except for iCa, which was slightly lower in plasma. Freezing at -20°C does not affect these parameters. Electrolytes (Na, K), glucose, lactate, and blood gases are stable in plasma and serum. These results facilitate the use of these analytes in the clinical diagnosis of equine diseases. On the other hand, caution must be exercised when using homemade heparin syringes, due to the potential interference of heparin.

Compared with the literature, values for iMg and iCa concentrations are similar to those described in adult horses.^{6,15,16} Berlin et al.¹⁵ determined the concentrations of iCa and iMg in whole blood, serum, and plasma, and tCa and tMg in the serum of healthy horses. These authors reported concentrations of iMg = 0.48–0.52 mmol/L depending on the sample type, iCa = 1.61–1.69 mmol/L, and ratios of iMg/tMg = $0.64 \pm 0.07\%$

and iCa/tCa = $0.52 \pm 0.02\%$ that were similar to our results. In the current work, similar concentrations were found for the three sample types (blood, plasma, and serum), except iCa concentration in plasma, which was slightly but significantly lower, similar to the previous authors. Other authors have found lower iMg concentrations in heparinized plasma compared with serum.¹⁶ The ratio of the ionized vs total form was 0.55 for calcium, similar to values previously described,^{1,2,15} and 0.80 for magnesium, slightly higher than with other studies.¹⁵

The use of serum and plasma instead of whole blood and the effect of the storage time on iCa and iMg are relevant questions, especially when dealing with horses since a clinical pathology laboratory is not always immediately available. The results presented here indicate that stability is high for the ionized forms of both calcium and magnesium. In samples kept at 4°C for 168 hours, a slight nonsignificant decrease in iMg (2.5%, 5.4%, and 8.5%) and iCa (1.9%, 1.7%, and 4.6%) concentrations, respectively, were observed. This is similar to results described by Lopez et al. in a micropartition study, where the authors found that refrigeration (4°C) did not affect iCa values, whereas iMg declined by 8% after 120 hours.¹⁷ For the total forms, there is a nonsignificant decrease of 3.9 and 3.4% at 168 h for tCa in plasma and serum, respectively, and no change for tMg.

It is well-known that iCa and iMg concentrations are influenced by the acid-base status of the patient. Acidosis increases iCa and iMg concentrations, whereas alkalosis reduces them.^{1,2} This property is clinically important when treating animals with respiratory or metabolic alkalosis, often observed after prolonged strenuous endurance exercise. iCa and iMg concentrations are also altered with changes in the pH of the collected samples, which can happen during processing. In our case, although preventive measures were taken to minimize the aerobic exposure of plasma in serum samples, an increased pH in serum and plasma was observed due to the loss of CO_2 over time, although the increase in pH was only significant after 8 hours. Whole blood is better able to maintain the pH due to its physiologic buffering systems. Our results show that, despite the steadily increased pH in serum and plasma, the influence on concentrations of the ionized forms of calcium and magnesium is not very relevant in this pH range ($\text{pH} = 7.6\text{--}7.8$). However, a close look at the correlation between iCa and pH revealed a significant negative correlation in plasma that was mild and nonsignificant in serum. On the other hand, there was no apparent correlation between iMg and pH. The negative correlation is explained because iCa and iMg bind to anionic protein binding sites and this binding increases at high pH, causing lower values for both ions. When looking at the iCa/tCa and iMg/tMg ratios, the negative correlation with pH is stronger, further supporting

