

Assessment of the effect of dilution of blood samples with sodium heparin on blood gas, electrolyte, and lactate measurements in dogs

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Objective—To evaluate the effect of dilution of blood samples with sodium heparin on blood gas, electrolyte, and lactate measurements in dogs.

Sample Population—Venous blood samples collected from 6 adult dogs of various breeds.

Procedure—Syringes were prepared with anticoagulant via 1 of 4 techniques, and the residual volume of liquid heparin in each type of prepared syringe was determined. Blood gas values and other selected clinicopathologic variables were measured in whole blood samples after collection (baseline) and after aliquots of the samples were diluted with heparin via 1 of the 4 manual syringe techniques. By use of a tonometer, whole blood samples were adjusted to 1 of 3 oxygen concentrations (40, 100, or 600 mm Hg) and the PO_2 values were measured at baseline and subsequent to the 4 heparin dilutions.

Results—The 4 syringe techniques resulted in 3.9%, 9.4%, 18.8%, and 34.1% dilutions of a 1-mL blood sample. Compared with baseline values, dilution of blood samples with liquid heparin significantly changed the measured values of PCO_2 , PO_2 , and base deficit and concentrations of electrolytes and lactate. Of the variables assessed, measurement of ionized calcium concentration in blood was most affected by heparin dilution.

Conclusions and Clinical Relevance—These findings in dogs indicate that dilution of blood samples with heparin can be a source of preanalytical error in blood gas, electrolyte, and lactate measurements. Limiting dilution of blood samples with heparin to < 4% by volume via an evacuation technique of syringe heparinization is recommended. (*Am J Vet Res* 2005;66:656–660)

Data obtained via blood gas and electrolyte analyses are important for informed decision making regarding the management of critically ill patients. Modern laboratory equipment for such analyses requires only very small blood samples, often < 1 mL. For repeated analyses, small blood samples are desirable to minimize blood loss from the patient. Liquid sodium heparin is a commonly used anticoagulant. The mixing of liquid heparin and the blood sample introduces 2 possible sources of preanalytical error: a simple dilution effect and an ion-binding effect on divalent cations such as calcium.¹

Among veterinary medical personnel attempting to obtain a blood sample from a patient, it is not an

uncommon practice to fill the dead space of a 3-mL syringe with heparin and then, because of difficulties during sample collection, collect somewhat < 1 mL of blood into it for the clinicopathologic analyses. As a consequence of this, the magnitude of heparin dilution of the blood sample can be large as well as variable. These variations in the magnitude of heparin dilution cause variations in the measured clinicopathologic variables. These measured variables are then compared with previously measured values to monitor change in the status of the patient; therefore, erroneous measurements serve only to confuse patient assessment. The purpose of the study reported here was to evaluate the effect of dilution of blood samples with sodium heparin on blood gas, electrolyte, and lactate measurements in dogs. We assessed this effect in blood samples collected by use of syringes that had been prepared via 4 techniques and contained different residual volumes of heparin.

Materials and Methods

Six client-owned dogs that were deemed healthy on the basis of history and findings of physical examinations were included in the study subsequent to obtaining informed consent. This study complied with the University of California, Davis, Institutional Animal Care and Use Committee guidelines for use of nonuniversity-owned animals. Ten milliliters of venous blood was collected from each of the 6 dogs into 12-mL syringes, the dead space of which had been pre-filled with 0.1 mL of liquid sodium heparin.^a Baseline values of pH and PCO_2 and concentrations of sodium, potassium, chloride, ionized calcium, and lactate were measured; bicarbonate concentration and standard base deficit were calculated in duplicate by use of a blood gas and electrolyte analyzer.^b Blood samples were then transferred into 3-mL syringes (to the 1-mL mark on the syringe) that had been preloaded with 4 different volumes of liquid sodium heparin (1,000 U/mL) via 4 preparation techniques. The blood gas and electrolyte analyses were then repeated on the diluted blood samples as quickly as possible; all samples were immersed in ice water prior to analysis. A maximum interval of 30 minutes elapsed from the time of blood collection to the completion of sample analysis, and all measurements were performed in duplicate.

