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# USE OF A PORTABLE POINT-OF-CARE (VETSCAN VS2) BIOCHEMICAL ANALYZER FOR MEASURING PLASMA BIOCHEMICAL LEVELS IN FREE-LIVING LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*)

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*Abstract:* The agreement of plasma biochemical values between a portable point-of-care analyzer and a veterinary diagnostic laboratory in wild caught loggerhead sea turtles (*Caretta caretta*) was tested. Banked plasma samples from presumptively healthy turtles collected for an on-going project that involves health assessments of sea turtles from the southeast coast of Florida were used for this study. Plasma biochemical analytes evaluated included albumin, aspartate aminotransferase, calcium, creatinine kinase, glucose, potassium, sodium, phosphorus, total protein, bile acids, and uric acid. Paired plasma samples were run in duplicates and compared between a point-of-care analyzer and a veterinary diagnostic laboratory (VDL). Overall, the precision was greater as measured within the point-of-care analyzer than within the VDL analyzer; however, agreement between the two testing methods was poor. Correlation ($r_i$) between the two analyzers was high for many of the analytes; however, the small *P*-value and high relative error led to the conclusion that the two analyzers were not equivalent. In addition, a comparison was made between the biochemical values obtained at the time of collection and after storage in an ultralow freezer for up to 2.5 yr. Plasma samples analyzed at the VDL, performed on different models of the same machine, were significantly lower after storage than those acquired near the time of collection. This difference was most likely because of sample degradation that occurred during storage. Whereas, statistically significant differences were observed within and between the analyzers, many of these differences may not be clinically significant. Even though this study has a few limitations, including a technical malfunction and the use of two different diagnostic laboratories, biochemical values for the given population are reported when using both a portable system and a diagnostic laboratory. Based on the findings of this study, the authors believe that point-of-care analyzers can provide valuable adjunctive diagnostics, especially in field situations.

*Key words:* *Caretta caretta*, plasma, loggerhead sea turtle, clinical biochemistry, point-of-care analyzer.

## INTRODUCTION

In situ health evaluations of wildlife species have become an increasingly valuable tool for monitoring free-living populations. Health evaluations often include physical examination data along with complete blood counts and biochemical analysis. Hematology and plasma or serum biochemistry analysis can provide important information about individuals as well as the population being studied. The ability to assess an individual's health status at the time of examination, especially in field studies, can be an important tool for data collection and treatment plan formulation. Plasma and serum biochemical values for clinically healthy species of reptiles are available for only a small number of the more than 7,500 species.[2,4,5,8,9,11,14,16,18,21,31,32,33,36] These values must be considered as guidelines and not absolute because of variability that can arise from species individuality, season, sex, age, temperature, nutritional and reproductive status, and captivity status.[3,6,8,9,15,19,25,31] In addition, sample handling and methodology of the analysis as well as sampling technique can impact results.[3]

Point-of-care analyzers have become common in both human and veterinary medicine, and can be a valuable tool in health assessments. They offer several benefits, including portability, small volume size, rapid results and direct control of the sample, and quality control.[17,20] There are several reports of their use in both domestic and exotic species.[1,15,17,18,20,36] Loggerhead sea turtles (LST; *Caretta caretta*) can be found in the temperate and tropical regions of the Pacific, Atlantic, and Indian oceans, with the majority of nesting taking place along the western rims of the

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Atlantic and Indian oceans.[7,29,34] They are currently listed as threatened in the state of Florida and endangered by the International Union for Conservation of Nature (www.redlist.org) and Convention on the International Trade in Endangered Species of Wild Fauna and Flora (www.cites.org).[24] Current threats include habitat destruction of nesting areas because of development, sand mining, and rising seas; pollution, intentional and incidental capture of juveniles and adults; egg loss to humans and to native and introduced predators; degradation of foraging habitat and prey; terrestrial and marine pollution and debris; bioaccumulation of metals; disease; and trauma.[7,10,26,27] A recent review of the literature has indicated a decline in most nesting areas by as much as 36% over the last decade, with complete cessation of nesting activity in several previously documented nesting sites.[27] Because of the status and size of loggerhead turtles, the ability to evaluate this species in the field would be beneficial by decreasing handling time and stress to the individual.

