Blood Values of Juvenile Northern Elephant Seals (*Mirounga angustirostris*) Obtained Using a Portable Clinical Analyzer

R. Scott Larsen, Martin Haulena, Carol B. Grindem, Frances M. D. Gulland

Background — Sick, injured, or orphaned juvenile northern elephant seals (*Mirounga angustisrostris*) treated at rehabilitation centers frequently present with abnormalities in blood sodium, potassium, chloride, BUN, and glucose concentrations, and HCT. These abnormalities could be detected rapidly using a portable blood analyzer, but the results with this analysis method do not necessarily equate with those obtained using other techniques. **Objective** — The objective of this study was to better assess the clinical relevance of blood values obtained from a portable analyzer and to compare the results with values obtained using more common methods of analysis. **Methods** — Heparinized whole blood samples were collected from 20 rehabilitated juvenile northern elephant seals. A portable clinical analyzer (i-STAT, i-STAT Corp, East Windsor, NJ, USA) was used to establish baseline values. Serum biochemical values were obtained using an automated chemistry analyzer (Olympus AU5200, Olympus America, Melville, NY, USA). HCT was determined using EDTA whole blood and a cell counter. **Results** — Using the portable analyzer, mean (minimum-maximum) values were obtained for sodium, 143 (132-146) mmol/L; potassium, 4.4 (3.9-5.8) mmol/L; chloride, 106 (101-109) mmol/L; BUN, 1.8 (1.1-2.4) mmol/L; glucose, 7.55 (5.99-8.49) mmol/L; and HCT, 0.55 (0.52-0.61) L/L. Average differences between methods were small for potassium (–0.45 mmol/L), BUN (0.1 mmol/L), and HCT (0.037 L/L) but were large for sodium (–6.8 mmol/L), chloride (–6.4 mmol/L), and glucose (–0.56 mmol/L). **Conclusions** — These results suggest that the i-STAT portable analyzer could be useful for clinically assessing juvenile elephant seals. However, when making medical decisions, the clinician should be aware of differences associated with various analyzers and sample types. (*Vet Clin Pathol.* 2002;31:106-110)

Key Words: Electrolytes, elephant seal, glucose, hematocrit, i-STAT, *Mirounga angustirostris*, point-of-care, portable analyzer

Every year, many sick, injured, or orphaned pinnipeds are treated at rehabilitation centers in the United States. Juvenile northern elephant seals (*Mirounga angustisrostris*) are one of the most common species treated.[1-3] Stranded pinnipeds often present with severe metabolic disease, including renal failure, hypoglycemia, electrolyte abnormalities, acidosis, dehydration, shock, starvation, gastrointestinal hemorrhage, and anemia.[2,4-6] Immediate identification of these disorders by rapid determination of electrolyte, glucose, BUN, and HCT levels could be life-saving. Small, portable point-of-care clinical analyzing systems have recently been developed that can be used to measure these values quickly and easily, expediting the acquisition of laboratory data in critical care situations. Results of studies in humans beings and domestic animals indicate that portable clinical analyzers can provide reliable data.[7-10]

Point-of-care analyzer systems commonly recommend the use of heparinized whole blood for analysis. Methods that use whole blood or plasma are preferred for emergency laboratory work because samples can be immediately processed without waiting for clot formation. However, the blood analysis methods reported for pinnipeds usually use serum for biochemical analysis and use blood mixed with EDTA for hematology. If portable analyzers are to be used for pinnipeds, it is important to identify differences in reference intervals that may result from the use of different analyzers or samples. We used a portable analyzer to determine sodium, potassium, chloride, glucose, BUN, and HCT values for juvenile elephant seals. These values were compared with those obtained with matched blood samples using serum for biochemistry analysis, and blood in EDTA for HCT. The mean difference (and associated SD) between the 2 methods was compared for each blood constituent.[11]

Materials and Methods

Between January and April 2000, approximately 70 stranded northern elephant seal pups were transported to the Marine Mammal Center (Sausalito, Calif, USA) for medical treatment, supportive care, and rehabilitation. In May 2000, 20 of these seals were evaluated to

From the Environmental Medicine Consortium, Department of Clinical Sciences (Larsen), and the Department of Microbiology, Pathology, and Parasitology (Grindem), College of Veterinary Medicine, North Carolina State University, Raleigh, NC; the North Carolina Zoological Park, Asheboro, NC (Larsen); and the Marine Mammal Center, Sausalito, Calif (Haulena, Gulland). Corresponding author: R. Scott Larsen, DVM, MS, Veterinary Medical Teaching Hospital, University of California, One Shields Ave, Davis, CA 95616 (slarsen@ucdavis.edu).

determine whether they were fit for release. Determination of an animal's suitability for release was based on clinical assessment, physical examination, and evaluation of CBC and serum chemistry results.

