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Comparison of i-STAT® with Traditional Laboratory Analysers in the Measurement of Blood Analytes from Field Captured Dugongs (*Dugong dugon*)

Arthur W. Wong,[1] Janet M. Lanyon,[1*] Helen L. Sneath,[1]
Graham R. Leggatt,[2] and Lucy Woolford[3*]

[1]*School of Biological Sciences, The University of Queensland, St Lucia, Queensland 4072, Australia*
E-mail: awong@marspecialists.org
[2]*The University of Queensland Diamantina Institute, Translational Research Institute, The University of Queensland, Brisbane, Queensland 4102, Australia*
[3]*School of Veterinary Sciences, The University of Adelaide, Roseworthy Campus, Roseworthy, South Australia 5371, Australia*
**Authors contributed equally as academic advisors*

Abstract

Point-of-care (POC) testing is useful in field health assessments of wildlife when the condition of the captured animal must be immediately assessed and/or the location is remote from analytical laboratories. However, prior to their incorporation into clinical health assessment, POC devices must be assessed for potential measurement biases. In this study, the i-STAT® portable blood analyser was used to evaluate electrolytes (Na, K, and Cl), glucose, creatinine, lactate, urea, and haematocrit (Hct) of 85 apparently healthy dugongs (*Dugong dugon*) during field health assessments off the coast of southern Queensland, Australia. Blood levels of analytes measured by i-STAT® were compared to values reported by the Beckman Coulter AU400® and AU680® automated chemistry analysers, and the Sysmex XT-2000i™ (for Hct). Lactate and urea values were outside i-STAT®'s detectable limits. Bland-Altman plots identified constant biases for all measurable analytes except Hct. For most analytes, i-STAT® measurements did not show strong agreement with laboratory analysers; differences between paired measurements fell within calculated precision-based acceptance limits less than 75% of the time. Reference intervals for electrolytes, glucose, creatinine, and haematocrit as measured by i-STAT® are reported; however, these must be interpreted in light of measurement biases detected when compared with reference analysers.

Key Words: blood, point of care, reference ranges, health, i-STAT®, electrolytes, haematocrit, dugong, *Dugong dugon*

Introduction

Point-of-care (POC) diagnostic testing utilises instruments outside the reference laboratory environment within close proximity to the patient (Flatland et al., 2013). Small portable POC analysers (POCA) are particularly advantageous for the monitoring of wild species as these can provide on-site analysis when examining animals in remote locations and/or when transport of samples to a reference laboratory is delayed. However, results from POCA are not always comparable with reference laboratory instrumentation due to different methodologies used (Dimeski et al., 2010), and results from POCA that have not been appropriately validated can present risks to the accuracy of diagnosis (Jacobs et al., 2006).

Portable POCA which produce rapid results (15 to 140 s) and require smaller sample volumes (2.5 to 190 µL) than laboratory bench top analysers (Louie et al., 2000) are advantageous for wildlife species when sampling is difficult and opportunistic or when multiple tests must be conducted on a single sample. The i-STAT® whole blood analyser (Abbott Point of Care Inc., Princeton, NJ, USA) is one of the most commonly used POC instruments in veterinary and wildlife programs (Suzuki et al., 2001; Pereira et al., 2012; Rettenmund et al., 2014; Stoot et al., 2014). Utilising biosensor cartridges, this POCA reports only those analyte values that meet pre-programmed criteria for sensor integrity, calibration data, and sample quality (Erickson & Wilding, 1993).

In marine mammals, i-STAT® has been used to assess and develop reference ranges for analytes including Hct, Hgb, HCO_3, TCO_2, and urea, which may serve as predictors of mortality (e.g., in stranded

common dolphins [*Delphinus delphis*]; Sharp et al., 2014). In rescued harbor seal (*Phoca vitulina*) pups, triglyceride levels measured by i-STAT® have been used to assess nutritional improvement during rehabilitation (Witte et al., 2014). The i-STAT® device is also being evaluated in Florida manatee (*Trichechus manatus latirostris*) health assessments for the measure of lactate as an indicator of capture-related exertion, and for the diagnostic utility of other analyte measurements (Bonde et al., 2012).

Prior to clinical use and as part of ongoing quality management, the POCA should be evaluated for the species and for analytes of interest through comparability testing against reference analysers based on paired measurement differences or other methodology (Greenacre et al., 2008; McCain et al., 2010; Flatland et al., 2013; Stoot et al., 2014). Comparisons of analyte values between i-STAT® and reference analysers have shown variable agreement between studies and species. For example, in some fish species, i-STAT® data showed good agreement for pH but highly significant differences for Na, PCO_2, HCO_3, and PO_2 levels (Harter et al., 2014). In multiple reptile species, i-STAT® results showed high correlations for levels of Na, K, and Ca, but proportional biases for Cl and glucose (McCain et al., 2010). In the bar-headed goose, i-STAT® was reliable for measures of pH, PO_2, PCO_2, and Hct, but not for sO_2 and Hb (Harter et al., 2015). In treadmill exercised horses, i-STAT® showed good clinical agreement for levels of base excess, PCO_2, PO_2, lactate, HCO_3, and pH (Silverman & Birks, 2002).

Significant differences between i-STAT® and laboratory reference analysers have been reported for marine mammals (Larsen et al., 2002; Varela et al., 2006; Sharp et al., 2014). Na values generated by i-STAT® are reported to be lower than from reference analysers in Atlantic bottlenose dolphins (*Tursiops truncatus*) (Varela et al., 2006) and northern elephant seals (*Mirounga angustirostris*) (Larsen et al., 2002), and there may be a tendency for i-STAT® to report falsely elevated Cl levels in these species (Larsen et al., 2002; Varela et al., 2006; Sharp et al., 2014).

