Article

Comparison of Hematocrit and Biochemical Analytes among Two Point-of-Care Analyzers (EPOC and i-STAT Alinity v) and a Veterinary Diagnostic Laboratory in the African Savanna Elephant (*Loxodonta africana*) and the Southern White Rhinoceros (*Ceratotherium simum simum*)

Hayley S. Stratton [1], Kimberly D. Ange-van Heugten [2,3] and Larry J. Minter [1,3,4,*]

[1] College of Veterinary Medicine, NC State University, 1060 William Moore Drive, Raleigh, NC 27606, USA
[2] Department of Animal Science, NC State University, 120 W Broughton Drive, Raleigh, NC 27695, USA
[3] Environmental Medicine Consortium, NC State University, Raleigh, NC 27606, USA
[4] Hanes Veterinary Medical Center, North Carolina Zoo, 4401 Zoo Parkway, Asheboro, NC 27205, USA
* Correspondence: jb.minter@nczoo.org

Abstract: This study compared hematocrit measured with the EPOC and i-STAT Alinity v point-of-care analyzers and manual measurement of packed cell volume in managed African savanna elephants (*Loxodonta africana*) and southern white rhinoceros (*Ceratotherium simum simum*). Biochemical analytes were also measured with the EPOC, i-STAT Alinity v, and a veterinary diagnostic laboratory in the same animals. Analytes assessed included blood urea nitrogen, chloride, creatinine, glucose, ionized calcium, potassium, and sodium. There were no differences for hematocrit values for African savanna elephants or southern white rhinoceros ($p \leq 0.05$). In African savanna elephants, there were no differences between the EPOC and i-STAT Alinity v analyzers for any measured analytes except ionized calcium. When compared to a veterinary diagnostic laboratory, there were differences for a majority of the biochemical analytes measured on the EPOC and i-STAT Alinity v analyzers in African savanna elephants. In southern white rhinoceros, there were differences for a majority of analytes among all three analyzers. While differences existed among the portable analyzers and a veterinary diagnostic laboratory for biochemical analytes in both species, these numerically small differences are unlikely to be clinically significant. For routine health care of African savanna elephants and southern white rhinoceros, these point-of-care analyzers may be a useful alternative to commercial analyzers for the parameters evaluated.

Keywords: biochemistry; *Ceratotherium simum simum*; EPOC analyzer; hematology; i-STAT Alinity v analyzer; *Loxodonta africana*; point-of-care analyzer

1. Introduction

The African savanna elephant (*Loxodonta africana*) and the southern white rhinoceros (*Ceratotherium simum simum*) are currently classified as endangered and near threatened, respectively, on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species [1,2]. Both of these populations are declining throughout their historical range due to poaching and increasing competition from expanding human populations [3–8]. Given their precarious populations, monitoring the health of both free-ranging animals and animals under human care is vital for the in situ and ex situ conservation of these species.

Hematological and biochemical analysis have proven to be useful tools for monitoring health and contributing to a complete clinical examination of elephants and rhinoceros [9–13]. The increased availability of portable point-of-care (POC) analyzers in veterinary medicine has facilitated plasma biochemistry and venous blood gas analysis in field settings in both

elephant and rhinoceros species [14–20]. POC analyzers perform blood analysis within minutes, allowing for more informed clinical decision making during veterinary procedures. Additionally, POC analyzers address some of the challenges of traditional benchtop analysis in these species including minimizing the effects of storage conditions and transport delays on results [21]. To maximize the utility of POC analyzers in clinical decision-making, POC analyzers should be validated for use in each species. Current guidelines published by the American Society of Veterinary Clinical Pathology (ASVCP) recommend comparison of test results from POC analyzers to those from a university, commercial, or reference laboratory [22]. Evaluation of POC analyzers for biochemical analysis has previously been performed in several wildlife species including American flamingos, Asian elephants, canvasbacks, cynomolgus macaques, loggerhead sea turtles, northern elephant seals, rockfish, Seminole killfish, and a variety of reptile species [19,23–31]. To the authors' knowledge, there are currently no reports comparing the Element POC (Heska, Loveland, CO 80538, USA) and i-STAT Alinity v (Zoetis, NJ 07054, USA) POC analyzers to a veterinary diagnostic laboratory for biochemical analysis in either the African savanna elephant or the southern white rhinoceros.

The Element POC or EPOC is a portable POC blood analyzer that is marketed for use in domestic animal species including dogs, cats, and horses. EPOC uses a single room-temperature test card to measure hematological, biochemical, and blood gas parameters [32]. The i-STAT Alinity v handheld analyzer is a handheld veterinary blood analyzer that is also marketed for use in domestic species including dogs, cats, and horses [33]. The i-STAT Alinity v utilizes refrigerated cartridges, and several different cartridges are available for measuring a range of analytes, including the CHEM8+ cartridge, which provides biochemical analysis (Zoetis, Parsippany, NJ 07054, USA) [33].