The purpose of this study was to assess the agreement between a point-of-care analyzer and values obtained at a veterinary diagnostic laboratory. In addition, blood chemistry profiles for a wild population of Atlantic loggerhead turtles during the months of November through February 2004 to 2006 are reported.

## MATERIALS AND METHODS

### Study population

This study used a repository of plasma from LST collected as part of an ongoing project (August 2004 to April 2008) that involves health assessments of LST and other sea turtle species that were removed from the intake canal of the Port St. Lucie Nuclear Power Plant in southeast Florida.[12] Sample collection was approved by the University of Florida (Gainesville, Florida) Institutional Animal Care and Use Committee (Protocol IACUC 722). All samples were collected under the authorization of the Florida Fish and Wildlife Conservation Commission Marine Turtle Permit 086.

The turtles that entered the intake canal of the Port St. Lucie Nuclear Power plant were captured, and a physical examination was performed that included morphologic measurements, weight, and blood collection. Turtles that were assessed to be healthy were subsequently tagged and released into the nearby Atlantic Ocean. Turtles that were determined to be ill or injured were relocated to state approved rehabilitation facilities for care and treatment.

Blood sampling involved collection of up to 5 ml of blood within 5–10 minutes of capture from the dorsal cervical sinus by established methods by using a 22-gauge Vacutainer needle (Beckton Dickinson, Franklin Lakes, New Jersey 07417, USA) for small LST and 20-gauge needle for subadult and adult LST.[28] Whole blood was drawn into sodium heparin Vacutainer tubes (Beckton Dickinson). If samples could not be processed immediately, then they were refrigerated until they could be processed, usually within $\leq$2 hr from the time of collection. Whole blood was centrifuged (Clay Adams Safety Head Centrifuge, Beckton Dickinson) for 5 minutes on the low setting at mid range speed. The plasma was then transferred to a sterile cryotube and stored in liquid nitrogen until it was shipped on dry ice to the University of Florida where it was stored at $-80^{\circ}$C. As part of the ongoing study, a standard reptile plasma biochemistry panel that consisted of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), glucose (Glu), blood urea nitrogen (BUN), uric acid (UA), cholesterol, total protein (TP), albumin (Alb), alkaline phosphatase, aspartate aminotransferase (AST), gamma-glutamyl transferase, creatinine kinase (CK), and carbon dioxide was performed at a veterinary diagnostic clinical pathology laboratory (VDL1) with a free-standing autoanalyzer (Hitachi 911/912, Roche Diagnostics Corporation, Indianapolis, Indiana 46250, USA) within 8 weeks of collection to establish reference intervals. Analyte concentrations obtained from this study are available online at http://accstr.ufl.edu/blood_chem.htm.

The sample population consisted of 100 presumptively clinically healthy LST collected during the months of November through February from 2004 to 2006. This time period was selected to minimize the effects of breeding and nesting on biochemical parameters (nesting of the North American Atlantic loggerhead population extends from May–August).[34] Average water temperature during the selected collection time was $21.6 \pm 2.52^{\circ}$C. Both juvenile and adult size classes were included based on straight carapace length from nuchal notch to tip (in centimeters) and were of unknown sex. Turtles ranged in size from 56 to 89 cm and weighed from 26 and 103 kg. Samples used for the current study had been stored in an ultralow freezer for up to 2.5 yr after initial collection.

### Analysis of samples

Plasma samples were removed from storage, allowed to thaw, manually mixed, and then partitioned into paired aliquots that were returned to the ultralow freezer where they were maintained at −80°C until removed for analysis. For analysis of samples at the diagnostic laboratory, aliquots of each sample were shipped overnight on dry ice to the Clinical Pathology Diagnostic Service (VDL2) at the University of Illinois, College of Veterinary Medicine (Champaign, Illinois, USA) for plasma biochemical analysis by using a Hitachi 917 chemistry analyzer. Samples were run in duplicate for each individual. The Hitachi 917 analyzer uses an ion selective electrode to measure Na, K, Cl, and a bichromatic photometric system for all other measurements, including AST, bile acids (BA), Ca, Glu, CK, P, TP, and UA.