The aim of this study was to assess i-STAT® for its utility in the measurement of electrolytes (Na, K, Cl, and anion gap), glucose, creatinine, lactate, urea, and Hct in blood samples collected from 85 live wild dugongs (*Dugong dugon*) during health assessments. i-STAT® analyte levels were compared with those from reference laboratory analysers (Beckman Coulter AU400® & AU680®, and Sysmex XT-2000i™ for Hct) and were assessed for potential biases. Differences between i-STAT® and laboratory analysers were assessed by Bland-Altman plots and reported in terms of calculated acceptable limits based on the inherent imprecision of analysers. Reference intervals are provided for i-STAT® analytes. The results of this study are intended to provide a critical assessment of a POCA that is becoming increasingly popular in marine mammal health assessment.

Methods

Blood Collection and Measurement of Analytes

Blood samples from apparently healthy, live wild dugongs from Moreton Bay, Australia, were collected to establish serum biochemistry and haematology reference intervals (RIs) (Lanyon et al., 2015; Woolford et al., 2015). Dugong sampling was conducted at times to avoid temperature and humidity extremes—that is, in austral autumn (May-June) over 5 y with mean ambient daily air temperatures ranging between 20.3 and 22°C (mean 21°C ± 0.29), which is within i-STAT®'s normal operating range of 16 to 30°C (Abbott Point of Care, 2014f). Analytes from each blood sample were measured using basic laboratory diagnostics and the i-STAT® portable blood analyser (Abbott Laboratories, Abbott Park, IL, USA.

During out-of-water health assessments, dugongs were captured and placed by crane onto the deck of a boat (Lanyon et al., 2010, 2015). Apparent health of dugongs was assessed based on factors such as demeanour, body girths, and skin condition, and then classified as having poor, fair, good, very good, and excellent body condition (after Lanyon et al., 2015). Whole blood was collected from the brachial arteriovenous plexus of the dugong using a 21-gauge needle and a 5-ml sterile draw-in syringe to initiate blood flow (Lanyon et al., 2010, 2015) while it was lying in a prone position in ventral recumbency on a foam mattress. Immediately upon collection, two to three drops of whole blood from the syringe were used to fill each i-STAT® cartridge. The analytes Na, K, Cl, Ca, anion gap, glucose, creatinine, lactate, urea, and Hct were measured using the i-STAT® cartridges *EC8+* or *Chem8+*, and *CG8+* or *CG4+*.

A standard suite of serum biochemistry values were determined from two different automated analysers using serum collected into BD Vacutainer® clot activator tubes, and fluoride oxalate tubes for lactate and glucose (Lanyon et al., 2015). From 2008 to 2009, serum biochemistry samples (n = 26) were analysed by a Beckman Coulter AU400® automated chemistry analyser (Beckman Coulter Inc., Brea, CA, USA) by the School of Veterinary Science, The University of Queensland. From 2010 to 2014, serum samples (n = 59) were analysed using a Beckman Coulter AU680® by IDEXX Laboratories (Brisbane, Queensland, Australia). Biochemical analysis of frozen serum was conducted within weeks of original collection so as

to avoid potential issues of analyte deterioration. Haematocrit values from 2010 to 2014 (n = 52) that were analysed in this study were obtained from blood samples collected in BD Vacutainer® potassium EDTA anticoagulant tubes, which were shipped chilled for same day analysis (i.e., within 12 h) using a Sysmex XT-2000i™ analyser (SXT-2000i™ ; Sysmex Corp., Kobe, Hyogo, Japan) by IDEXX Laboratories in Brisbane (Woolford et al., 2015).

Data Analysis

Only apparently healthy dugongs were used in this study. Dugongs in poor to fair body condition were excluded from this analysis because an objective was to develop i-STAT® RIs for healthy dugongs, and their inclusion in the dataset did not improve the range of analyte measurements compared between analysers. Only analytes directly measured by i-STAT® and the laboratory analysers were compared in this study. i-STAT® values of Na, K, Cl, anion gap, glucose, and creatinine from healthy dugongs were compared to those obtained by the Beckman Coulter AU400® and AU680®. i-STAT® values for Hct were compared to those obtained from the SXT-2000i™ .

Statistical analyses were conducted using *STATISTICA*©, Version 12.5 (StatSoft, Inc., Tulsa, OK, USA). Pearson's correlation analyses were conducted to assess the significance of the linear relationships between i-STAT® analyte values and those obtained by the laboratory analysers to provide a basic measure of association between the two methods (Bland & Altman, 1999). Differences between analyser measurements were evaluated by Bland-Altman plots to identify mean differences or biases which may be indicative of systematic error (Bland & Altman, 1999; Hanneman, 2008). When a majority of i-STAT® measurements were greater or less than the laboratory analyser values, this was considered a constant bias. Standard deviation (SD) and confidence interval (CI) of the bias were considered measures of random error or relative imprecision of the i-STAT® compared to the laboratory analysers (Hanneman, 2008). Limits of agreement were defined by ± SD from the mean difference, and a comparison was deemed acceptable when 95% of differences fell within these limits (Flatland et al., 2013). A pattern of increasing or decreasing difference between the values generated by each analyser with increasing or decreasing combined mean of the two values were indicative of a proportional bias.

To determine the level of agreement between results based on imprecision data, acceptance limits for the difference between the i-STAT® and laboratory analysers were established for each analyte based on the combined inherent imprecision of the i-STAT® and the laboratory analyser to which it was compared. Imprecision of the analysers was determined using the coefficient of variation (CV) for each measured analyte. For i-STAT®, a high mean and low mean CV were provided for each analyte by the manufacturer based on aqueous controls. As per the manufacturer's recommendation, the i-STAT® CV which was most closely associated with the mean analyte value established from RIs of dugong serum biochemistry (Lanyon et al., 2015) and haematology (Woolford et al., 2015) was chosen. For the AU400® analyser and the SXT-2000i™ , CV values were provided by the manufacturers; and for the AU680® analyser, CV values were provided by IDEXX Laboratory based on internal quality control testing. For each analyte, a combined CV value was then calculated by the following formula: $\sqrt{CV^2_{\text{i-STAT}} + CV^2_{\text{lab analyser}}}$ (Jensen & Kjelgaard-Hansen, 2006). The acceptance limit was then calculated for each paired analyte measurement using the formula, Acceptance limit = $0 \pm (1.96 * CV_{\text{combined}} * \text{mean}_{\text{paired measurement}})$ (Jensen & Kjelgaard-Hansen, 2006). If the difference between i-STAT® and the laboratory analyser was within the calculated acceptance limit, then the i-STAT® analyte measurement was deemed to be acceptable. If ≥ 95% of measured differences fell within the acceptance limits, the methods were considered comparable with regards to inherent imprecision.