The objective of this research was to compare biochemical values obtained using the EPOC and i-STAT Alinity v POC analyzers to values obtained at a veterinary diagnostic laboratory for two zoological species under managed care: the African savanna elephant and the southern white rhinoceros. Additionally, this research compared hematocrit values obtained using the EPOC and i-STAT Alinity v POC analyzers to manual measurement of packed cell volume. We hypothesized that there would be no significant differences for biochemical values measured among the POC analyzers and the veterinary diagnostic laboratory or for hematocrit measured among the POC analyzers and manual measurement of packed cell volume for either species.

2. Materials and Methods

2.1. Animals and Sample Collection

Six African savanna elephants (3 males and 3 females; age range 18–49 years) and 9 southern white rhinoceros (9 females; age range 1–53 years) managed by the North Carolina (NC) Zoo, Asheboro, NC, USA had blood collected during routine quarterly wellness examinations between August 2021 and October 2021. All animals included in this study were deemed to be clinically healthy based on physical examination and routine diagnostic testing at the time of sample collection. This research was conducted under the guidelines and approval of the North Carolina Zoo Research Committee.

All blood samples used in this study were collected voluntarily using positive re-inforcement behavioral training techniques. For the African savanna elephants, blood was collected from the auricular vein using a 20-gauge butterfly needle with a vacutainer attachment (Becton, Dickinson, and Company, NJ 07417, USA). For the southern white rhinoceros, blood was collected from the medial radial vein using the same methodology. Blood samples were collected into serum separator vacutainer tubes (Becton, Dickinson, and Company, NJ 07417, USA) and vacutainer tubes containing the anticoagulant lithium heparin (Becton, Dickinson, and Company, NJ 07417, USA). Following collection at the animal enclosures, samples were transported to the NC Zoo veterinary hospital laboratory within 5 min of sample collection for further processing.

2.2. Hematological and Biochemical Analysis

At the NC Zoo veterinary hospital laboratory, non-heparinized hematocrit tubes were filled from the lithium heparin-coated vacutainer tubes and centrifuged at a g-force of 11,359 for 5 min to allow for the manual measurement of packed cell volume for each animal.

The blood samples collected into serum separator vacutainer tubes were centrifuged at a g-force of 2840 for 10 min. The serum samples were shipped overnight on ice in insulated packaging to a commercial veterinary diagnostic laboratory (Antech Diagnostics, Inc., Fountain Valley, CA 92708 USA). Serum samples were analyzed using an AU5800 Series Clinical Chemistry Analyzer (Beckman Coulter, Brea, CA 92821, USA) within 24 h of venipuncture.

Whole blood samples collected into lithium heparin vacutainer tubes were utilized for biochemical analysis at the NC Zoo veterinary hospital laboratory using both the EPOC and i-STAT Alinity v POC analyzers. The EPOC cartridge measured the following analytes: pH, partial pressure of carbon dioxide, partial pressure of oxygen, sodium, chloride, potassium, ionized calcium, creatinine, blood urea nitrogen (BUN), glucose, lactate, and hematocrit. The EPOC also calculated the following analytes: total carbon dioxide, bicarbonate, base excess of extracellular fluid, base excess of blood, anion gap, oxygen saturation, and hemoglobin. The i-STAT CHEM8+ cartridge was used for sample analysis on the i-STAT Alinity v analyzer. Analytes measured by the CHEM8+ cartridge include sodium, potassium, chloride, total carbon dioxide, ionized calcium, glucose, BUN, creatinine, and hematocrit. The CHEM8+ cartridge also calculated anion gap and hemoglobin. Samples were analyzed using the EPOC and i-STAT Alinity v analyzers within 15 min of venipuncture.

Only analytes that could be measured by both handheld analyzers were assessed in this study. Specific analytes assessed include BUN, chloride, creatinine, glucose, hematocrit, ionized calcium, potassium, and sodium. Hematocrit was compared between the EPOC analyzer, the i-STAT Alinity v analyzer, and manual measurement of packed cell volume. Six biochemical analytes (BUN, chloride, creatinine, glucose, ionized calcium, potassium, and sodium) were compared between the veterinary diagnostic laboratory, the EPOC analyzer, and the i-STAT Alinity v analyzer. For ionized calcium, only comparison between the EPOC analyzer and the i-STAT Alinity v analyzer was performed, as ionized calcium was not measured by the veterinary diagnostic laboratory.

2.3. Statistical Analysis

All statistical analyses were performed in RStudio version 1.4.1717 (Boston, MA 02210, USA). Given the small sample sizes for both the African savanna elephant and the southern white rhinoceros, non-parametric testing was utilized. A Wilcoxon signed-rank test was used in both species for pairwise comparison of the specific analyzers used to measure each analyte. Statistical significance for all analyses was set at $p \leq 0.05$. Accuracy and precision were not evaluated for any of the analyzers assessed in this study.