Analysis of samples by a portable point-of-care analyzer, the Vetscan VS2 (Abaxis Inc., Union City, California 94587, USA) used commercially available avian–reptile reagent disks, which includes analytes for Alb, AST, BA, Ca, CK, Globulin (Glob), Glu, K, Na, P, TP, and UA. The VS2 analyzer uses a photometric system for analysis. Samples were removed from storage the day of analysis, thawed under refrigerated conditions and processed. The reagent disk was loaded with 0.1 ml of plasma by using the manufacturer supplied loading pipette and placed into the analyzer according to the manufacturer's instructions. Detailed methodologies used in each of the analyzer types, point-of-care and automated laboratory systems, have been previously reported and are not discussed in this review.[17,36]

In addition to comparing the two analyzer types, a comparison was made between the biochemical values obtained from the two diagnostic laboratories by using data acquired at the time of collection before long-term storage and data acquired when using the stored plasma samples. The original biochemical data were obtained by using a Hitachi 911 autoanalyzer at the University of Florida (Gainesville, Florida, USA), Veterinary Medical Center Clinical Pathology Service (VDL1), near the time of sample arrival from the St. Lucie Power Plant. The biochemical values obtained for the present study evaluated stored samples by using the Hitachi 917 at VDL2. Total sample storage time ranged from 2 to 3 yr and had been through a minimum of 3 freeze–thaw cycles. The Hitachi 917 analyzer is an updated model of the Hitachi 911 analyzer, but the analyzers use the same principles and techniques.

### Statistical analysis

Statistical analysis was performed with a commercially available statistical program (SAS, SAS Institute Inc., Cary, North Carolina 27513, USA). Variables were tested for normality and, when necessary, were log-transformed for analysis. Reproducibility (precision) was evaluated by using the intraclass correlation ($r_i$), Bland-Altman statistics, mean difference, limits of agreement, Wilcoxon signed rank test, and the paired *t*-test, when appropriate. For $r_i$, values $> 0.9$ were considered to have excellent agreement, values 0.8–0.9 had good agreement, values of 0.7–0.8 had fair agreement, and values $< 0.7$ had poor agreement. Significance was defined as $P < 0.05$. Agreement (as a measure of accuracy)[35] between analyzers was evaluated by Bland-Altman statistics, mean difference with corresponding limits of agreement and relative error, $r_i$, paired *t*-test, when appropriate, and the Wilcoxon signed rank test.

## RESULTS

### Data analysis

A total of 215 Vetscan disks were run. Because of the internal quality control system used by the Vetscan VS2 analyzer, 12 panels (5.6%) were cancelled. The system also reported 83 instances (38.6%) in which an analyte was outside the linear range of the analyzer, but the remaining analytes were correctly analyzed and reported. Typically, this finding was noted in the bile acids, UA, or Ca analytes. In addition, after the first 45 paired samples were analyzed, the VS2 analyzer experienced technical difficulties and was replaced by a new analyzer. To minimize the effect of an additional variable on the results, only results from 55 of the 100 samples analyzed with the Vetscan analyzer were used for comparison and in determining precision and agreement. The number of error messages encountered during the data acquisition from the Hitachi biochemical analyzer was not available for comparison.

### Precision of instruments

Repeatability of measurements was excellent for some values and poor for others, both within a given analyzer and between analyzers. Fifty-five samples were included in the assessment of the Vetscan analyzer (Table 1) by using the avian-reptilian profile rotors. The repeatability, within