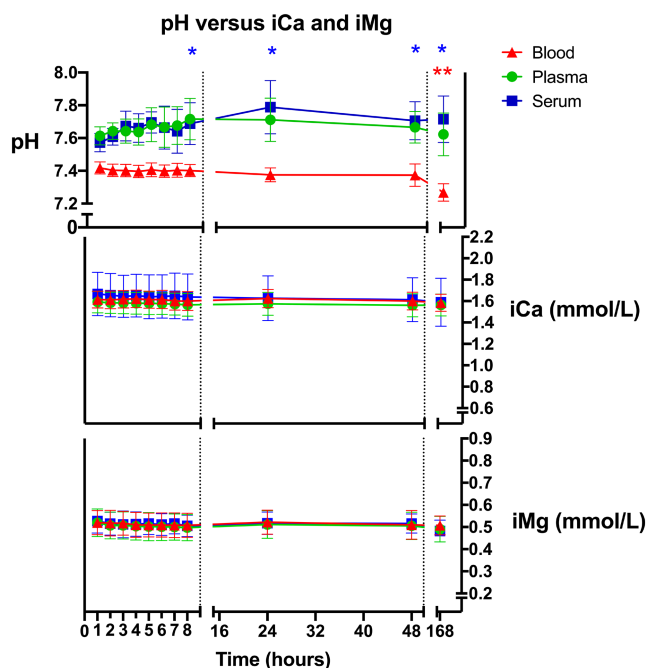
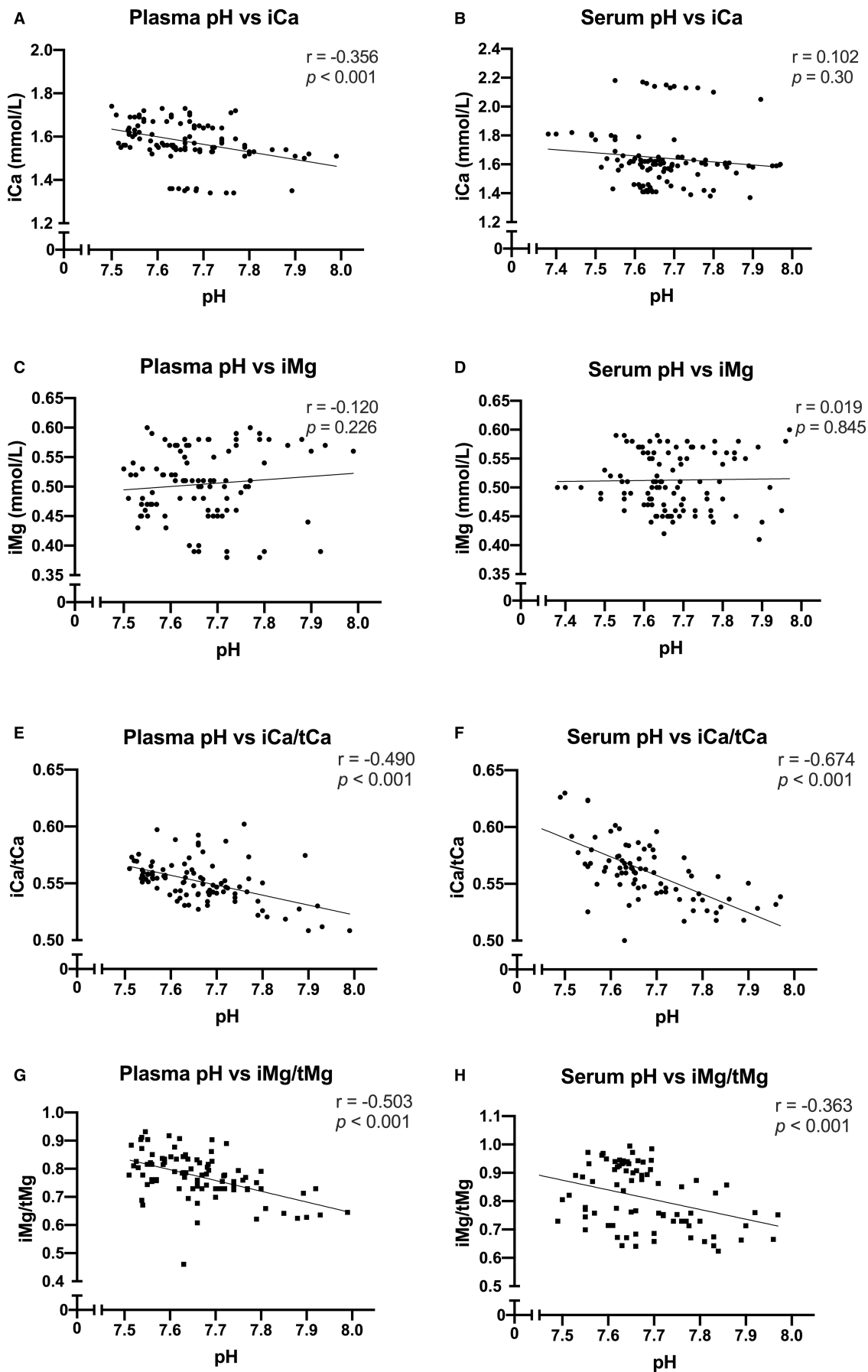