3. Results

There were no significant differences for hematocrit values for African savanna elephants or southern white rhinoceros when comparing the EPOC and i-STAT Alinity v analyzers to the manual measurement of packed cell volume or when comparing the EPOC and i-STAT Alinity v analyzers to each other (Tables 1–3).

Table 1. Descriptive statistics of hematocrit and biochemical values in African savanna elephants (*Loxodonta africana*; n = 6) and southern white rhinoceros (*Ceratotherium simum simum*; n = 9) measured by the EPOC analyzer, manual measurement of packed cell volume (PCV), and a veterinary diagnostic laboratory (VDL).

Analyte	Analyzer	African Savannah Elephants				Southern White Rhinoceros			
		Mean ± SEM	Median	IQR (Q1–Q3)	Min–Max	Mean ± SEM	Median	IQR (Q1–Q3)	Min–Max
Hematocrit (%)	PCV	34.7 ± 0.56	35.0	33.5–35.8	33–36	34.8 ± 1.38	35.0	33.0–36.0	28–42
	EPOC	37.3 ± 1.41	37.0	35.5–38.5	33–43	36.1 ± 2.02	36.0	32.0–37.0	27–47
Sodium (mmol/L)	VDL	130.2 ± 0.79 [a]	130.5	128.5–131.0	128–133	132.3 ± 0.58	132.0	131.0–134.0	130–135
	EPOC	129.0 ± 0.73 [b]	128.5	128.0–129.8	127–132	133.4 ± 0.73	134.0	132.0–135.0	130–137
Potassium (mmol/L)	VDL	4.5 ± 0.14 [a]	4.6	4.4–4.6	4.0–5.0	4.3 ± 0.07	4.4	4.2–4.4	4.0–4.6
	EPOC	4.3 ± 0.18 [b]	4.3	4.1–4.5	3.6–4.9	4.3 ± 0.05	4.4	4.2–4.4	4.1–4.5
Chloride (mmol/L)	VDL	88.0 ± 1.03	87.0	87.0–89.3	85–92	91.7 ± 1.00 [a]	91.0	91.0–93.0	86–97
	EPOC	90.2 ± 1.08	89.5	88.3–90.8	88–95	96.0 ± 1.20 [b]	96.0	94.0–99.0	89–100
Glucose (mg/dL)	VDL	88.7 ± 6.96 [a]	87.5	76.3–99.5	68–113	66.4 ± 4.21 [a]	68.0	61.0–76.0	47–82
	EPOC	96.8 ± 7.23 [b]	93.5	85.0–108.0	76–123	73.3 ± 4.28 [b]	79.0	64.0–82.0	55–89
Blood Urea Nitrogen (mg/dL)	VDL	7.7 ± 0.33 [a]	7.5	7.0–8.0	7–9	17.6 ± 0.93 [a]	18.0	16.0–20.0	13–21
	EPOC	5.7 ± 0.61 [b]	6.0	5.3–6.8	3–7	16.1 ± 0.99 [b]	16.0	15.0–18.0	12–20
Creatinine (mg/dL)	VDL	1.7 ± 0.21 [a]	1.6	1.4–1.8	1.3–2.7	1.2 ± 0.08 [a]	1.3	1.0–1.3	0.9–1.5
	EPOC	2.1 ± 0.23 [b]	1.9	1.8–2.3	1.7–3.1	1.5 ± 0.12 [b]	1.6	1.1–1.8	1.0–1.9

[a,b] Denotes statistically significant difference between the PCV or VDL and the EPOC analyzer based on Wilcoxon signed-rank analysis test ($p \leq 0.05$).

Table 2. Descriptive statistics of hematocrit and biochemical values in African savanna elephants (*Loxodonta africana*; *n* = 6) and southern white rhinoceros (*Ceratotherium simum simum*; *n* = 9) measured by the i-STAT Alinity v analyzer, manual measurement of packed cell volume (PCV), and a veterinary diagnostic laboratory (VDL).