**Table 1.** Mean (± SD) values of selected plasma biochemical values.

| | VDL2[a] | | VS20 | |
|---|---|---|---|---|
| Biochemical parameter | Hit1 | Hit2 | Ab1 | Ab2 |
| Alb (g/dl) | 1.04 ± 0.18 | 1.06 ± 0.185 | 1.26 ± 0.244 | 1.23 ± 0.21 |
| AST (IU/L) | 90.95 ± 59.15 | 88.49 ± 57.95 | 92.1 ± 52.152 | 91.44 ± 51.81 |
| BA (μmol/L) | 1.11 ± 1.01 | 0.91 ± 1.005 | <LD | <LD |
| Ca (mg/dl) | 3.82 ± 1.24 | 3.66 ± 1.209 | 4.43 ± 0.725 | 4.426 ± 0.61 |
| CK (IU/L) | 302 ± 423.5 | 293 ± 414.5 | 327 ± 440.92 | 321.4 ± 434.37 |
| Glu (mg/dl) | 89.11 ± 26.39 | 90.62 ± 26.87 | 96.8 ± 25.853 | 94.76 ± 24.45 |
| K (mmol/L) | 3.54 ± 0.46 | 3.58 ± 0.458 | 4.6 ± 0.624 | 4.489 ± 0.55 |
| Na (mmol/L) | 154.8 ± 2.72 | 155.9 ± 3.024 | 154 ± 7.038 | 152.2 ± 5.35 |
| P (mg/dl) | 6.31 ± 1.12 | 6.29 ± 1.099 | 6.76 ± 1.226 | 6.715 ± 1.14 |
| TP (g/dl) | 3.27 ± 0.76 | 3.31 ± 0.777 | 3.42 ± 0.823 | 3.329 ± 0.79 |
| UA (mg/dl) | <LD | <LD | 0.67 ± 0.383 | 0.655 ± 0.42 |

[a] VDL, veterinary diagnostic laboratory; Hit, Hitachi 917; Ab, Abaxis; Alb, albumin; AST, aspartate aminotransferase; BA, bile acids; <LD = less than limits of detection; Ca, calcium; CK, creatinine kinase; Glu, glucose; K, potassium; Na, sodium; P, phosphorus; TP, total protein; UA, uric acid. The diagnostic laboratory values were obtained with the Hit (VDL2). The point-of-care analyzer values were obtained with the Ab VS20. Hit1 ($N = 55$), Hit2 ($N = 55$), Ab1 ($N = 55$), and Ab2 ($N = 55$) represent the paired sample runs on each machine. Bile acids were not available for the VS20, and UA levels were not available for the Hit because the values were outside of the dynamic range of the testing method.

the Vetscan analyzer, determined by using Bland-Altman statistics, $r_i$, and the corresponding assessment of relative error, was poor for Na and CK; fair for Alb and K; good for Glu, P, and TP; and excellent for AST and Ca (Table 2).

Repeatability of the VDL2 (Hitachi 917 analyzer) ($n = 100$) by using the same statistical analysis was poor for AST, Ca, CK, Na, and P; good for Glu; and excellent for Alb, K, and TP.

### Agreement between the point-of-care analyzer and VDL2

Because of the change of Vetscan VS2 analyzers during the study, the first 45 of the 100 samples that were chosen for this comparative study were not included in the comparison between analyzer types so to minimize additional variables. Based on 55 paired samples, agreement between the two analyzers, the Vetscan and the Hitachi 917, varied among analytes as assessed by mean difference with corresponding limits of agreement, relative error, $r_i$, paired *t*-test, when appropriate, and the Wilcoxon signed rank test. There was poor agreement between Alb, CK, Glu, K, Na, and P; mixed agreement between analyzers for AST and TP; and only fair agreement for Ca (Table 2). Bile acids and UA levels were not tested for agreement because of limitations of the individual systems. Bile acid values on the Vetscan analyzer were always reported as <35 μmol/L, which was below the limit of detection of the analyzer. The Hitachi analyzer produced results that ranged from 0 to 6, with a mean of 0.85 mmol/L, which is below the limit of detection for the Vetscan analyzer. The UA values for the Hitachi 917 analyzer were consistently reported as <2.2 mg/dl and, therefore, were not included in the evaluation.

### VDL1 versus VDL2

Comparison between the baseline historical Hitachi data and recent Hitachi analysis included 100 samples (Table 3). Agreement between the recent Hitachi 917 analyzer (VDL2) values and the baseline data from the Hitachi 911 analyzer (VDL1) show poor agreement for all analytes except Glu, which had good agreement (Table 3). Summary results of all 3 biochemistry analyzers are provided in Table 4.