FIGURE 2 Effect of storage at 4°C up to 168 hours on pH and its relation with ionized calcium (iCa) and ionized magnesium (iMg) in whole blood (red), plasma (blue), and serum (green). Samples of 10 horses were measured with Prime Vet Plus (Nova Biomedical) analyzer. Data are presented as mean and standard deviation. For each type of sample, Rout tests with $Q = 1\%$ were carried out to identify outliers. Data distribution was evaluated using Shapiro–Wilk tests. Two-way ANOVA tests were performed to compare variations within each sample and between sample types. Factor “time” and “type of sample” and their interaction were evaluated. For multiple comparisons, Dunnett tests were performed using a sample at time 1 as the control. Serum pH increased significantly after 8 hours of sample collection ($P < 0.05$, blue asterisks). Blood pH decreased significantly after 168 hours ($P < 0.01$, red asterisk).

FIGURE 3 Pearson correlations of ionized Ccalcium (iCa) and ionized magmnesium (iMg) concentrations, and the iCa/tCa and iMg/tMg ratios versus pH in plasma and serum. Correlations were calculated using a 95% confidence interval. Pearson coefficient (r) and P -values are described in each graph. (A) Correlation of plasma pH versus iCa; (B) Correlation of serum pH versus iCa; (C) Correlation of plasma pH versus iMg; (D) Correlation of serum pH versus iMg; (E) Correlation of plasma pH versus iCa/tCa ratio; (F) Correlation of serum pH versus iCa/tCa ratio; (G) Correlation of plasma pH versus iMg/tMg ratio; (H) Correlation of serum pH versus iMg/tMg ratio.



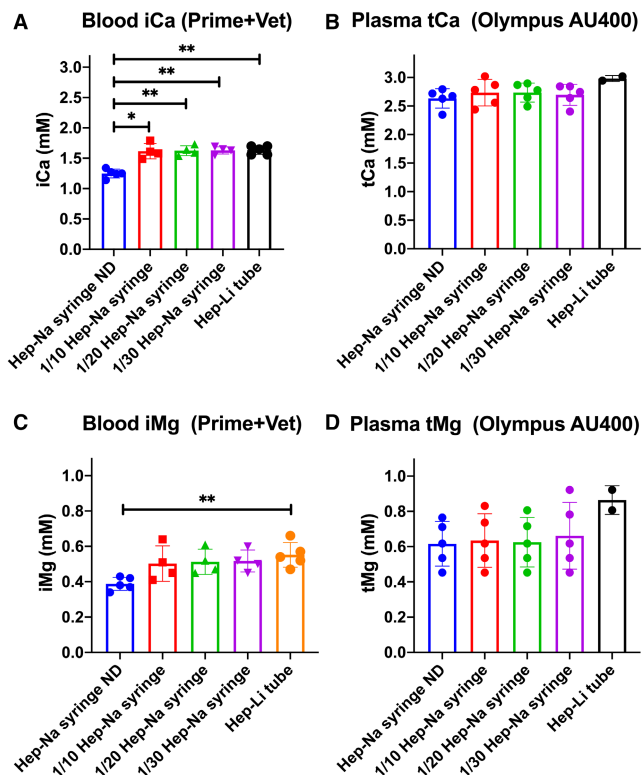


FIGURE 4 Effect of heparin concentration in plasma on measured concentrations of (A) ionized Ca (iCa), (B) total Ca (tCa), (C) ionized Mg (iMg), and (D) total Mg (tMg) in equine heparinized plasma samples obtained from 5 different horses, using the Prime Plus Vet (Nova Biomedical) analyzer (iCa and iMg) and the AU400 analyzer (tCa and tMg). Blood samples were collected in commercial Li-heparin tubes (Hep-Li; heparin concentration 20 UI/mL), and heparin-flushed syringes at different Na-heparin concentrations (blue, Na-heparin syringe nondiluted 297 IU/mL; red, Na heparin 29.7 IU/mL; green, Na heparin 14.9 IU/mL; purple, Na heparin 9.9 IU/mL). Samples were analyzed immediately after collection. Rout tests with $Q = 1\%$ were carried out to identify outliers. Data distribution was analyzed using Shapiro–Wilk tests. Brown–Forsythe and Welch ANOVA tests and Dunnett T3 tests were performed to evaluate possible differences between groups and to make multiple comparisons, respectively. * $P < 0.05$; ** $P < 0.01$.