| Analyte | Analyzer | African Savannah Elephants | | | | Southern White Rhinoceros | | | |
		Mean ± SEM	Median	IQR (Q1–Q3)	Min–Max	Mean ± SEM	Median	IQR (Q1–Q3)	Min–Max
Hematocrit (%)	PCV	34.7 ± 0.56	35.0	33.5–35.8	33–36	34.8 ± 1.38	35.0	33.0–36.0	28–42
	i-STAT	35.0 ± 0.86	35.0	34.0–36.0	32–38	35.3 ± 1.57	35.0	33.0–37.0	28–44
Sodium (mmol/L)	VDL	130.2 ± 0.79	130.5	128.5–131.0	128–133	132.3 ± 0.58	132.0	131.0–134.0	130–135
	i-STAT	129.2 ± 0.87	128.5	128.0–129.8	127–133	133.0 ± 0.67	133.0	132.0–134.0	129–136
Potassium (mmol/L)	VDL	4.5 ± 0.14 [a]	4.6	4.4–4.6	4.0–5.0	4.3 ± 0.07 [a]	4.4	4.2–4.4	4.0–4.6
	i-STAT	4.2 ± 0.15 [b]	4.2	4.0–4.4	3.6–4.7	4.2 ± 0.06 [b]	4.3	4.1–4.4	3.9–4.4
Chloride (mmol/L)	VDL	88.0 ± 1.03	87.0	87.0–89.3	85–92	91.7 ± 1.00 [a]	91.0	91.0–93.0	86–97
	i-STAT	88.7 ± 1.09	88.5	86.5–89.8	86–93	94.3 ± 1.08 [b]	94.0	93.0–97.0	88–98
Glucose (mg/dL)	VDL	88.7 ± 6.96 [a]	87.5	76.3–99.5	68–113	66.4 ± 4.21 [a]	68.0	61.0–76.0	47–82
	i-STAT	94.5 ± 6.32 [b]	91.0	82.8–106.0	78–116	73.4 ± 4.62 [b]	77.0	66.0–84.0	50–89
Blood Urea Nitrogen (mg/dL)	VDL	7.7 ± 0.33 [a]	7.5	7.0–8.0	7–9	17.6 ± 0.93	18.0	16.0–20.0	13–21
	i-STAT	6.0 ± 0.37 [b]	6.0	5.3–6.8	5–7	18.0 ± 1.00	19.0	15.0–20.0	14–22
Creatinine (mg/dL)	VDL	1.7 ± 0.21 [a]	1.6	1.4–1.8	1.3–2.7	1.2 ± 0.08 [a]	1.3	1.0–1.3	0.9–1.5
	i-STAT	2.0 ± 0.28 [b]	1.8	1.6–2.1	1.5–3.3	1.3 ± 0.12 [b]	1.5	1.0–1.6	0.9–1.8

[a,b] Denotes statistically significant difference between the PCV or VDL and the i-STAT Alinity v analyzer based on Wilcoxon signed-rank analysis test ($p \leq 0.05$).

Table 3. Descriptive statistics of hematocrit and biochemical values in African savanna elephants (*Loxodonta africana*; *n* = 6) and southern white rhinoceros (*Ceratotherium simum simum*; *n* = 9) measured by the i-STAT Alinity v and EPOC analyzers.

Analyte	Analyzer	African Savannah Elephants				Southern White Rhinoceros			
		Mean ± SEM	Median	IQR (Q1–Q3)	Min–Max	Mean ± SEM	Median	IQR (Q1–Q3)	Min–Max
Hematocrit (%)	i-STAT	35.0 ± 0.86	35.0	34.0–36.0	32–38	35.3 ± 1.57	35.0	33.0–37.0	28–44
	EPOC	37.3 ± 1.41	37.0	35.5–38.5	33–43	36.1 ± 2.02	36.0	32.0–37.0	27–47
Sodium (mmol/L)	i-STAT	129.2 ± 0.87	128.5	128.0–129.8	127–133	133.0 ± 0.67	133.0	132.0–134.0	129–136
	EPOC	129.0 ± 0.73	128.5	128.0–129.8	127–132	133.4 ± 0.73	134.0	132.0–135.0	130–137
Potassium (mmol/L)	i-STAT	4.2 ± 0.15	4.2	4.0–4.4	3.6–4.7	4.2 ± 0.06 [a]	4.3	4.1–4.4	3.9–4.4
	EPOC	4.3 ± 0.18	4.3	4.1–4.5	3.6–4.9	4.3 ± 0.05 [b]	4.4	4.2–4.4	4.1–4.5
Chloride (mmol/L)	i-STAT	88.7 ± 1.09	88.5	86.5–89.8	86–93	94.3 ± 1.08 [a]	94.0	93.0–97.0	88–98
	EPOC	90.2 ± 1.08	89.5	88.3–90.8	88–95	96.0 ± 1.20 [b]	96.0	94.0–99.0	89–100
Glucose (mg/dL)	i-STAT	94.5 ± 6.32	91.0	82.8–106.0	78–116	73.4 ± 4.62	77.0	66.0–84.0	50–89
	EPOC	96.8 ± 7.23	93.5	85.0–108.0	76–123	73.3 ± 4.28	79.0	64.0–82.0	55–89
Blood Urea Nitrogen (mg/dL)	i-STAT	6.0 ± 0.37	6.0	5.3–6.8	5–7	18.0 ± 1.00 [a]	19.0	15.0–20.0	14–22
	EPOC	5.7 ± 0.61	6.0	5.3–6.8	3–7	16.1 ± 0.99 [b]	16.0	15.0–18.0	12–20
Creatinine (mg/dL)	i-STAT	2.0 ± 0.28	1.8	1.6–2.1	1.5–3.3	1.3 ± 0.12 [a]	1.5	1.0–1.6	0.9–1.8
	EPOC	2.1 ± 0.23	1.9	1.8–2.3	1.7–3.1	1.5 ± 0.12 [b]	1.6	1.1–1.8	1.0–1.9
Ionized Calcium (mmol/L)	i-STAT	1.29 ± 0.023 [a]	1.30	1.25–1.33	1.21–1.35	1.54 ± 0.035 [a]	1.59	1.47–1.62	1.35–1.64
	EPOC	1.22 ± 0.033 [b]	1.21	1.18–1.28	1.10–1.32	1.47 ± 0.032 [b]	1.47	1.40–1.53	1.34–1.63