## DISCUSSION

Variability in hematology and plasma biochemistry values is known to exist both within and between reptilian species. This variation has been attributed to environmental conditions such as climate, season, and toxins, as well as nutrition, age, sex, population dynamics, methods of collection, sample handling, and biochemical assay methods.[11,25,31] When evaluating a clinical test or methodology, limits of allowable error for accuracy and precision need to be determined. Factors that can affect limits of allowable error include reference values, clinician opinion, biologic variation, state of the art, and

**Table 2.** Compiled results of statistical analysis for precision and agreement of the VetscanVS2 and the Hitachi 917 chemistry analyzer.

| Biochemical parameter[a,b] | Mean difference | Relative error (%) | $r_i$ | Paired *t*-test | Wilcoxon signed rank test | Power of paired *t*-test | Precision | Agreement |
|---|---|---|---|---|---|---|---|---|
| Alb_A | 0.03 | 1.6 | 0.734 | 0.31 | 0.146 | 0.17 | Fair | |
| Alb_A vs. H1 | −0.127 | −9.5 | 0.616 | 0.001 | 0 | 0.955 | | Poor |
| Alb_A vs. H2 | −0.07 | −5.5 | 0.821 | 0.002 | 0.001 | 0.893 | | Poor |
| AST_A | 0.636 | 1.0 | 0.992 | 0.468 | 0.507 | 0.111 | Excellent | |
| AST_A vs. H1 | −1.127 | 3.8 | 0.991 | 0.411 | 0.101 | 0.129 | | Excellent |
| AST_A vs. H2 | −2.945 | 5.9 | 0.988 | 0.044 | 0 | 0.526 | | Poor |
| Ca_A | 0.006 | −0.2 | 0.96 | 0.876 | 0.843 | 0.053 | Excellent | |
| Ca_A vs. H1 | −0.032 | −2.6 | 0.704 | 0.808 | 0.735 | 0.057 | | Fair |
| Ca_A vs. H2 | −0.194 | −6.6 | 0.782 | 0.109 | 0.05 | 0 | | Fair |
| CK_A | 5.509 | 8.6 | 0.998 | 0.107 | 0.026 | 0.363 | Poor | |
| CK_A vs. H1 | −24.873 | −35.5 | 0.998 | 0 | 0 | 1 | | Poor |
| CK_A vs. H2 | −28.327 | −29.8 | 0.999 | 0 | 0 | 1 | | Poor |
| Glu_A | 1.982 | 1.9 | 0.953 | 0.067 | 0.023 | 0.45 | Good | |
| Glu_A vs. H1 | −7.655 | −11.7 | 0.97 | 0 | 0 | 1 | | Poor |
| Glu_A vs. H2 | −4.164 | −8.4 | 0.99 | 0 | 0 | 1 | | Poor |
| K_A | 0.105 | 2.1 | 0.702 | 0.093 | 0.244 | 0.391 | Fair | |
| K_A vs. H1 | −1.058 | −25.9 | 0.792 | 0 | 0 | 1 | | Poor |
| K_A vs. H2 | −0.905 | −22.5 | 0.906 | 0 | 0 | 1 | | Poor |
| Na_A | 2.218 | 1.4 | −0.022 | 0.071 | 0.073 | 0.44 | Poor | |
| Na_A vs. H1 | 0.436 | 0.4 | −0.044 | 0.674 | 0.077 | 0.07 | | Poor |
| Na_A vs. H2 | 3.764 | 2.5 | −0.085 | 0 | 0 | 0.99 | | Poor |
| Phos_A | 0.045 | 0.5 | 0.89 | 0.55 | 0.708 | 0.091 | Good | |
| Phos_A vs. H1 | −0.0447 | −6.9 | 0.851 | 0 | 0 | 0.999 | | Poor |
| Phos_A vs. H2 | −0.429 | −6.7 | 0.879 | 0 | 0 | 1 | | Poor |
| TP_A | 0.093 | 2.6 | 0.936 | 0.022 | 0.015 | 0.641 | Good | |
| TP_A vs. H1 | −0.155 | −4.4 | 0.943 | 0 | 0 | 0.984 | | Poor |
| TP_A vs. H2 | −0.024 | −0.7 | 0.985 | 0.199 | 0.28 | 0.248 | | Excellent |