All measurements were performed by use of a 3-mL syringe with a 22-gauge needle attached, and this will be referred to as the syringe for the rest of this report. For the dilution assessments, 0.5 mL of heparin was aspirated into each of the syringes. For each syringe, the plunger was drawn back to the 3-mL mark to allow coating of the inner surface of the syringe with heparin. The preparation of syringes was continued such that each syringe contained 1 of 4 volumes of heparin. For some syringes, all of the air and heparin was expelled; 3 mL of air was then drawn into each syringe and again forcibly expelled from it. This was repeated 3 times in an attempt to remove as much heparin from these syringes as

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possible; these were designated as evacuated syringes. For other syringes, all of the air and heparin was expelled from each syringe, so that the dead space of the syringe and the hub of the 22-gauge needle remained filled with heparin; these were designated as DS syringes. In a third group of syringes, all of the air and much of the heparin were expelled from the syringe (the plunger was depressed down to the 0.1-mL mark on the syringe); these were designated as 0.1-DS syringes. For the remaining syringes, all of the air and some of the heparin were expelled from the syringe (the plunger was depressed down to the 0.25-mL mark on the syringe); these were designated as 0.25-DS syringes.

To determine the mean volume of heparin in syringes in each of the 4 dilution groups, 5 additional syringes and needles were prepared via each of the 4 techniques as described (total, 20 syringes and 20 needles). These were weighed before and after the addition of heparin on a digital gram scale to 0.1-mg accuracy (precision, ± 0.005 mg). The difference in syringe weight represented the volume of heparin contained in the syringe. The mean of these results was used to calculate the percentage dilution by volume attributable to heparin. All measurements were performed in duplicate.

All measurements were also performed in duplicate on a sample of liquid sodium heparin.^a The expected change in measured variables in each heparin-blood mixture was calculated from the volume of heparin at each dilution and the value of each variable in the heparin sample versus the volume of blood at each dilution and the baseline value of those variables. The expected changes were not calculated for pH or base deficit. Such calculations for pH would require accurate calculations of total hydrogen ion content of the samples of whole blood. Base deficit is a calculated value based on measurements of pH and PCO_2 and is also difficult to calculate accurately because it is a titratable variable.

Measurements of PO_2 —On a separate occasion, venous blood samples (10 mL) were collected from the same 6 dogs into 12-mL syringes in which the dead space had been prefilled with 0.1 mL of liquid sodium heparin.^a The hemoglobin concentration was measured^c in each sample prior to dividing it evenly among 3 glass flasks of a rotating tonometer.^d The samples were processed in the tonometer for 1 hour with 1 of 3 oxygen concentrations (5%, 14%, or 95%) to produce PO_2 values of approximately 40, 100, and 600 mm Hg (designated as PO_2 -40, PO_2 -100, and PO_2 -600). The carbon dioxide concentration in all tanks was 5%, and the remaining gas was nitrogen. The water bath of the tonometer was maintained at 37°C throughout the experimental period. After the 1-hour equilibration at each oxygen concentration, a blood sample was removed from the tonometer and the PO_2 value was measured in duplicate by use of a blood gas analyzer^e; this represented the baseline PO_2 measurement for each oxygen level. Each tonometered blood sample was then drawn into a syringe containing 1 of the 4 volumes of heparin, and the PO_2 value was immediately remeasured in duplicate.

Expected changes in PO_2 values were calculated from the cumulative total oxygen contents of the heparin, plasma, and hemoglobin present in the diluted sample. The oxygen contents of heparin and plasma were calculated as 0.003 multiplied by the PO_2 . The oxygen content of the hemoglobin was calculated by use of the following equation:

$$\text{Oxygen content of hemoglobin} = 1.36 \times \text{hemoglobin concentration (g/dL)} \times \text{percentage hemoglobin saturation.}$$

Hemoglobin saturation was determined from the measured PO_2 value via a published equation² of the canine oxygen hemoglobin dissociation curve. The sum of the heparin, plasma, and hemoglobin oxygen contents gave the total oxy-

gen content of the combined heparin-blood mixture. The postdilution hemoglobin concentration was calculated, and the predicted PO_2 of the diluted blood sample was determined via the canine oxygen hemoglobin dissociation curve.

Analyzers—All analyzers used in this study met the recommended quality control standards. All variables were measured and recorded at 37°C. The intra-assay repeatability of the blood gas and electrolyte analyzer^b was as follows: pH, ± 0.002 ; PCO_2 , ± 0.4 mm Hg; potassium concentration, ± 0.04 mEq/L; sodium concentration, ± 0.04 mEq/L; chloride concentration, ± 0.5 mEq/L; ionized calcium concentration, ± 0.008 mmol/L; and lactate concentration, ± 0.1 mmol/L. The intra-assay repeatability of the blood gas analyzer^e varied with the PO_2 and was as follows: PO_2 -40, 38 ± 0.9 mm Hg; PO_2 -100, 126 ± 1.4 mm Hg; and PO_2 -600, 562 ± 7.4 mm Hg.