[a,b] Denotes statistically significant difference between the i-STAT Alinity v analyzer and the EPOC analyzer based on Wilcoxon signed-rank analysis test ($p \leq 0.05$).

In the African savanna elephants, significant differences were found when comparing the EPOC analyzer to the veterinary diagnostic laboratory for 5 of the 6 biochemical analytes compared (sodium, potassium, glucose, BUN, and creatinine) (Table 1). When comparing the i-STAT Alinity v analyzer to the veterinary diagnostic laboratory, there were significant differences for 4 of the 6 biochemical analytes compared (potassium, glucose, BUN, and creatinine) (Table 2). There was no significant difference between the EPOC and i-STAT Alinity v analyzers for any of the measured biochemical analytes except for ionized calcium (Table 3).

In the southern white rhinoceros, significant differences were found when comparing the EPOC analyzer to the veterinary diagnostic laboratory for 4 of the 6 biochemical analytes compared (chloride, glucose, BUN, and creatinine) (Table 1). When comparing the i-STAT Alinity v analyzer to the veterinary diagnostic laboratory, there were significant differences for 4 of the 6 biochemical analytes compared (potassium, chloride, glucose, and creatinine) (Table 2). When comparing the EPOC and i-STAT Alinity v analyzers, there were significant differences for 5 of the 7 biochemical analytes compared (potassium, chloride, BUN, creatinine, and ionized calcium) (Table 3).

4. Discussion

There were no significant differences for hematocrit values for African savanna elephants or southern white rhinoceros when comparing the EPOC and i-STAT Alinity v analyzers to the manual measurement of packed cell volume. Prior studies in other species have identified significant differences between manual and automated measurements of hematocrit [34,35]. This may be due to plasma trapping within erythrocytes in centrifuged samples for manual measurement [34–36]. Despite these prior studies, manual measurement of packed cell volume was chosen for comparison to the POC analyzers as it is the typical method of analysis performed at the NC Zoo veterinary hospital laboratory. Manual measurement of packed cell volume is a widely used diagnostic tool due to its low cost, availability of equipment, ease of performance, and ability to obtain rapid analysis. The results of this study suggest that the difference between manual and automated measurement of hematocrit may be minimal in the African savanna elephant and southern white rhinoceros.

In the African savanna elephants analyzed in this study, there were no statistically significant differences for any of the assessed biochemical analytes between the EPOC and i-STAT Alinity v analyzers except for ionized calcium.

Ionized calcium is a clinically significant analyte in elephants. There is emerging evidence that hypocalcemia is a common problem in managed populations of elephants [37]. For example, in Asian elephants, providing calcium-rich diets has been shown to increase total and ionized calcium levels, suggesting that subclinical hypocalcemia may be prevalent in managed herds [37,38]. Both clinical and subclinical hypocalcemia in elephants may lead to complications during the periparturient period, including dystocia and metabolic bone disease [37,39,40]. To prevent these complications, calcium supplementation is recommended during parturition if ionized calcium drops below 1.20 mmol/L [37,39]. Thus, confidence in an ionized calcium concentration measured by a POC analyzer is valuable in the routine health management of elephants under human care.

Ionized calcium was higher when measured on the i-STAT Alinity v compared to the EPOC for every individual analyzed in this study, suggesting an intrinsic difference in analysis between the two analyzers; however, while there was a statistically significant difference for ionized calcium measurements between the EPOC and i-STAT Alinity v analyzers, this difference is unlikely to be clinically significant. Results of a recent study report mean plasma ionized calcium levels in captive African elephants as 1.23 ± 0.05 mmol/L in summer and 1.19 ± 0.06 mmol/L in winter [38]. The values provided by both POC analyzers in this study were clinically similar to the summer value reported by van Sonsbeek et al. (2013) [38]. For the EPOC analyzer, 3 out of 6 individuals measured within one standard deviation of the summer value reported by

van Sonsbeek et al., 2013 (1.18 to 1.28 mmol/L), with one individual measuring below this range and two individuals measuring above this range (Table 3) [38]. For the i-STAT Alinity v analyzer, 3 out of 6 individuals measured within one standard deviation of the van Sonsbeek et al. (2013) summer value, with the other three individuals measuring above this range (Table 3) [38]. While the blood samples in this study were collected over a time frame spanning summer and fall (August to October 2021), the summer value reported by van Sonsbeek at al., 2013 was chosen for comparison due to the subtropical climate in North Carolina, USA where samples were collected.