At the time of sampling, the seals weighed 67 ± 6 kg and were estimated to be 4-5 months old. They were manually restrained, and blood was drawn from the epidural intravertebral vein into a collection tube coated with lithium heparin (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) using Monoject needles (20 ga by 3.8 cm) and adapters (Sherwood Medical, St Louis, Mo, USA). Lithium heparin was chosen over sodium heparin so electrolyte analysis would not be altered by the electrolytes in the heparin medium. Samples were analyzed within 10 minutes with the portable clinical analyzer (i-STAT, i-STAT Corporation, East Windsor, NJ, USA) by introducing heparinized whole blood (approximately 65 µL) into a disposable cartridge (i-STAT 6+, i-STAT) designed for simultaneous assay of electrolytes, glucose, BUN, and HCT. Each cartridge contains a series of thin-film electrodes (biosensors) that contact the blood sample and send signals to an electronic system. The system compares these signals with calibration signals contained within the cartridge and processes the results.[12] Sodium (mmol/L), potassium (mmol/L), and chloride (mmol/L) values were measured by direct ion-selective electrode potentiometry.[12] Glucose was measured by oxidation with glucose oxidase and amperometric measurement of hydrogen peroxide.[12] Urea (BUN) was hydrolyzed to ammonium in a reaction catalyzed by urease, and the resultant ammonium ions were measured amperometrically.[11] Values for glucose and BUN were reported by the analyzer as mg/dL; results were then converted to mmol/L. HCT (L/L) was determined by conductivity.[12]

At the same time that the heparinized sample was taken, a second blood sample was collected from each patient into a plain red-top glass tube (Vacutainer) and into a tube containing EDTA (Vacutainer). These samples were kept refrigerated and were processed by trained laboratory personnel within 1 hour of sample collection. The samples in the plain tubes were allowed to clot, centrifuged at 3100*g* for 10 minutes, and then serum was extracted using a pipette. Serum concentrations of sodium, potassium, chloride, BUN, and glucose were measured using an automated analyzer (Olympus AU5200, Olympus America, Melville, NY, USA). Sodium, potassium, and chloride concentrations were determined by indirect ion-selective electrode potentiometry. Glucose was measured using the hexokinase method.[13] BUN was determined colorimetrically with reductive amination of 2-oxoglutarate by glutarate dehydrogenase oxidation using ammonia generated by urea degradation with urease. The samples were processed by trained laboratory personnel. Laboratory quality control (QC) was performed, with calibration on every lot of samples and also every 24 hours. Once per month, blind studies were conducted, and QC values, methods, and ranges were reviewed. Blood from the EDTA tube was used to determine HCT using an automated cell counter (Vet ABC, Heska Corporation, Fort Collins, Colo, USA). The cell counter had previously been calibrated for marine mammal RBCs. Calibration had been established by comparing cell counter results from juvenile elephant seals (n = 19) with HCT values obtained using microcentrifugation. For elephant seals, values from the cell counter were 0.011 (± 0.011) L/L higher than manually spun results (J. Lawrence, Marine Mammal Center, personal communication).