Statistical analyses—Mean and SD values of all variables at baseline and at each level of dilution were calculated. Changes in each variable following dilution were evaluated by use of a repeated-measures ANOVA, applying the Geisser-Greenhouse-Epsilon probability and the Box-Epsilon probability. When a significant effect was identified, the Bonferroni all-pairwise multiple comparison test was used to identify the significant differences ($P < 0.05$). All statistical analyses were performed by use of commercially available software.^f

Results

By actual weight, the evacuated syringes contained 0.039 ± 0.0076 mL of heparin and the DS syringes contained 0.094 ± 0.0096 mL of heparin. The 0.1-DS syringes contained 0.188 ± 0.0126 mL of heparin, and the 0.25-DS syringes contained 0.341 ± 0.008 mL of heparin. When syringes were filled to 1 mL, this represented 3.9%, 9.4%, 18.8%, and 34.1% dilution of the blood sample, respectively.

The PO_2 value of the pure liquid sodium heparin was 161 mm Hg, and the pH value was 6.655. In this control sample, the value of PCO_2 (4.6 mm Hg) and the concentrations of bicarbonate (0 mEq/L), ionized calcium (0.02 mEq/L), potassium (0.1 mEq/L), and lactate (0 mEq/L) were negligible; the sodium and chloride concentrations were 160 and 166 mEq/L, respectively.

Dilution with heparin did not significantly change the measured pH or PO_2 -40 values of blood samples at any of the 4 levels of dilution (Table 1). The PCO_2 value and bicarbonate and potassium concentrations in blood samples after 9.4%, 18.8%, and 34.1% heparin dilution were significantly lower than the baseline measurements. Compared with baseline values, blood lactate concentration was significantly decreased after the 18.8% and 34.1% dilutions of blood with heparin; however, the PO_2 -100 value of blood samples was significantly increased by the 18.8% and 34.1% dilutions with heparin, whereas the PO_2 -600 value was significantly reduced by only the 34.1% dilution. Base deficit and sodium concentration were increased significantly from the baseline measurements by the 9.4%, 18.8%, and 34.1% dilutions of blood with heparin. At all levels of dilution, blood chloride concentration was significantly increased and ionized calcium concentration was significantly decreased from the baseline value. The mean hemoglobin concentration for the 6 dogs was 14.9 ± 1.0 g/dL.

Table 1—Actual and expected measurements (mean \pm SD) of pH, blood gas, and base deficit values and electrolyte and lactate concentrations in blood samples obtained from 6 dogs after collection (baseline) and after aliquots of the samples had been diluted with liquid sodium heparin via 1 of 4 syringe techniques.