Based on the results of this study, it is reasonable to consider the EPOC and i-STAT Alinity v analyzers as alternative POC analyzers to each other for the majority of biochemical analytes in African savanna elephants.

While statistically significant differences existed between the portable analyzers and the veterinary diagnostic laboratory for the majority of biochemical analytes in both species, the information provided by these analyzers is likely still clinically useful based on similarities to previously published values from other healthy individuals; however, due to the small sample populations utilized in this study, the values in this current study are not reference intervals.

In African elephants, published reference interval studies have previously been performed; however, the analytical methods used in most of these studies are outdated or minimally described [41,42]. A recent study by Steyrer et al. (2021) developed reference intervals for hematology and select clinical chemistry values in free-ranging African elephants [43]; however, the biochemical analytes evaluated by Steyrer et al. (2021) (albumin, alkaline phosphatase, aspartate aminotransferase, calcium, creatinine kinase, gamma glutamyl transferase, globulin, magnesium, phosphorous, total protein, and urea) did not include any of the biochemical analytes measured in this study. Another recent study by Wood et al. (2020) evaluated circulating plasma biochemical concentrations in a population of managed African elephants (*n* = 6) [44]. The mean values for all analytes in this current study population of African savanna elephants were clinically similar to the mean values published by Wood et al. (2020) for sodium, potassium, glucose, BUN, and creatinine [44]. Chloride values were not reported by Wood et al. (2020) [44]. Of note, significant bias may exist when comparing these values as the majority of animals in the sample population utilized by Wood et al. (2020) were also included in this study's population.

For southern white rhinoceros, there were significant differences for a majority of biochemical analytes among the analyzers evaluated. For southern white rhinoceros, the published reference interval for ionized calcium measured on the i-STAT Alinity v is 1.36–1.56 mmol/L [20]. The mean values for ionized calcium on the two portable analyzers in this study were both within this reference range (1.47 and 1.54 mmol/L for the EPOC and i-STAT Alinity v analyzers, respectively). Trivedi et al. (2021) also published values for sodium, potassium chloride, glucose, BUN, and creatinine measured on the i-STAT Alinity v in managed southern white rhinoceros (*n* = 10) [20]. The mean values for sodium, potassium, chloride, and creatinine measured on all three analyzers in this study were comparable to the values published by Trivedi et al. (2021) (132.5 mmol/L, 4.24 mmol/L, 92.6 mmol/L, and 1.29 mg/dL, respectively) [20]. All individual measured values for glucose in the rhinoceros in this study were lower than the mean value (89.5 $\pm$ 11.05 mg/dL) published by Trivedi et al. (2021). The range reported by Trivedi et al. (2021) indicates a right-skewed distribution (70–181 mg/dL) with the animal having a glucose of 181 mg/dL increasing the mean compared to the median. As the median value reported by Trivedi et al. (2021) (74.5 mg/dL) is more similar to the values obtained in this study, the authors suspect that the right-skewed distribution is the most likely explanation for this discrepancy [20]; however, other possibilities could be considered including diet and stress.

For BUN, the mean value published by Trivedi et al. (2021) for managed animals was 8.1 mg/dL [20]. All individual BUN measurements for southern white rhinoceros in this study were higher than the published value with a mean of 16.1, 18.0, and 17.6 mg/dL for

the EPOC analyzer, i-STAT Alinity v, and veterinary diagnostic laboratory, respectively. Interestingly, the mean values for BUN obtained in this study were more closely aligned with the value reported by Trivedi et al. (2021) for free-ranging southern white rhinoceros ($n = 30$; 15.8 ± 0.51 mg/dL). A reason for the difference in BUN measurements for managed southern white rhinoceros between studies is not clear. Of note, 6 of the 9 southern white rhinoceros utilized in this study were also included in the sample population of 10 southern white rhinoceros utilized by Trivedi et al. (2021) [20]; thus, external factors such as environment, diet, seasonal, or reproductive changes should be considered as potential causes for the differences in measured BUN values. Interestingly, in the study conducted by Trivedi et al. (2021), samples were collected from southern white rhinoceros at the North Carolina Zoo in February. In this study, samples were collected between August through October. This may suggest a seasonal change to BUN. In February, the animals were likely housed in the barns more frequently due to colder temperatures, and they would have been in close proximity to water sources. In contrast, during August through October, the animals spend more time on their outdoor exhibit. The outdoor exhibit encompasses 40 acres, and the animals would have had to walk further to reach their water sources. The authors hypothesize that BUN may be lower in the winter months due to easier access to water and increased water consumption. This hypothesis may also explain why the mean values for BUN obtained in this study were more similar to the values obtained by Trivedi et al. (2021) for free-ranging southern white rhinoceros, as the outdoor exhibit is meant to mirror the animals' natural habitat [20]. Based on this hypothesis, if concerns for elevated BUN exist, management should be evaluated to ensure that animals have adequate access to water sources.