For proportional biases observed from the Bland-Altman analyses of i-STAT® Na measurements, additional correlation analyses were conducted to determine if these differences were due to possible interference from an associated increase in concentration of other blood analytes (Zoppi et al., 1993; Dimeski et al., 2010; Abbott Point of Care, 2014e). Correlation analyses were conducted between i-STAT® and AU680® measurement differences observed for Na by comparing these differences with AU680® anion gap values. Anion gap provides a measure of the increase in unmeasured anions (Stockham & Scott, 2008), which could possibly contribute to interference of Na values (Zoppi et al., 1993).

RIs were determined using *Reference Value Advisor* (*Ref Val*), Version 2.1 (Ecole Nationale Vétérinaire de Toulouse, 2012). RIs and CIs for dugong blood analytes measured by i-STAT® were established according to recommendations by the International Federation for Clinical Chemistry and the Clinical and Laboratory Standards Institute and adopted by the American Society for Veterinary Clinical Pathology (Friedrichs et al., 2012).

Results

Assessment of i-STAT® analyte measurements was conducted using blood collected from 85 apparently healthy dugongs. Not all blood analyte data were normally distributed. Descriptive statistics of the analytes that were assessed are summarized in Table 1. Lactate values in dugongs always exceeded the i-STAT® maximum measurable limit of 20 mmol/L, and these levels were confirmed as elevated above this value by reference laboratory analysers. Furthermore, for 72 of the 85 dugongs measured for urea, the analyser reported values that were below the minimum detection limit of 1 mmol/L. When compared to reference laboratory values, 62 of the 85 i-STAT® urea values were reported as below the minimum limit when the reference laboratory values were ≤ 1.8 mmol/L (Lanyon et al., 2015), suggesting the possibility of a constant negative bias. Due to these measurement limitations, lactate and urea levels indicated by i-STAT® were omitted from further analysis.

Correlation Analyses

For all tested analytes (except lactate and urea), there was a significant positive correlation with respective paired values between those measured by i-STAT® and those from the laboratory analysers (see Table 2 for details on sample sizes and statistical relationships). The measure of association between i-STAT® and laboratory analyser values varied depending on the analyte being measured. The Pearson's correlation coefficient value was lowest for Na (n = 56) between the i-STAT® and the AU680® (r = 0.3). This comparison also showed a wide range in 95% CIs of 0.04 to 0.56 mmol/L (Table 2). The greatest correlation coefficient value was for glucose measured by i-STAT® (n = 11) and the AU400® (r = 0.93, p < 0.001, 95% CI = 0.66 to 1.2; Table 2). All other i-STAT® and laboratory analyte value comparisons (i.e., K, Cl, creatinine, and Hct) indicated a moderate level of association (r = 0.54 to 0.71). However, the significance of the relationships between i-STAT® and laboratory analyte values was examined further by Bland-Altman analysis.

Table 1. Descriptive statistics of blood analytes from apparently healthy dugongs measured by i-STAT® in comparison to the AU400®, AU680®, and SXT-2000i™ analysers

Analytes	Machine	n	Mean	± SD	Min	Max
i-STAT® and AU400®						
Na (mmol/L)	i-STAT®	26	158	2.87	153	162
	AU 400®		162	3.30	156	168
K (mmol/L)	i-STAT®	26	5.5	0.50	4.7	6.5
	AU400®		5.9	0.49	5.0	6.8
Cl (mmol/L)	i-STAT®	26	117	3.42	111	123
	AU400®		109	3.99	101	115
Glucose (mmol/L)	i-STAT®	11	6.3	1.00	4.4	7.4
	AU400®		6.9	1.16	4.6	8.0
i-STAT® and AU680®						
Na (mmol/L)	i-STAT®	56	159	3.41	151	165
	AU680®		163	6.09	153	183
K (mmol/L)	i-STAT®	56	5.0	0.52	4.6	6.9
	AU680®		5.9	0.58	4.8	7.6
Cl (mmol/L)	i-STAT®	56	115	4.44	102	126
	AU680®		109	4.65	102	121
Glucose (mmol/L)	i-STAT®	57	5.9	1.00	3.4	8.0
	AU680®		6.5	1.17	4.0	8.7
Creatinine (mmol/L)	i-STAT®	37	0.06	0.01	0.03	0.09
	AU680®		0.07	0.01	0.05	0.10
i-STAT® and SXT-2000i™						
Hct (L/L)	i-STAT®	52	0.30	0.03	0.29	0.46
	SXT-2000i™		0.38	0.02	0.32	0.43

Bland-Altman Analyses and Calculated Acceptance Limits

Based on acceptance limits calculated from analyser CV values (Table 3), none of the i-STAT® measurements examined by Bland-Altman plots showed strong agreement with those obtained by laboratory analysers (Table 4). Analyte values from i-STAT® exhibited a variable range of biases in comparison to reference laboratory analysers, limiting the possibility for simple correction factors. The most measurements (75%) falling within the calculated precision-based acceptance limits were observed for Hct when i-STAT® values were compared with the SXT-2000i™ analyser.

Sodium (Na)—Bland-Altman analysis suggested that Na as measured by the i-STAT® (n = 26) was underestimated compared to AU400® values, with a constant bias of -3.81 ± 2.5 mmol/L (Table 4; Figure 1A). There was no proportional bias in differences in Na values over the combined mean range from 154.5 to 164.5 mmol/L (Figure 1A). The number of i-STAT® Na values within the acceptance limit for the AU400® was eight of 26 (30.8%) (Table 4).