[a] Alb, albumin; AST, aspartate aminotransferase; Ca, calcium; CK, creatinine kinase; Glu, glucose; K, potassium, Na, sodium; P, phosphorus; TP, total protein. [b] _A is the intraclass within the VS20; _A vs. H1 compares the VS20 with the Hitachi 917 1st run; _A vs. H2 compares the VS2 with the Hitachi 917 2nd run. $N = 55$, $P < 0.05$. Data for Alb, AST, Ca, CK, Glu, K, Na and TP were log transformed.

assessment of the effect of error on clinical use.[35] The National Committee for Clinical Laboratory Standards, known as Clinical Laboratory Improvements Amendments (CLIA), established guidelines in human laboratory medicine to help standardize results and to determine reliability and accuracy of new testing methods.[35] No equivalent standards have been established for veterinary medicine. If we use the CLIA guidelines as a starting point for determining clinical relevance in our study, then very few parameters meet the acceptable performance requirements for both the point-of-care analyzer and the VDL. In the current study, only Na and K met CLIA standards for the Hitachi systems used at the VDL, and only Ca met CLIA standards for the VS2 analyzer. However, when compared with previously reported biochemical values from wild caught loggerhead turtles, the results in this study closely resemble those values, with the exceptions of Ca and TP levels, which were found to be notably lower in the current study.[2,9,14,32,36]

When compared with a recent study by Wolf et al.,[36] which evaluated five different chemistry analyzers for use in health assessments in sea turtle species, similar biochemistry results for two analytes, Na and TP, were found. However, notable differences were seen between the two studies for K, Ca, P, AST, and CK values. Several differences between the two studies exist. Twenty-two individuals were tested, 18 loggerhead, 3 green, and 1 Kemp's Ridley. Paired samples were not compared in that study, and whole blood was used for analysis in the Vetscan analyzer within 2 hr of collection as opposed to stored plasma samples that were used in the present study.

Although there are significant statistical differences detected within and among the various biochemical analyzers in this study, many of the

**Table 3.** Compiled results of statistical analysis for precision and agreement of the Hitachi 917 and the Hitachi 911 (original) chemistry analyzer.

| Blood parameter[a] | Mean difference | Relative error (%) | $r_i$ | Paired *t*–test | Wilcoxon signed rank test | Power of paired *t*–test | Precision | Agreement |
|---|---|---|---|---|---|---|---|---|
| Alb_H | −0.004 | 9.8 | 0.963 | 0.31 | 0.146 | 0.17 | Excellent | |
| Alb_old vs. H1 | −0.024 | 63.6 | 0.497 | 0.001 | 0 | 0.955 | | Poor |
| Alb_old vs. H2 | −0.02 | 63.9 | 0.499 | 0.002 | 0.001 | 0.893 | | Poor |
| AST_H | 5.7 | 5.8 | 0.994 | 0 | 0 | 1 | Poor | |
| AST_old vs. H1 | −135.776 | −78.6 | 0.71 | 0 | 0 | 1 | | Poor |
| AST_old vs. H2 | −141.316 | −83.2 | 0.715 | 0 | 0 | 1 | | Poor |
| Ca_H | 0.325 | 7.3 | 0.9 | 0 | 0 | 1 | Poor | |
| Ca_old vs. H1 | −1.645 | −33.7 | 0.279 | 0 | 0 | 1 | | Poor |
| Ca_old vs. H2 | −1.973 | −40.6 | 0.19 | 0 | 0 | 1 | | Poor |
| CK_H | −2.707 | 1.3 | 0.921 | 0.865 | 0 | 0.053 | Poor | |
| CK_old vs. H1 | −803.835 | −129.2 | 0.472 | 0 | 0 | 1 | | Poor |
| CK_old vs. H2 | −810.071 | −129.9 | 0.451 | 0 | 0 | 1 | | Poor |
| Glu_H | −0.01 | −0.1 | 0.98 | 0.983 | 0.028 | 0.05 | Good | |
| Glu_old vs. H1 | −0.827 | −1.5 | 0.8 | 0.548 | 0.023 | 0.092 | | Good |
| Glu_old vs. H2 | −0.816 | −1.4 | 0.791 | 0.561 | 0.072 | 0.089 | | Good |
| K_H | 0.001 | −0.1 | 0.965 | 0.938 | 0.673 | 0.051 | Excellent | |
| K_old vs. H1 | −0.048 | −1.4 | 0.844 | 0.065 | 0 | 0.455 | | Poor |
| K_old vs. H2 | −0.049 | −1.3 | 0.86 | 0.039 | 0 | 0.543 | | Poor |
| Na_H | 1.51 | 0.9 | 0.931 | 0 | 0 | 0.954 | Poor | |
| Na_old vs. H1 | 1.392 | 1.0 | −0.193 | 0.197 | 0.566 | 0.25 | | Poor |
| Na_old vs. H2 | −0.041 | 0.1 | 0.121 | 0.964 | 0 | 0.5 | | Poor |
| Phos_H | 0.231 | 3.9 | 0.959 | 0 | 0 | 1 | Poor | |
| Phos_old vs. H1 | −1.222 | −17.3 | 0.564 | 0 | 0 | 1 | | Poor |
| Phos_old vs. H2 | −1.447 | −21.0 | 0.589 | 0 | 0 | 1 | | Poor |
| TP_H | 0.004 | 0.1 | 0.994 | 0.661 | 0.408 | 0.072 | Excellent | |
| TP_old vs. H1 | 0.089 | 2.7 | 0.92 | 0.009 | 0.004 | 0.756 | | Poor |
| TP_old vs. H2 | 0.084 | 2.5 | 0.921 | 0.013 | 0.004 | 0.711 | | Poor |