this mechanism. These results reinforce the data reported in horses on the influence of pH, which is quite robust for iCa¹ but scarcer for iMg.²

Our results show that heparin interferes with the determination of iCa and iMg at high concentrations (~200–300 IU/mL) causing decreased values of both parameters of around 20%. Although this has been alerted previously,¹⁸ and commercial heparin tubes are readily available, homemade syringes are commonly used in some settings. Thus, caution is advised when collecting samples in homemade heparin syringes, which always should use a diluted concentration of the anticoagulant. This interference is similar to that described in humans, where a concentration of 100–150 IU/mL heparin may cause up to an 11–28% decrease in ionized calcium concentration^{19,20} and 150 U/mL heparin a 13% decrease in ionized magnesium.²⁰

Finally, regarding the other biochemical parameters, the concentrations of electrolytes (Na, K, Cl) were similar to that described previously.^{21,22} Our results confirm that the three electrolytes were stable in plasma and serum when cells have been removed from the sample, and there was no hemolysis. However, the levels of K in whole blood markedly increased due to hemolysis after collection since K is mostly an intracellular cation.

Glucose and lactate were also stable in plasma and serum, and as expected, glucose decreased in whole blood throughout the time since it was converted to lactate mainly by the glycolytic pathway in erythrocytes (which lack mitochondria and are very dependent on glucose as a nutrient). As a consequence, lactate increased in whole blood with time.

Regarding blood gases, pO₂, pCO₂ and total and oxygenated hemoglobin had good stability throughout the time. It has to be remarked that samples were kept in the cold and tightly closed and consequently in anaerobic conditions.

5 | CONCLUSIONS

Samples stored at 4°C can be used to determine iCa, iMg, and other parameters up to 8 hours after collection. Heparin interference gives lower values for iCa and iMg and should be considered if using homemade heparin syringes instead of commercial heparinized syringes or evacuated tubes.

ACKNOWLEDGMENTS

The authors thank Nova Biomedical Corporation for providing the Stat Profile Prime Plus Vet analyzer and associated reagents used for this study.

DISCLOSURE

The authors have indicated that they have no affiliations or final involvement with any organization or entity with a financial interest in, or in financial competition with the subject matter discussed in this article.

ORCID

Anna Bassols  <https://orcid.org/0000-0003-4213-2274>

REFERENCES

- Toribio RE. Disorders of calcium and phosphate metabolism in horses. *Vet Clin North Am - Equine Pract.* 2011;27:129-147. doi:10.1016/j.cveq.2010.12.010
- Stewart AJ. Magnesium disorders in horses. *Vet Clin North Am - Equine Pract.* 2011;27:149-163. doi:10.1016/j.cveq.2010.12.009
- Newhouse I, Johnson K, Montepare W, McAuliffe J. Variability within individuals of plasma ionic magnesium concentrations. *BMC Physiol.* 2002;2:1-7. doi:10.1186/1472-6793-2-6
- Constable P, Trefz FM, Stämpfli H. Effects of pH and the plasma or serum concentrations of total calcium, chloride, magnesium, l-lactate, and albumin on the plasma ionized calcium concentration in calves. *J Vet Intern Med.* 2019;33:1822-1832. doi:10.1111/jvim.15509