Compared to the AU680®, i-STAT® underestimated levels of Na (n = 56) by -4.7 ± 6.01 mmol/L (Table 4; Figure 2). i-STAT® and AU680® had a combined mean range of Na from 152 to 171 mmol/L (Figure 1B). This wider combined mean range of Na levels revealed that for mean values beyond 165 mmol/L, i-STAT® develops a proportional bias, increasingly underestimating Na at higher mean values (Figure 1B). In comparison to the AU680®, 25 of 56 Na values by i-STAT® (44.6%) were within the calculated acceptance limit (Table 4). Increasing underestimation of Na levels by i-STAT® was associated with an increase in anion gap (r = -0.583, $p < 0.001$) (Figure 2).

Potassium (K)—K values obtained by i-STAT® (n = 26) compared to AU400® had a negative bias of -0.34 ± 0.47 mmol/L. i-STAT® and AU400® had a combined mean range of K from 4.85 to 6.65 mmol/L (Figure 3A). In comparison to the AU400®, three of 26 K measurements by i-STAT® (11.5%) fell within acceptance limits (Table 4). i-STAT® and AU680® had a combined mean range of K from 4.75 to 7.1 mmol/L (Figure 3B). K levels obtained by i-STAT® (n = 56) compared to

Table 2. Correlation analysis of i-STAT® measured in comparison to laboratory analysers

Analytes	i-STAT® comparison	*n*	*r*	*p*	95% CI	y-intercept	Slope
Na (mmol/L)	AU400®	26	0.68	< 0.001	0.37-0.99	62.20	0.59
	AU680®	56	0.30	0.023	0.04-0.56	130.90	0.17
K (mmol/L)	AU400®	26	0.55	0.004	0.19-0.90	2.30	0.55
	AU680®	56	0.60	< 0.001	0.38-0.82	2.36	0.53
Cl (mmol/L)	AU400®	26	0.61	0.001	0.27-0.94	59.95	0.52
	AU680®	56	0.54	< 0.001	0.31-0.77	58.20	0.52
Glucose (mmol/L)	AU400®	11	0.93	< 0.001	0.66-1.20	0.70	0.81
	AU680®	57	0.70	< 0.001	0.51-0.89	2.02	0.60
Creatinine (mmol/L)	AU680®	37	0.61	< 0.001	0.34-0.88	0.02	0.65
Hct (L/L)	SXT-2000i™	52	0.71	< 0.001	0.51-0.91	0.04	0.88

Abbreviations: Na = sodium, K = potassium, Cl = chloride, and Hct = haematocrit

Table 3. Machine imprecision for measuring different blood analytes as determined by the coefficient of variation (CV), and the calculated combined CV of i-STAT® with different laboratory analysers

Analytes	CV % i-STAT®	CV % AU400®	CV % AU680®	CV % SXT-2000i™	Combined CV i-STAT® and AU400®	Combined CV i-STAT® and AU680®	Combined CV i-STAT® and SXT-2000i™
Na	0.3	0.64	1.0	--	0.007	0.010	--
K	0.6	0.76	1.6	--	0.010	0.017	--
Cl	0.5	0.71	1.3	--	0.009	0.014	--
Glucose	0.8	0.97	1.2	--	0.013	0.014	--
Creatinine	4.8	2.00	4.0	--	0.052	0.062	--
Hct	1.5	--	--	1.50	--	--	0.021

Abbreviations: Na = sodium, K = potassium, Cl = chloride, Hct = haematocrit, and Combined CV = $\sqrt{}$ (CV2i-STAT + CV2lab analyser)

Table 4. Summary of Bland-Altman analysis of measured differences between i-STAT® and laboratory analyser results. The number of measurements falling within acceptance limits on the basis of the combined inherent imprecision of analysers is also provided.

Analytes	Machine compared to i-STAT®	*n*	Bias	SD	Bias confidence limit	Lower agreement limit (1.96 SD)	Upper agreement limit (+1.96SD)	i-STAT total % error	CB	PB	# within acceptance limit	% within acceptance limit
Na (mmol/L)	AU400®	26	-3.81	2.5	-9.79	-8.70	1.09	6.04	Y	N	8/26	30.8
	AU680®	56	-4.7	6.01	-14.1	-16.48	7.09	14.4	N	Y	25/56	44.6
K (mmol/L)	AU400®	26	-0.34	0.47	1.84	-1.26	0.58	31.4	Y	N	3/26	11.5
	AU680®	56	-0.41	0.5	1.94	-1.38	0.56	33.1	Y	N	16/56	28.6
Cl (mmol/L)	AU400®	26	7.85	3.32	13.00	1.34	14.40	11.9	Y	N	1/26	3.9
	AU680®	56	5.34	4.36	17.1	-3.21	13.90	15.6	Y	N	11/56	19.6
Glucose (mmol/L)	AU400®	11	-0.62	0.42	1.66	-1.45	0.21	24.0	Y	N	1/11	9.1
	AU680®	57	-0.56	0.85	3.33	-2.23	1.10	51.2	Y	N	10/57	17.5
Creatinine (mmol/L)	AU680®	37	-0.01	0.01	0.04	-0.03	0.01	54.4	Y	N	21/37	56.7
Hct (L/L)	SXT-2000i™	52	-0.005	0.02	0.09	-0.05	0.04	23.7	N	N	39/52	75.0

Abbreviations: Na = sodium, K = potassium, Cl = chloride, Hct = haematocrit, CB = Constant Bias, and PB = Proportional Bias; N = no, Y = yes.

AU680® had a negative bias of -0.41 ± 0.5 mmol/L (Table 4; Figure 3B). In comparison to the AU680®, 16 of 56 (28.6%) of K values by i-STAT® fell within the acceptance limit (Table 4).