Mean, SD, median, 25th percentile, 75th percentile, and minimum and maximum values for each blood analyte using each method of analysis were determined. The mean (± SD) difference between values obtained for the same sample (value from the portable analyzer minus the value from the automated analyzer) were determined for all analytes. The intervals of values from the 2 methods were compared with previously reported intervals for juvenile elephant seals.[4]

Results and Discussion

Sodium values from the portable analyzer were slightly lower than those previously reported, but values from the automated analyzer were slightly higher (Table 1).[4] Portable analyzer values were 4-10 mmol/L less than those from the automated laboratory instrument, suggesting that sodium values obtained with the 2 techniques cannot be considered equal. Equating the 2 measurements could cause a clinician to make an erroneous diagnosis of pinniped hyponatremia (sodium < 147 mmol/L)[6] and could lead to inappropriate treatment. The cause for the discrepancy probably is related to the use of heparinized whole blood rather than serum in the portable analyzer. Elephant seal RBCs have relatively high intracellular sodium concentrations relative to terrestrial mammals,[14] and some of this sodium is released when blood samples are spun for serum extraction. However in a previous study in domestic dogs, there was poor correlation for sodium values between the i-STAT and an automated chemistry analyzer, even when heparinized whole blood was used for both machines.[10] Therefore it is prudent to interpret laboratory values, especially sodium values, using appropriate specimen and instrument-derived reference intervals.

Both techniques produced potassium values comparable to previously reported serum potassium values for elephant seals (Table 1).[4] However, potassium concentrations from the portable analyzer were 0.45 (± 0.43)

Table 1. Biochemical values for juvenile northern elephant seals obtained from whole blood using a portable analyzer (i-STAT) and from serum using an automated analyzer (Olympus AU5200). Hematocrit values were obtained from whole blood using the portable analyzer and from EDTA blood using a cell counter (Vet ABC).

Analyte	n	Method	Mean ± SD	Difference ± SD*	Median	25th Percentile	75th Percentile	Min–Max	Published Min–Max†
Sodium (mmol/L)	20	i-STAT	143±3		144	143	145	132-146	
		Serum	151±2	–6.8±3.2	150	149	152	147-156	143-154
Potassium (mmol/L)	20	i-STAT	4.7±0.6		4.4	4.1	5.1	3.9-5.8	
		Serum	5.1±0.5	–0.45±0.43	5.0	4.8	5.3	4.4-6.2	4.7-6.1
Chloride (mmol/L)	19‡	i-STAT	106±3		106	103	108	101-109	
		Serum	100±2	6.4±3.2	100	98	100	97-104	98-107
BUN (mmol/L)§	20	i-STAT	1.8±0.4		1.7	1.5	2.1	1.1-2.4	
		Serum	1.7±0.3	0.1±0.2	1.7	1.5	1.9	1.1-2.1	1.2-2.1
Glucose (mmol/L)§	20	i-STAT	7.55±0.67		7.60	7.27	7.77	5.99-8.49	
		Serum	8.55±1.67	–0.56±1.06	8.05	7.55	8.60	6.33-10.43	7.10-10.49
HCT (L/L)	20	i-STAT	0.55±0.04		0.54	0.53	0.57	0.52-0.61	
		Serum	0.52±0.03	0.037±0.004	0.52	0.50	0.54	0.48-0.54	0.46-0.61

*Difference = mean value from the portable analyzer – mean value from the automated analyzer.
†Results from 25 juvenile elephant seals.[4]
‡A chloride value was not obtained for 1 sample.
§Glucose and BUN values were converted from mg/dL to mmol/L.

mmol/L less than those obtained from the automated analyzer. In humans, whole blood potassium concentrations are typically 0.1 to 0.7 mmol/L lower than serum concentrations due to the release of potassium from ruptured platelets during coagulation.[15] The difference between sample types likely accounts for the difference in potassium values observed in the elephant seals, however it should be noted that in a previous report of portable analyzer use in domestic animals a 0.5-1.5 mmol/L difference in potassium also was observed.[8]

Chloride values for both techniques were similar to previously reported values,[4] although results from the portable analyzer were consistently higher than those from the automated analyzer (Table 1). The cause of the large difference in chloride values in this investigation is unknown, but may relate to the different sample types used. The portable analyzer has been reported to overestimate whole blood chloride in other species.[7-9] The mean difference was larger in elephant seals (6.4 mmol/L) than that reported in human beings (1.9-5.5 mmol/L), dogs (3.6 mmol/L), cats (2.3 mmol/L), and horses (2.0 mmol/L).[7-9] Discrepancies in chloride values have been attributed to the narrow range of values for chloride[7] and to the sensitivity of chloride ion-selective electrode systems to the effects of protein.[9] Thus the large difference in chloride observed here may be due to both sample and machine effects.