5. Hurcombe SDA, Toribio RE, Slovis NM, et al. Calcium regulating hormones and serum calcium and magnesium concentrations in septic and critically ill foals and their association with survival. *J Vet Intern Med*. 2009;23:335-343. doi:10.1111/j.1939-1676.2009.0275.x
6. Toribio RE, Kohn CW, Chew DJ, Sams RA, Rosol TJ. Comparison of serum parathyroid hormone and ionized calcium and magnesium concentrations and fractional urinary clearance of calcium and phosphorus in healthy horses and horses with enterocolitis. *Am J Vet Res*. 2001;62:938-947. doi:10.2460/ajvr.2001.62.938
7. de Baaij JHF, Hoenderop JGJ, Bindels RJM. Magnesium in man: implications for health and disease. *Physiol Rev*. 2015;95:1-46. doi:10.1152/PHYSREV.00012.2014
8. Rodríguez-Ortiz ME, Canalejo A, Herencia C, et al. Magnesium modulates parathyroid hormone secretion and upregulates parathyroid receptor expression at moderately low calcium concentration. *Nephrol Dial Transplant*. 2014;29:282-289. doi:10.1093/NDT/GFT400
9. Brown EM, Macleod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*. 2001;81:239-297. doi:10.1152/PHYSREV.2001.81.1.239
10. Endres D, Rude R. Mineral and bone metabolism. In: Burtis C, Ashwood E, Bruns D, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 4th ed. Elsevier Saunders; 2006:1891-1965.
11. Velissaris D, Karamouzos V, Pierrakos C, Aretha D, Karanikolas M. Hypomagnesemia in critically ill sepsis patients. *J Clin Med Res*. 2015;7:911-918. doi:10.14740/JOCMR2351W
12. Humphrey S, Kirby R, Rudloff E. Magnesium physiology and clinical therapy in veterinary critical care. *J Vet Emerg Crit Care (San Antonio)*. 2015;25:210-225. doi:10.1111/VEC.12253
13. Garcia-Lopez JM, Provost PJ, Rush JE, Zicker SC, Burmaster H, Freeman LM. Prevalence and prognostic importance of hypomagnesemia and hypocalcemia in horses that have colic surgery. *Am J Vet Res*. 2001;62:7-12. doi:10.2460/ajvr.2001.62.7
14. Sanmartí J, Armengou L, Troya-Portillo L, et al. Plasma-ionized magnesium in hospitalized horses with gastrointestinal disorders and systemic inflammatory response syndrome. *Animals*. 2022;12:1479. doi:10.3390/ani12121479
15. Berlin D, Aroch I. Concentrations of ionized and total magnesium and calcium in healthy horses: effects of age, pregnancy, lactation, pH and sample type. *Vet J*. 2009;181:305-311. doi:10.1016/j.tvjl.2008.03.014
16. Schumacher SA, Yardley J, Bertone AL. Ionized magnesium and calcium concentration and their ratio in equine plasma samples as determined by a regulatory laboratory compared to a clinical reference laboratory. *Drug Test Anal*. 2019;11:455-460. doi:10.1002/DTA.2509
17. Lopez I, Estepa JC, Mendoza FJ, Mayer-Valor R, Aguilera-Tejero E. Fractionation of calcium and magnesium in equine serum. *Am J Vet Res*. 2006;67:463-466. doi:10.2460/ajvr.67.3.463
18. Tappin S, Rizzo F, Dodkin S, Papasouliotis K, Tasker S, Murphy K. Measurement of ionized calcium in canine blood samples collected in prefilled and self-filled heparinized syringes using the i-STAT point-of-care analyzer. *Vet Clin Pathol*. 2008;37:66-72. doi:10.1111/j.1939-165X.2008.00001.x
19. Sachs C, Rabouine P, Chaneac M, Kindermans C, Dechaux M, Falch-Christiansen T. Preanalytical errors in ionized calcium measurements induced by the use of liquid heparin. *Ann Clin Biochem*. 1991;28:167-173. doi:10.1177/000456329102800208
20. Ritter C, Ghahramani M, Marsoner HJ. More on the measurement of ionized magnesium in whole blood. *Scand J Clin Lab Invest Suppl*. 1996;56:275-280. doi:10.3109/00365519609088647
21. Rose RJ. Electrolytes: clinical applications. *Vet Clin North Am Equine Pract*. 1990;6:281-294. doi:10.1016/S0749-0739(17)30542-4
22. Tasker JB. Fluid and electrolyte studies in the horse. 1. Blood values in 100 normal horses. *Cornell Vet*. 1966;56:67-76.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sanmartí J, Robles-Guirado JA, Jose-Cunilleras E, Bassols A. Sample stability and heparin interference in ionized calcium and ionized magnesium measurements in horses using the Stat Profile Prime Plus co-oximetry electrolyte analyzer. *Vet Clin Pathol*. 2023;00:1-9. doi:10.1111/vcp.13200