Chloride (Cl)—Cl values determined by i-STAT® (n = 26) in comparison to AU400® had a positive bias of 7.85 ± 3.32 mmol/L (Table 4; Figure 4A). i-STAT® and AU400® had a combined mean range of Cl values from 107.5 to 118 mmol/L (Figure 4A). In comparison to the AU400®, one of 26 Cl measurements by i-STAT® (3.9%) were within the acceptable limit. Cl levels by i-STAT® (n = 56) in comparison to AU680® had a positive bias of 5.34 ± 4.36 mmol/L (Table 4; Figure 4B). i-STAT® and AU680® had a combined mean range of Cl levels from 102 to 120.5 mmol/L (Figure 4B). In comparison to the AU680®, 11 of 56 (19.6%) Cl values by i-STAT® were within the acceptance limit (Table 4).

Glucose—Glucose values measured by i-STAT® (n = 11) in comparison to AU400® were underestimated by -0.62 ± 0.42 mmol/L (Table 4; Figure 5A). i-STAT® and AU400® had a combined mean range of glucose levels from 4.5 to 7.55 mmol/L. In comparison to the AU400®, one of 11 (9.1%) measurements by i-STAT® was within the acceptable limit. Glucose levels by i-STAT® (n = 57) in comparison to the AU680® were underestimated by -0.56 ± 0.85 mmol/L (Table 4; Figure 5b). i-STAT® and AU680® had a combined mean range from 3.8 to 8.1 mmol/L (Figure 5B). When compared to the AU680®, 10 of 57 (17.5%) values by i-STAT® were within the acceptance limit (Table 4).

Creatinine—Creatinine values determined by i-STAT® (n = 37) in comparison to the AU680® were underestimated by -0.01 ± 0.01 mmol/L (Table 4; Figure 6). i-STAT® and AU680® had a combined mean range of creatinine levels from 0.4 to 0.9 mmol/L (Figure 6). i-STAT® values were within the acceptance limit of the AU680® on 21 of 37 (56.7%) occasions (Table 4).

Haematocrit (Hct)—Values of Hct determined by i-STAT® (n = 52) in comparison to the SXT-2000i™ had a negative measurement bias of -0.005 ± 0.02 L/L (Table 4; Figure 7). The i-STAT® and SXT-2000i™ had a combined mean range of Hct measurements from 0.31 to 0.42 L/L (Figure 7). i-STAT® Hct levels were within the acceptance limit for 39 of 52 (75%) times (Table 4).

Reference Intervals

As per recommendations of the American Society for Veterinary Clinical Pathology (ASVCP) guidelines for quality assurance for POC testing in veterinary medicine, instrument-specific RIs were established for each analyte examined using i-STAT® (Table 5). Paired sample comparison between i-STAT® and reference laboratories indicated bias and suboptimal correlation when compared with reference analysers. These biases may be of a magnitude to affect clinical interpretation of results, particularly for those animals whose measurements fall near the upper or lower limits of the RI.

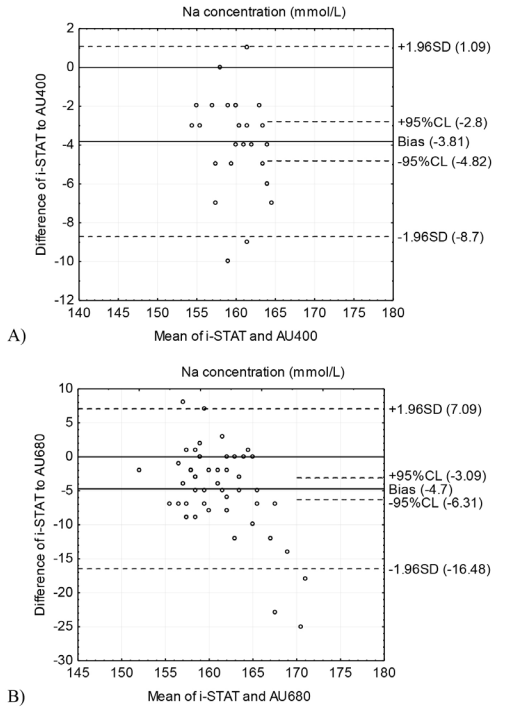

Figure 1. Bland-Altman plot of blood sodium (Na) levels measured by i-STAT® compared to the automated laboratory analysers Beckman Coulter AU400® (A) and AU680® (B), with mean (bias) and ± 1.96 SD, and 95% confidence limits (CL) based on single blood samples fraom wild dugongs. Na levels measured by i-STAT® in comparison to AU400® (n = 26) indicate a negative constant bias (A). In comparison to AU680®, i-STAT Na measurements (n = 56) show a negative proportional bias as sample Na concentration increases.

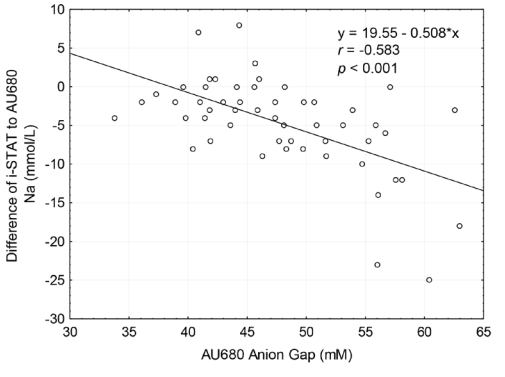

Figure 2. Correlation analysis of differences in blood Na levels measured by i-STAT® and AU680® blood in relation to increasing AU680® anion gap measurements. Differences between machine measurements become increasingly negative in relation to AU680® anion gap (r = -0.583, p < 0.001) for field-captured wild dugongs (n = 41).