Although the majority of measured values were comparable to previously published values for both African savanna elephants and southern white rhinoceros, clinicians should use their own clinical judgment when interpreting values produced by the EPOC and i-STAT Alinity v POC analyzers in these species. Based on the statistically significant differences for biochemical analytes between both the EPOC and i-STAT Alinity v analyzers compared to a veterinary diagnostic laboratory in both species, unexpected measurements on the EPOC and i-STAT Alinity v analyzers may warrant additional investigation and analysis on an alternative analyzer. University, commercial, and reference laboratories are considered the standard of comparison for POC analyzers in the current guidelines published by the American Society of Veterinary Clinical Pathology (ASVCP) based on the type of analysis performed by these analyzers [22]. While the EPOC and i-STAT Alinity v POC analyzers utilize electrochemistry for biochemical analysis, many larger laboratories including the commercial veterinary diagnostic laboratory utilized in this study employ photometry for biochemical analysis [32,33,45].

One limitation of the current study is the small sample sizes utilized ($n = 6$ elephants and $n = 9$ rhinoceros). The recommended sample size for method comparison studies is conventionally 40–100 samples [46]. In particular, analytes with a narrow normal physiologic range, such as sodium, potassium, chloride, creatinine, and ionized calcium, require a larger sample size than utilized in this study to accurately assess differences between analyzers. The small sample size also precluded the assessment of precision and accuracy of the analyzers.

Another limitation of this study is that the EPOC and i-STAT Alinity v analyzers utilized whole blood for analysis, while the veterinary diagnostic laboratory utilized serum. The red blood cells present in whole blood that are not present in serum may impact commonly measured biochemical parameters. For example, in whole blood samples, glucose concentration decreases over time due to glycolysis [47]. In the human literature, glucose has been shown to decrease by 5–7% per hour [47]. The human literature has also demonstrated that use of whole blood for potassium measurement can mask hypokalemia [48]. In hemolyzed samples, ruptured erythrocytes can falsely increase potassium levels due to potassium present within erythrocytes [48]. When whole blood is utilized, hemolyzed

samples may go unrecognized [48]. Difference in sample types is a general limitation in comparing POC analyzers to reference analyzers.

Finally, all of the animals included in this study were deemed to be clinically healthy at the time of sample collection; therefore, we have limited ability to make conclusions about the performance of the EPOC and i-STAT Alinity v analyzers comparatively in African savanna elephants and southern white rhinoceros that are clinically unwell or have biochemical abnormalities.

5. Conclusions

Although there were statically significant differences for a majority of analytes between the EPOC and i-STAT Alinity v POC analyzers when compared to the veterinary diagnostic laboratory, the clinical difference was minimal. Ideally, reference intervals should be generated for each analyzer and accuracy and precision should be assessed; however, for the purposes of routine health care of African savanna elephants and southern white rhinoceros, POC analyzers can be considered a useful alternative to commercial analyzers for the select parameters evaluated in this study. Further study is warranted to assess the utility of POC analyzers in the face of disease and in managed compared to free-ranging populations.