Variable	Measurement	Baseline	Syringe technique*			
			Evacuation	DS	0.1-DS	0.25-DS
Dilution of blood with heparin (%)		0	3.9	9.4	18.8	34.1
pH	Actual	7.413 \pm 0.028	7.413 \pm 0.027	7.412 \pm 0.028	7.41 \pm 0.028	7.389 \pm 0.049
Pco ₂ (mm Hg)	Actual	36.8 \pm 6.5 ^a	35.5 \pm 6.3 ^{a,b}	33.4 \pm 6.3 ^b	29 \pm 4.5 ^c	23.5 \pm 3.9 ^d
	Expected		35.5	33.8	30.7	25.8
Po ₂ -40† (mm Hg)	Actual	40.7 \pm 0.8	40.4 \pm 0.8	40.2 \pm 0.8	40 \pm 0.8	40 \pm 1.2
	Expected		40.7	40.8	41.0	41.2
Po ₂ -100† (mm Hg)	Actual	103 \pm 4 ^a	104 \pm 4 ^a	104 \pm 2 ^a	109 \pm 4 ^b	108 \pm 4 ^b
	Expected		103	104	106	105
Po ₂ -600† (mm Hg)	Actual	571 \pm 58 ^{a,b}	607 \pm 38 ^a	590 \pm 52 ^a	547 \pm 41 ^b	459 \pm 34 ^c
	Expected		555	535	499	424
Bicarbonate (mEq/L)	Actual	23 \pm 3.1 ^a	22.1 \pm 3.1 ^a	20.7 \pm 3.1 ^b	17.9 \pm 2.2 ^c	14.4 \pm 2.0 ^d
	Expected		22.1	20.8	18.7	15.2
Base deficit	Actual	-1.1 \pm 2.9 ^a	-1.9 \pm 2.8 ^a	-3.2 \pm 2.9 ^b	-5.9 \pm 2.1 ^c	-9.5 \pm 2.1 ^d
Potassium (mEq/L)	Actual	4.6 \pm 0.1 ^a	4.4 \pm 0.1 ^a	4.2 \pm 0.1 ^b	3.6 \pm 0.2 ^c	2.7 \pm 0.2 ^d
	Expected		4.4	4.2	3.8	3.1
Sodium (mEq/L)	Actual	147 \pm 2 ^a	147 \pm 2 ^a	149 \pm 1 ^b	151 \pm 1 ^c	153 \pm 1 ^d
	Expected		148	148	150	152
Chloride (mEq/L)	Actual	117 \pm 2 ^a	119.5 \pm 3 ^b	122.2 \pm 3 ^c	127.5 \pm 2 ^d	134.7 \pm 2 ^e
	Expected		120	122	127	134
Ionized calcium (mmol/L)	Actual	1.1 \pm 0 ^a	1.0 \pm 0.1 ^b	0.8 \pm 0 ^c	0.5 \pm 0 ^d	0.3 \pm 0 ^e
	Expected		1.1	1.0	0.9	0.7
Lactate (mmol/L)	Actual	2.0 \pm 0.8 ^a	1.9 \pm 0.7 ^a	1.8 \pm 0.7 ^{a,b}	1.5 \pm 0.5 ^b	1.1 \pm 0.4 ^c
	Expected		1.9	1.8	1.6	1.3

*Syringes were prepared via aspiration of 0.5 mL of heparin into each of the syringes to coat the inner surface of the casing. The preparation of syringes was continued such that the residual heparin was forcibly expelled from some syringes (evacuation), heparin remained only within the dead space of the syringe and the hub of a 22-gauge needle (DS syringes), heparin was expelled from the syringe as the plunger was depressed down to the 0.1-mL mark on the syringe (0.1-DS syringes), or heparin was expelled from the syringe as the plunger was depressed down to the 0.25-mL mark on the syringe (0.25-DS syringes). †Blood samples were processed on a tonometer for 1 hour with 1 of 3 oxygen concentrations (5%, 14%, or 95%) to produce Po₂ values of approximately 40, 100, and 600 mm Hg (designated as Po₂-40, Po₂-100, and Po₂-600).
^{a-e}Values in rows without superscripts are not significantly ($P < 0.05$) different from each other. Within a row, means with superscripts in common are not significantly ($P < 0.05$) different from each other. Within a row, means with different superscripts are significantly ($P < 0.05$) different from each other.

Discussion

To avoid the clotting of blood samples in vitro, collected samples have to contain an anticoagulant. In the present study, we used liquid sodium heparin as an anticoagulant in the initial blood samples obtained from the 6 study dogs and thereby established a defined set of baseline values for the variables of interest. There is no doubt that these baseline values were diluted (compared with the actual values of the variables in the dogs) by use of the anticoagulant, but this was not relevant to our study. For any of the variables assessed, we were interested in the magnitude of the change from a defined set of baseline values that was associated with dilution of blood with liquid sodium heparin. For this reason, changes caused by the use of anticoagulant in the initial blood samples were not incorporated into our calculations.

Compared with baseline values, dilution of a whole blood sample with liquid heparin significantly

changed the measured values of PCO₂, PO₂, and base deficit and concentrations of bicarbonate, potassium, sodium, chloride, ionized calcium, and lactate. The physical dilution of a blood sample with a solution that has a different electrolyte and blood gas composition than that of blood will change the net electrolyte and acid-base measurements of the sample in a manner that is proportional to the degree of dilution and the relative concentration of each component in the blood and in the diluent.

Liquid sodium heparin is very acidic, and the solution used in the present study had a pH value of 6.655. However, no significant change in blood pH value from baseline was detected in samples after dilution with heparin, even at 34.1% dilution. This was attributed to the fact that liquid sodium heparin contains no buffers and has no titratable acidity; the stoichiometric or total concentration of hydrogen ions in the heparin solution is quite small. In comparison, whole blood contains

many buffers that minimize the impact on pH from the addition of acidic solutions; in humans, even a 50% dilution of blood with liquid sodium heparin is reported^{3,4} not to change the pH of the sample.