[a] _H is the intraclass measurement of each parameter within the Hitachi 917; _old vs. H1 compares the Hitachi 911 with the Hitachi 917 1st run (H1);_old vs. H2 compares the Hitachi 911 with the Hitachi 917 2nd run (H2). $N = 98$. $P < 0.05$. Samples were potentially stored up to 2.5 yr between analysis. Alb, albumin; AST, aspartate aminotransferase; Ca, calcium; CK, creatinine kinase; Glu, glucose; K, potassium; Na, sodium; P, phosphorus; TP, total protein.

detected differences were not considered to be clinically significant. These include AST, CK, K, Na, TP, Glu, and P. Although correlation ($r_i$) was good for many of the biochemical parameters, agreement was determined to be poor between analyzers when looking at measures of statistical significance ($P < 0.05$). Reference intervals can be impacted by the diversity of the population being examined; the greater the diversity, the wider the reference intervals will be.[23] In this case, the population being examined is from an open ocean system, with potentially very different environmental exposure and nutrient ingestion. As mentioned previously, there can be significant variation between species and within a species, values can vary according to geographic location, the age, and its activity.[13] In addition, the degree of lipemia, hemolysis, and icterus can affect the outcome of the results. These factors have been shown to impact the performance of auto analyzers and can affect the results of a given test across testing methods and can vary significantly across species.[19]

The significant differences seen between the two Hitachi analyzers may be explained by changes in sample quality because of extended time in storage or from repeated freeze–thaw cycles. In the present study population ($n = 100$), the samples went through a minimum of three freeze–thaw cycles. This number may be higher because of additional studies that may have been performed with these samples. The discrepancy observed between the two Hitachi analyzers warrants additional investigation of the effects of long-term storage on sample integrity. However, a recent study that evaluated the effects of repeated freeze–thaw cycles in canine plasma did not result in clinically relevant changes in routine plasma biochemical constituents.[30] Another study that looked at the effects of storage and processing on nitrogenous compounds in ovine blood, found that plasma BUN, globulins, and