BUN values obtained with the 2 techniques were similar to each other and similar to previously reported values in elephant seals (Table 1).[4] Differences between BUN values obtained with these 2 techniques were small, and the values were considered equivalent.

The glucose values obtained using the portable analyzer and the automated analyzer were both slightly lower than previously reported values for healthy elephant seals (Table 1).[4] However, values from the automated analyzer were slightly higher overall than those from the portable analyzer. Whole blood samples were processed on the portable analyzer within minutes of sampling, whereas serum samples were processed on the automated analyzer up to 1 hour later. With human samples, glucose values would be expected to be lower in serum than in immediately processed whole blood, because of glycolysis during prolonged contact of blood cells with serum.[4] However in elephant seals, glucose uptake and glycolytic rates are slow and heparinized blood samples can be stored for several hours without significant decreases in plasma glucose concentration.[16] In fact, glucose concentration may actually be higher in plasma or serum than in whole blood. Although the glucose concentration is the same in the water of plasma and the water of RBCs, the RBCs contain more protein and consequently have a lower concentration of water.[13] This lower concentration of water may cause a dilutional effect, making the concentration of glucose in whole blood lower than that in serum or plasma.[13] We do not

know whether this dilution effect accounts for the discrepancy between the portable and automated analyzer results; however, in human beings and domestic animals, portable analyzer results for glucose differ substantially from the results of automated analyzers.[17-19]

HCT results from the 2 methods were similar and were within reference intervals previously reported for juvenile elephant seals (Table 1),[4] however values from the portable analyzer were an average of 0.037 L/L higher than those obtained from the cell counter. A 0.04 L/L difference between methods has been reported for human blood,[9] and a 0.05 L/L difference has been reported for dogs, cats, and horses.[10] Other investigators have reported on the variability of pinniped HCT values, which can be affected by laboratory methodology, age, and animal handling technique.[6,20] For example, electronic cell counters calculate the HCT using the measured RBC count and MCV. Most pinnipeds have relatively large RBCs, and elephant seals have MCV values of 170-185 fl.[4] As a result, the RBC count, MCV, and HCT values are accurate for pinnipeds only if those parameters are measured using a calibrated cell counter to account for the larger size of marine mammal RBCs.[4] In a previous study, HCT measurements by a cell counter were 4-15% higher than those obtained by microcentrifugation; however, the cell counter used in that investigation had not been calibrated for pinniped RBCs.[20] In contrast, the cell counter used in this investigation had previously been calibrated for pinniped RBCs, and the HCT measurements obtained were only 0.011 (± 0.011) L/L higher than values obtained by microcentrifugation (J. Lawrence, personal communication). Both the cell counter and the portable analyzer rely on the rheologic properties of RBCs, and the conductivity of blood is greatly dependent on the volume fraction of RBCs. These properties are quite different for seal and human blood,[21] so the higher volume fraction of elephant seal RBCs results in increased conductivity and, consequently, increased HCT values.[10]

The results of this study suggest that the i-STAT portable clinical analyzer could be useful for evaluating elephant seals in critical care situations. However, some values obtained with the portable analyzer depart substantially from those determined with serum processed on an automated analyzer. Much of this variation may be associated with differences in sample type. It is more appropriate to use a reference interval from portable analyzer values that are obtained from healthy animals of a given species rather than directly comparing portable analyzer values to those obtained using other methods. Individual laboratory-derived reference intervals are not generally available for marine mammals and other wildlife species, as they are for domestic animals; therefore, appropriate use of published intervals is very important. The minimum and maximum values reported in this investigation are narrow because the animals were of similar ages and were kept in similar environments. However, these values serve as a starting point for defining reference intervals for portable analyzers in young elephant seals. Values reported here are only for juvenile elephant seals, but seals of this age group are most commonly encountered in clinical settings.[2] ◊

Acknowledgments

The authors thank the veterinary technicians, husbandry staff, and volunteers of the Marine Mammal Center, including L. Phoenix, J. Lawrence, J. Sicree, D. Wickham, and P. Labelle.