In our study, the PCO_2 values in blood samples from dogs decreased from baseline in a manner that would be predicted by simple volumetric dilution with a solution that had a PCO_2 of near zero. It has been reported³ in humans that PCO_2 would decline 1% for each 1% of dilution, and our results concur with that suggestion. Although the value of PCO_2 in the blood samples in our study was significantly decreased from baseline after 9.4% dilution with heparin, a clinically important decrease in PCO_2 value was only noted with dilutions of $\geq 18.8\%$. In humans, it has been suggested⁶ that there has to be at least 25% dilution of blood with heparin to produce a clinically important change in PCO_2 value.

Blood bicarbonate concentration and base deficit values decreased from baseline values after dilution of blood samples with sodium heparin; the extent of the decrease in bicarbonate concentration was similar to that predicted by simple dilution. In our opinion, this metabolic acidosis would become clinically important at $\geq 18.8\%$ dilution of blood with heparin. The decrease in base deficit in the blood samples could be explained by the dilution of bicarbonate. At 34.1% dilution with heparin, the magnitude of the decrease in base deficit from baseline was -8.4 ; the decrease in bicarbonate concentration from baseline was 8.6 mEq/L. The quantitative acid-base enthusiast will also note the decrease in apparent strong ion difference⁷ (ie, [sodium concentration + potassium concentration + {ionized calcium concentration \times 2} - chloride concentration - lactate concentration]) from 32.1 mEq/L at baseline to 20.7 mEq/L after 34.1% dilution of blood with heparin, which is indicative of a metabolic acidosis.

The relationship between the value of PO_2 and hemoglobin saturation (or oxygen content) is described by a hemoglobin oxygen dissociation curve. Low PO_2 values (40 mm Hg) are located on the steep portion of the curve, where large changes in hemoglobin saturation or oxygen content are associated with small changes in PO_2 . At low PO_2 values, a large change in oxygen content would be required to cause a significant change in PO_2 in blood. Despite the fact that the pure liquid sodium heparin evaluated in our study had a PO_2 value of 161 mm Hg, the addition of liquid heparin to a blood sample that had a low PO_2 value would be expected to have little impact on the net oxygen content and no impact on the PO_2 value of the blood in that sample.

The region of the hemoglobin oxygen dissociation curve corresponding to a PO_2 value of 100 mm Hg is flatter than the region associated with a PO_2 value of 40 mm Hg; therefore, compared with blood samples that have low PO_2 values, greater changes in PO_2 will result from a given change in oxygen content in samples that have high PO_2 values. In the samples of blood evaluated in our study that had a PO_2 value of 100 mm Hg, the 18.8% and 34.1% dilutions of those samples with heparin resulted in significant increases in the mea-

sured PO_2 (from a baseline value of 103 mm Hg to 109 and 108 mm Hg, respectively). These changes were very similar to the calculated expected changes. In a previous investigation⁸ in humans, a baseline PO_2 value of 113 mm Hg was not significantly increased after dilution of blood samples with heparin until samples underwent $> 25\%$ dilution by volume.

In our study, blood samples with very high PO_2 values, representing the upper flat portion of the oxygen hemoglobin dissociation curve, were expected to have a marked decrease in PO_2 subsequent to heparin dilution because pure liquid sodium heparin had a PO_2 value of 161 mm Hg. However, a significant decrease in PO_2 values in blood samples with very high PO_2 values was detected only with the 18.8% and 34.1% dilutions of blood with heparin.

Sodium heparin contains higher concentrations of sodium (160 mEq/L) and chloride (166 mEq/L) than serum samples from clinically normal dogs⁹; therefore, the concentrations of these variables increase in blood samples in proportion to the volume of dilution with heparin. There is almost no potassium in liquid sodium heparin, and dilution of blood samples with heparin can result in substantial decreases in the measured potassium concentration. In the blood samples evaluated in the present study, mean potassium concentration decreased from 4.6 mEq/L at baseline to 2.7 mEq/L after 34.1% dilution with heparin. In a clinical evaluation of a dog, such an erroneous measurement might trigger aggressive, unnecessary treatment with supplemental potassium, which can have serious consequences. The changes in the lactate concentration (from the baseline value) detected in blood samples in our study could also be predicted by simple dilution with a solution that contained no lactate.