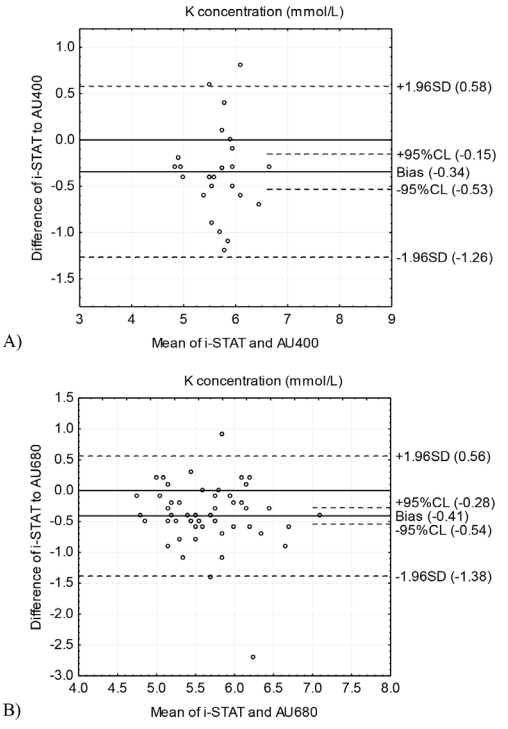

Figure 3. Bland-Altman plot of potassium (K) levels measured by i-STAT® compared to AU400® (A) and AU680® (B), with mean (bias) and ± 1.96 SD, and 95% CL based on single blood samples from wild dugongs. K levels measured by i-STAT® compared to AU400® (n = 26) indicate a negative constant bias (A). In comparison to AU680®, i-STAT® K values (n = 56) also show a negative constant bias (B).

Discussion

This assessment of the i-STAT® blood analyser as a point-of-care (POC) tool for the health assessment of captured wild dugongs identified a number of analytical biases, as well as a variable number of measurements falling within calculated acceptance criteria of laboratory analysers. Reasons for incongruity between some analyte values in this study are most likely due to differences in analytical methods and analytic parameters. Other factors such as differences in calibration and precision, and pre-analytical effects on samples must be considered. However, the information yielded through statistical comparison with reference analysers, combined with the generation and use of instrument-specific RIs, will assist researchers and laboratorians to maximize diagnostic utility and understanding of the i-STAT® for field examination of dugongs.

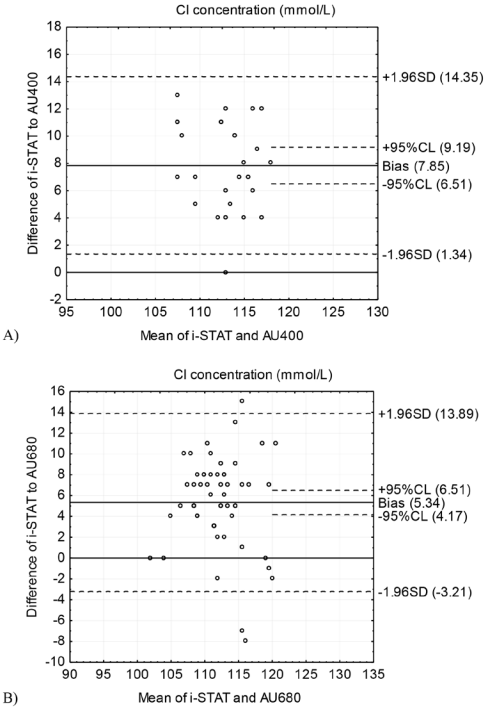

Figure 4. Bland-Altman plot of chloride (Cl) levels measured by i-STAT® compared to AU400® (A) and AU680® (B), with mean (bias) and ± 1.96 SD, and 95% CL based on single blood samples from wild dugongs. Cl levels measured by i-STAT® in comparison to AU400® ($n = 26$) indicate a positive constant bias (A). In comparison to AU680®, i-STAT® Cl values ($n = 56$) also show a positive constant bias (B).

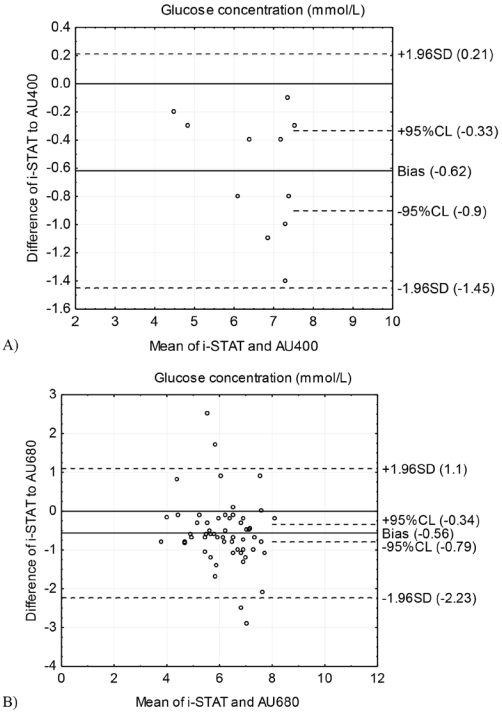

Figure 5. Bland-Altman plot of glucose levels measured by i-STAT® compared to AU400® (A) and AU680® (B), with mean (bias) and ± 1.96 SD, and 95% CL based on single blood samples from wild dugongs. Glucose values by i-STAT® compared to AU400® ($n = 11$) indicate a negative constant bias (A). In comparison to AU680®, i-STAT® glucose values ($n = 57$) show a negative constant bias (B).

Differences in analyte values between i-STAT® and laboratory analysers were apparent based on correlation analyses and Bland-Altman plots. i-STAT® measurements of Na, K, Cl, glucose, creatinine, and Hct did not correlate strongly with values determined from each of the AU400®, AU680®, and SXT-2000i™ analysers. i-STAT® Na measurements showed a proportional bias compared with the AU680®, while Na (AU400®), K, Cl, glucose, and creatinine had constant biases. Hct did not show a pronounced bias. These differences will likely also affect calculated values such as anion gap and haemoglobin (Abbott Point of Care, 2014d).