**Table 4.** Summary results of all three biochemical analyzers (mean ± SD), Hitachi 911, 917 and Abaxis Vetscan.

| Biochemical parameter | Hitachi 911 (VDL1)[a] | Hitachi 917 (VDL2) | | Abaxis Vetscan | |
|---|---|---|---|---|---|
| | Mean ± SD | Mean A ± SD | Mean B ± SD | Mean A ± SD | Mean B ± SD |
| Alb (g/dl) | 1.09 ± 0.38 | 1.06 ± 0.19 | 1.06 ± 0.19 | 1.26 ± 0.24 | 1.23 ± 0.21 |
| AST (IU/L) | 283.16 ± 118.8 | 102.15 ± 54.84 | 96.45 ± 52.29 | 92.1 ± 52.15 | 91.44 ± 51.8 |
| BA (μmol/L) | | 1 ± 0.82 | 0.7 ± 0.88 | <LD | <LD |
| Ca (mg/dl) | 6.01 ± 1.12 | 4.35 ± 1.41 | 4.03 ± 1.28 | 4.43 ± 0.73 | 4.43 ± 0.61 |
| CK (IU/L) | 1102.4 ± 737.2 | 285.71 ± 392.5 | 287.41 ± 398.85 | 327 ± 440.92 | 321.4 ± 434 |
| Glu (mg/dl) | 93.89 ± 21.26 | 91.84 ± 23.11 | 91.85 ± 23.01 | 96.8 ± 25.85 | 94.76 ± 24.4 |
| K (mmol/L) | 3.78 ± 0.45 | 3.71 ± 0.49 | 3.71 ± 0.46 | 4.6 ± 0.62 | 4.49 ± 0.55 |
| Na (mmol/L) | 155.85 ± 8.94 | 157.17 ± 4.18 | 155.68 ± 3.03 | 154 ± 7.04 | 152.2 ± 5.35 |
| P (mg/dl) | 7.49 ± 1.42 | 6.28 ± 1.13 | 6.05 ± 1.15 | 6.76 ± 1.23 | 6.72 ± 1.14 |
| TP (g/dl) | 3.25 ± 0.81 | 3.31 ± 0.83 | 3.31 ± 0.84 | 3.42 ± 0.82 | 3.33 ± 0.79 |
| UA (mg/dl) | | <LD | <LD | 0.67 ± 0.38 | 0.66 ± 0.42 |

[a] VDL, veterinary diagnostic laboratory; SD, standard deviation; Alb, albumin; AST, aspartate aminotransferase; BA, bile acids; LD, limits of detection; Ca, calcium; CK, creatinine kinase; Glu, glucose; K, potassium; Na, sodium; P, phosphorus; TP, total protein; UA, uric acid. Paired samples were run for on the Hitachi 917 and Vetscan, A = 1st run, B = 2nd run. Total samples numbers ($n$): Hitachi 911, $n = 98$ for all parameters, Hitachi 917, $n = 100$ for all parameters. Total sample numbers with the Abaxis Vetscan Alb ($n = 58$), AST ($n = 100$ and 98), Ca ($n = 72$ and 71), CK ($n = 99$ and 97), Glu ($n = 100$ and 98), K ($n = 100$ and 97), Phos ($n = 100$ and 98), TP ($n = 100$ and 98), UA ($n = 97$ and 89). Values that were below the limits of detection are represented by <LD.

ammonia values increased, whereas TP and other plasma proteins declined with length in storage.[22] Notably, the studies that have been reported in the literature have been short term, comparing values over a span of a few days, and would not account for the extended period of time these samples were stored.

Overall, the precision of the point-of-care analyzer, the Vetscan VS2 analyzer, was greater than that of the VDL analyzer. The point-of-care analyzer in this study may be a useful diagnostic tool for the health evaluation of loggerhead sea turtles when measuring AST, Ca, Glu, P, and TP. Based on measurements of precision, results for Alb, K, Na, and CK may need to be evaluated or confirmed with additional testing methods. Evaluation of bile acids is not recommended for the point-of-care analyzer at this time, because the values appear to be below the lower limits of measurement of the analyzer. Both analyzer types had poor levels of precision for Na and CK.

Based on the findings of this study, point-of–care analyzers can provide valuable adjunctive diagnostics, especially in field situations when rapid results are needed. The technical difficulty that was encountered with the point-of-care analyzer during this study has been resolved and is not considered to be a concern. As previously discussed, values should be interpreted with consideration given to the variability that can arise from demographic, geographic, and method variation, and, ideally, normal reference intervals should be established for a given species.

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