References

1. Goldstein T, Johnson SP, Werner LJ, Nolan S, Hilliart BA. Causes of erroneous white blood cell counts and differentials in clinically healthy young northern elephant seals (*Mirounga angustirostris*). *J Zoo Wildl Med.* 1998;29:408-412.
2. Roletto J. Hematology and serum chemistry values for clinically healthy and sick pinnipeds. *J Zoo Wildl Med.* 1993;24:145-157.
3. Schroeder RJ, Quandri D, McIntyre RW, Walker WA. Marine mammal disease surveillance program in Los Angeles County. *J Am Vet Med Assoc.* 1973;163:580-581.
4. Bossart GD, Reidarson TH, Dierauf LA, Dufeld DA. Clinical pathology. In: Dierauf LA, Gulland FMD, eds. *CRC Handbook of Marine Mammal Medicine.* 2nd ed. Boca Raton, Fla: CRC Press; 2001:383-436.
5. Gulland FMD, Haulena M, Dierauf LA. Seals and sea lions. In: Dierauf LA, Gulland FMD, eds. *CRC Handbook of Marine Mammal Medicine.* 2nd ed. Boca Raton, Fla: CRC Press; 2001:907-926.
6. Medway W, Geraci JR. Clinical pathology of marine mammals. In: Fowler ME, ed. *Zoo and Wild Animal Medicine.* 2nd ed. Philadelphia, Pa: WB Saunders; 1986:791-797.
7. Erickson KA, Wilding P. Evaluation of a novel point-of-care system, the i-STAT portable clinical analyzer. *Clin Chem.* 1993; 39:283-287.
8. Grosenbaugh DA, Gadawski JE, Muir WW. Evaluation of a portable clinical analyzer in a veterinary hospital setting. *J Am Vet Med Assoc.* 1998;213:691-694.

9. Jacobs E, Vadasdi E, Sarkozi L. Analytical evaluation of i-STAT portable clinical analyzer and use by nonlaboratory healthcare professionals. *Clin Chem.* 1993;39:1069-1074.

10. Looney AL, Ludders J, Erb HN, Gleed R, Moon P. Use of a handheld device for analysis of blood electrolyte concentrations and blood gas partial pressures in dogs and horses. *J Am Vet Med Assoc.* 1998;213:526-530.

11. Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. *Statistician.* 1983;32:307-317.

12. *i-Stat System Manual.* Waukesha, Wis: Sensor Devices, 1996.

13. Linne JJ, Ringsrud KM. Chemistry. In: *Clinical Laboratory Science.* 4th ed. St Louis, Mo: Mosby; 1999:229-262.

14. Eadie JB, Kirk RL. The Na^+ and K^+ concentration in blood cells and plasma of the elephant seal. *Aust J Sci.* 1952;15:26-27.

15. Tietz NP, Pruden EL, Siggaard-Andersen O. Electrolytes. In: Burtis CA, Ashwood ER, eds. *Tietz Fundamentals of Clinical Chemistry.* 4th ed. Philadelphia, Pa: WB Saunders; 1996:497-505.

16. Castellini MA, Castellini JM, Kirby VL. Effects of standard anticoagulants and storage procedures on plasma glucose values in seals. *J Am Vet Med Assoc.* 1992; 201:145-148.

17. Wess G, Reusch C. Assessment of five portable blood glucose meters for use in cats. *Am J Vet Res.* 2000;61:1587-1592.

18. Wess G, Reusch C. Evaluation of five portable blood glucose meters for use in dogs. *J Am Vet Med Assoc.* 2000;216:203-209.

19. Cohn LA, McCaw DL, Tate DJ, Johnson JC. Assessment of five portable blood glucose meters, a point-of-care analyzer, and color test strips for measuring blood glucose concentration in dogs. *J Am Vet Med Assoc.* 2000;216:198-202.

20. Castellini JM, Meiselman HJ, Castellini MA. Understanding and interpreting hematocrit measurements in pinnipeds. *Mar Mammal Sci.* 1996;12:251-264.

21. Wickam LL, Bauersachs RM, Wenby RB, et al. Red cell aggregation and viscoelasticity of blood from seals, swine and man. *Biorheology.* 1990;27:191-204.