Dilution of blood samples with heparin had the greatest impact on the measured ionized calcium concentration; this effect was in excess of that which could be accounted for by dilution alone. Heparin directly chelates calcium and other divalent cations, such as magnesium, resulting in lower measured ionized calcium and ionized magnesium concentrations.¹⁰ This is a heparin-specific phenomenon; the reduction in ionized calcium concentration is proportional to the concentration of heparin in the blood sample and will occur with use of liquid or lyophilized monovalent salts of heparin, such as sodium or lithium heparin.^{1,11} Some commercially available syringes containing heparin may considerably affect the measurement of ionized calcium concentration in blood because the manufacturer intended the syringe to be used only for blood gas analysis and has not restricted the amount of lyophilized heparin in the syringe.¹² For measurement of ionized calcium concentration in blood samples, it has been recommended to ensure that the final heparin concentration in the sample is < 15 U/mL of blood.¹³ In the blood samples evaluated in the present study, the smallest volume of dilution (3.9%) corresponded to approximately 40 U of heparin/mL of blood.

Although it is important to minimize the extent of heparin-associated dilution of blood samples, it is also important to minimize iatrogenic blood loss from the patient. Collection of large-volume blood samples to

minimize dilution is usually not a viable option. Heparin is a potent anticoagulant, and theoretically, only 1 unit of heparin is required to prevent the clotting of 1 mL of blood.¹⁴ The evacuated syringe technique used in the present study resulted in a residual quantity of approximately 40 units of heparin in each syringe; this far exceeds the amount of heparin required to prevent clotting of 1 to 3 mL of blood. This method of manual syringe heparinization represented a 3.9% dilution of a 1-mL blood sample and is statistically acceptable for measurement of all the clinicopathologic variables evaluated in our study, except ionized calcium concentration and, perhaps, chloride concentration. The evacuated syringe technique should represent the preferred technique in syringe preparation for collection of blood samples for blood gas and electrolyte measurements; the volume of heparin (0.039 mL) remaining in the evacuated syringe prior to blood withdrawal would represent 7.8%, 3.9%, 2.0%, and 1.3% dilutions of 0.5-, 1-, 2-, or 3-mL blood samples, respectively. Commercially available syringes and blood collection tubes containing lyophilized heparin will also avoid this dilutional source of error in blood gas analysis but may not be suitable for ionized calcium evaluation unless so stated. For analyses of ionized calcium and magnesium concentrations in blood samples, it is important to consider the impact of both the volume and concentration of the heparin.

Heparin dilution can alter measured values of variables in blood samples in a significant and clinically important manner. For most variables, these changes can be predicted on the basis of the degree of dilution and the differences in content between the original blood sample and the constituents of the heparin solution. In our study of blood samples obtained from dogs, the changes in pH and P_{O_2} -40 values from baseline were generally less than predicted, whereas the change in ionized calcium concentration was greater than predicted. Typically, it is suggested¹⁵ to limit heparin dilution of a blood sample to < 10%. In general, the results of the present study support this guideline, but it would seem preferable and achievable to limit dilution of blood samples with heparin to < 4%. As indicated by our data obtained in dogs, this can be attained by use of a technique in which heparin is drawn into the syringe to be used for collection of the blood sample; then, the heparin is evacuated from the syringe and the syringe is filled with at least 1 mL of blood. The use of dry lyophilized commercially heparinized syringes will prevent the dilutional effects on blood gas values and serum electrolyte and lactate measurements but can still introduce preanalytical errors in measurement of ionized calcium concentration. For ionized calcium analysis of blood samples,

cautious use of liquid heparin or use of commercial syringes containing heparin that are produced specifically for that purpose is recommended. Individual clinical practices need to establish a standard protocol for blood gas, electrolyte, and lactate analysis of blood samples that will minimize preanalytical errors; if sample analysis is consistent, then changes in measured values detected in samples over time can be interpreted as changes in the animal being evaluated and will not reflect variations in sample-handling techniques.

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- a. Elkins-Sinn Inc, Cherry Hill, NJ.
 - b. ABL 705, Radiometer Medical A/S, Copenhagen, Denmark.
 - c. OSM 3 hemoximeter, Radiometer Medical A/S, Copenhagen, Denmark.
 - d. 3-flask rotating tonometer, Modified Instrumentation Laboratory, Lexington, Mass.
 - e. ABL 5, Radiometer Medical A/S, Copenhagen, Denmark.
 - f. NCCS statistical software system, Kaysville, Utah.
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