To accurately assess potential biases between machines, the recommended sample size of paired measurements is 40 to 100, with as wide a range in measurements as possible (Linnet, 1999). In this study, sample sizes for comparison between i-STAT® and AU680® fell within this range; however, for comparisons between i-STAT® and AU400® ($n \leq 26$) and for creatinine measurement comparison between i-STAT® and AU680® ($n = 37$), sample sizes were below the preferred minimum. Ranges of measurements can potentially be limited by sampling apparently healthy animals only since sick animals may show values significantly higher or lower than healthy animals (Greenacre et al., 2008; McCain et al., 2010). In this study, the range of analyte levels was not limited by sampling healthy dugongs only; however, because values for dugongs in poor to fair body condition were found to be within the ranges for healthy animals, these cohorts were not included in the final comparative analysis.

Due to logistical constraints, this study did not develop species-specific CV values for dugong blood analytes as is ideally recommended (Harr et al., 2013). However, as with similar studies, using species-specific CV values instead of manufacturer's CV values may not have significantly altered our overall findings (Greenacre et al., 2008; McCain et al., 2010). Manufacturer's CV

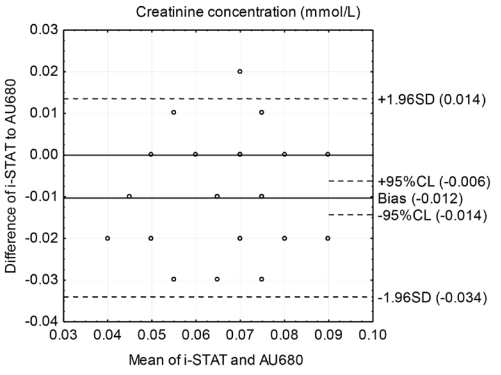

Figure 6. Bland-Altman plot of creatinine levels measured by i-STAT® and laboratory analyser Beckman Coulter AU680®, with mean (bias) and ± 1.96 SD, and 95% CL based on single blood samples taken from n = 37 wild dugongs. Distribution of data indicates a negative constant bias.

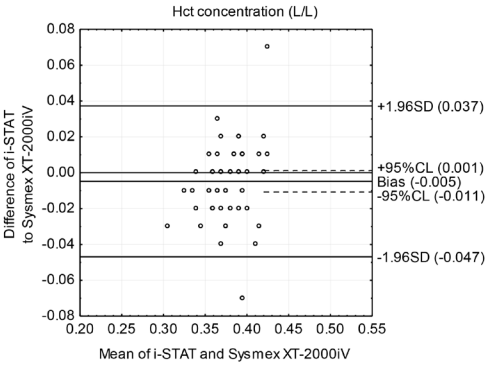

Figure 7. Bland-Altman plot of haematocrit (Hct) levels measured by i-STAT® and SXT-2000i™, with mean (bias) and ± 1.96 SD, and 95% CL based on single blood samples taken from n = 52 wild dugongs

values for POC and laboratory analysers are a logistical and cost-effective option for researchers. They provide an easily accessible point of reference for comparison of results between studies of different species when evaluating the utility of commercially available analysers.

Electrolytes: Na, K, and Cl

Observed differences in electrolyte levels between analysers likely reflect instrument methodology. Measurement of blood levels of the electrolytes Na, K, and Cl by i-STAT® are calculated by the Nernst equation through direct ion selective electrode (ISE) potentiometry with a reference calibrant solution (Abbott Point of Care, 2014f). This methodology differs from that of the AU400® and AU680® analysers, which both use an indirect ISE method (Beckman Coulter Inc., 2013). Factors apart from analyser methodology must also be considered to explain differences. Heparin-treated blood samples may cause i-STAT® to report lower Na levels (Vuillaume et al., 1999). However, this study used pure whole blood samples with no additives so avoided possible interference from heparin. The increasing underestimation of Na by i-STAT® with increasing concentrations of Na (compared to AU400®, the mean bias = -9.79 mmol/L; and compared to AU680®, the mean bias = -14.1 mmol/L) may have been due to interference by an associated increase in ionic strength. High ionic strength can reduce the recovery of measurable Na by direct potentiometry (Zoppi et al., 1993). The anion gap reference range for pursued and captured dugongs is between 36 to 63 mmol/L (Lanyon et al., 2015)—that is, higher and wider than that reported for most domestic mammalian species (Carlson & Bruss, 2008) and humans (3 to 11 mmol/L; Wilczynski, 2014).

Table 5. i-STAT® reference intervals (RIs) of blood analytes from field-captured dugongs in Moreton Bay, Australia. RI and confidence intervals (CIs) were calculated using nonparametric methods. For analytes for which n < 40, only descriptive statistics are provided.

Analytes	*N*	Mean	± SD	Min	Max	LRI (90% LCI)	URI (90% UCI)
Na (mmol/L)	82	158.5	3.2	151.0	165.0	152.1 (151-153.1)	164.9 (163-165)
K (mmol/L)	82	5.47	0.51	4.6	6.9	4.61 (4.6-4.8)	6.5 (6.3-6.9)
Cl (mmol/L)_	82	115.4	4.2	102.0	126.0	104.2 (102-111)	123.9 (122-126)
Glucose (mmol/L)	83	5.88	1.02	3.4	8.0	3.9 (3.4-4.2)	7.96 (7.29-8)
Creatinine (mmol/L)	37	0.63	0.01	0.03	0.09	--	--
Hct (L/L)	84	0.38	0.03	0.29	0.46	0.32 (0.29-0.32)	0.44 (0.423-0.46)

Abbreviations: Na = sodium, K = potassium, Cl = chloride, and Hct = haematocrit

The high levels of lactate reported in dugongs as a result of capture exertion (Lanyon et al., 2012) may contribute to the proportional bias of Na measurements that we observed in this study. While lactate levels were significantly elevated in dugongs, differential electrolyte measurements by i-STAT® can be affected by factors other than lactate.

Overall, K values measured by i-STAT® were lower than those by laboratory analysers (compared to AU400®, the mean bias = -0.34 mmol/L; and compared to AU680®, the mean bias = -0.41 mmol/L). This difference may be due to the more rapid time to processing from blood collection by i-STAT® compared to the laboratory analysers. Following collection of whole blood samples, there is an initial decrease in K levels as active live cells continue to uptake K to maintain osmotic equilibrium with the surrounding plasma (Penney, 2008). Over time, this cellular activity declines, and K begins to leak out of cells (Dimeski et al., 2010).

The relative overestimation of Cl by i-STAT® (mean bias of 7.85 and 5.34 mmol/L for AU400® and AU680®, respectively) was similar to previous studies (Pinckard et al., 2001; Varela et al., 2006; Sharp et al., 2014). Previously, the higher levels of Cl have been variously attributed to analytical error due to elevated blood urea from renal dysfunction, capture response, or dehydration (Pinckard et al., 2001; Varela et al., 2006; Sharp et al., 2014). However, in this study, laboratory urea levels were within the normal reference range, and the majority of i-STAT® urea levels were below the minimum detectable limit. The observed differences in Cl measurements between i-STAT® and laboratory analysers may simply be due to fundamental differences in method of measurement (i.e., direct ISE and indirect ISE, respectively).

Glucose

Glucose values by i-STAT® were lower compared to those by laboratory analysers (mean bias of -0.62 and -0.56 mmol/L for AU400® and AU680®, respectively). Again, this may reflect methodology. i-STAT® conducts an enzyme oxidation of glucose and determines glucose concentration via amperometric measurement relative to hydrogen peroxide freed in the reaction (Abbott Point of Care, 2014c). In contrast, laboratory analysers measure glucose through the process of phosphorylation and then reduction of phosphorylated products to NADH, which is proportional to the glucose concentration (Stein, 1965).

Creatinine

i-STAT® values of creatinine were slightly lower than the AU680® (mean bias = -0.01 mmol/L). i-STAT® measures creatinine amperometrically through a series of hydrolytic and oxidative reactions which free hydrogen peroxide molecules from creatinine (Abbott Point of Care, 2014b). The proportional levels of hydrogen peroxide molecules are then measured by the electrical current they produce when oxidized by the i-STAT®'s electrode (Abbott Point of Care, 2014b). In contrast, laboratory analysers measure creatinine using a modification of the Jaffe method which uses substances containing alkaline picrate to create a proportionally measurable colour reaction with creatinine (Jaffe, 1886).

Haematocrit (Hct)

i-STAT® values of Hct were slightly lower compared to the SXT-2000i™ (mean bias = -0.005 L/L). i-STAT® measures Hct conductometrically and corrects for electrolyte concentration (Abbott Point of Care, 2014d). In contrast, the SXT-2000i™ unit calculates Hct based on the measure of red blood cells using impedance technology (Abbott Laboratories, 2007; Hill et al., 2009). The slightly higher Hct levels measured by SXT2000i™ compared to i-STAT® may be due to differences in dugong erythrocyte osmolality in relation to the sample diluent used by the SXT-2000i™ . Dugongs, like other fully marine mammals, have serum osmolalities that are higher (345.2 ± 18.2 mOsm/kg, n = 13; Lanyon, unpub. data) than terrestrial mammals, including humans (reference range is 275 to 295 mOsm/kg; Waymouth, 1970; Tuazon et al., 2013). Haematocrit analysis of dugong cells by SXT-2000i™ may cause dugong erythrocytes to swell slightly when they are placed in the relatively hypotonic sample diluent designed for the analysis of human blood cells (Lilliehöök & Tvedten, 2009). Swelling of erythrocytes when measured by SXT-2000i™ may contribute to higher Hct when compared with i-STAT®.

Range Limitation: Lactate and Urea

i-STAT® is inappropriate for measurement of blood lactate in captured dugongs. Lactate levels are high in captured dugongs as a result of exertion due to pursuit associated with capture (Lanyon et al., 2015) and reach levels that are above the i-STAT®'s detectable range. Lactate measurement by i-STAT® may be useful in other settings where dugongs are not pursued prior to sampling such as during captive rehabilitation. i-STAT® amperometrically measures peroxide molecules derived from the enzymatic conversion of lactate to pyruvate by lactate oxidase (Abbott Point of Care, 2014d). Laboratory analysers typically use the

same reaction method but measure the free peroxide molecules photometrically based on a relative colour reaction (Sacks, 2012). For future consideration, a possible method to establish lactate within the measurable range of the i-STAT® could be to conduct sample dilution and subsequent dilution factor correction, but this approach would forfeit some simplicity of use of the POC device.

Blood urea measurements determined by i-STAT® had a negative constant bias in relation to laboratory analysers. All AU680® measurements of urea that were ≤ 1.8 mmol/L were below i-STAT®'s minimum detectable level of 1 mmol/L. To measure urea, each of the automated analysers in this study and the i-STAT® use an enzymatic reaction to hydrolyse urea and then measure the corresponding molecules of nicotinamide adenine dinucleotide (NADH) which are freed as a result of the reaction (Talke & Schubert, 1965; Abbott Point of Care, 2014a). The results of this study confirm the manufacturer's claim that i-STAT® reports urea at lower levels compared to automated analysers (Abbott Point of Care, 2014a).

Conclusion

The results from this study are intended to improve awareness of potential biases which may occur when using i-STAT® in field studies of dugongs or other wildlife species. The decision to use i-STAT® as a POC analyser should be determined by the clinician or researcher for their particular study setting based on comparative evaluation of analysers and identification of any biases that may be present. Lactate and urea as measured by i-STAT® are not applicable for dugongs under this study's capture conditions due to high levels of lactate occurring outside the i-STAT®'s detectable range. We recommend that i-STAT® results for field-captured dugongs be interpreted using the i-STAT®-specific RIs generated in this study; however, these should also be interpreted in light of the biases and correlations identified. The i-STAT® results from this study may serve as a point of reference for other marine mammal health studies considering the use of a POC device.

Acknowledgments

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