References

1. Emslie, R. *Ceratotherium simum*. The IUCN Red List of Threatened Species. 2012, e.T4185A16980466.en. Available online: http://dx.doi.org/10.2305/IUCN.UK.2012.RLTS.T4185A16980466.en (accessed on 20 March 2022).
2. Gobush, K.S.; Edwards, C.T.T.; Balfour, D.; Wittemyer, G.; Maisels, F.; Taylor, R.D. *Loxodonta africana* (amended version of 2021 assessment). The IUCN Red List Threat Species. 2021, e.T181008073A204401095. Available online: https://dx.doi.org/10.2305/iucn.uk.2021-2.rlts.t181008073a204401095.en (accessed on 17 April 2022).
3. Chanyandura, A.; Muposhi, V.K.; Gandiwa, E.; Muboko, N. An analysis of threats, strategies, and opportunities for African rhinoceros conservation. *Ecol. Evol.* **2021**, *11*, 5892–5910. [CrossRef] [PubMed]
4. Compaore, A.; Sirima, D.; Hema, E.M.; Doamba, B.; Ajong, S.N.; Di Vittorio, M.; Luiselli, L. Correlation between increased human-elephant conflict and poaching of elephants in Burkina Faso (West Africa). *Eur. J. Wildl. Res.* **2020**, *66*, 24. [CrossRef]
5. Fritz, H. Long-term field studies of elephants: Understanding the ecology and conservation of a long-lived ecosystem engineer. *J. Mammal.* **2017**, *98*, 603–611. [CrossRef]
6. Mpakairi, K.S.; Ndaimani, H.; Tagwireyi, P.; Zvidzai, M.; Madiri, T.H. Futuristic climate change scenario predicts a shrinking habitat for the African elephant (*Loxodonta africana*): Evidence from Hwange National Park, Zimbabwe. *Eur. J. Wildl. Res.* **2020**, *66*, 1. [CrossRef]
7. Nhleko, Z.N.; Ahrens, R.; Ferreira, S.M.; McCleery, R.A. Poaching is directly and indirectly driving the decline of South Africa's large population of white rhinos. *Anim. Conserv.* **2021**, *25*, 151–163. [CrossRef]
8. Penny, S.G.; White, R.L.; Scott, D.M.; MacTavish, L.; Pernetta, A.P. Using drones and sirens to elicit avoidance behaviour in white rhinoceros as an anti-poaching tactic. *Proc. R. Soc. B* **2019**, *286*, 20191135. [CrossRef] [PubMed]

9. Candra, D.; Riyanto, M.A.C.T.; Barry, J.; Radcliffe, R.W. Hematology and serum biochemistry of Sumatran rhinoceros (*Dicerorhinus sumatrensis*) in a rainforest sanctuary in Way Kambas National Park, Indonesia. *J. Zoo Wildl. Med.* **2013**, *44*, 280–284. [CrossRef]

10. Miller, M.A.; Buss, P.E. Rhinoceridae (Rhinoceroses). In *Fowler's Zoo and Wild Animal Medicine*, 8th ed.; Miller, R.E., Fowler, M.E., Eds.; Elsevier: St. Louis, MO, USA, 2015; pp. 538–547. [CrossRef]

11. Silva, I.D.; Kuruwita, V.Y. Hematology, plasma, and serum biochemistry values in domesticated elephants (*Elephas maximus ceylonicus*) in Sri Lanka. *J. Zoo Wildl. Med.* **1993**, *24*, 440–444.

12. Thapa, J.; Mikota, S.K.; Gaihre, K.P.; Paudel, S.; Singh, D.K.; Dhakal, I.P.; Nakajima, C.; Suzuki, Y. Tuberculosis seroprevalence and comparison of hematology and biochemistry parameters between seropositive and seronegative captive Asian elephants in Nepal. *J. Vet. Med. Sci.* **2021**, *83*, 1278–1283. [CrossRef]

13. Wiedner, E. Proboscidea. In *Fowler's Zoo and Wild Animal Medicine*, 8th ed.; Miller, R.E., Fowler, M.E., Eds.; Elsevier: St. Louis, MO, USA, 2015; pp. 517–532. [CrossRef]

14. Buss, P.; Miller, M.; Fuller, A.; Haw, A.; Stout, E.; Olea-Popelka, F.; Meyer, L. Postinduction butorphanol administration alters oxygen consumption to improve blood gases in etorphine-immobilized white rhinoceros. *Vet. Anaesth. Analg.* **2018**, *45*, 57–67. [CrossRef]

15. Buss, P.; Olea-Popelka, F.; Meyer, L.; Hofmeyr, J.; Mathebula, N.; Kruger, M.; Brüns, A.; Martin, L.; Miller, M. Evaluation of cardiorespiratory, blood gas, and lactate values during extended immobilization of white rhinoceros (*Ceratotherium simum*). *J. Zoo Wildl. Med.* **2015**, *46*, 224–233. [CrossRef] [PubMed]

16. Hooijberg, E.H.; Steenkamp, G.; Buss, P.; Goddard, A. Method comparison and generation of plasma biochemistry RIs for the white rhinoceros on a point-of-care and wet chemistry analyzer. *Vet. Clin. Pathol.* **2017**, *46*, 287–298. [CrossRef] [PubMed]

17. Hooijberg, E.H.; Steenkamp, G.; du Preez, J.P.; Goddard, A. Analytic and quality control validation and assessment of field performance of a point-of-care chemistry analyzer for use in the white rhinoceros. *Vet. Clin. Pathol.* **2017**, *46*, 100–110. [CrossRef]

18. Horne, W.A.; Loomis, M.R. Elephants. In *Zoo Animal and Wildlife Immobilization and Anesthesia*, 2nd ed.; West, G., Heard, D., Caulkett, N., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2014; pp. 703–719. [CrossRef]

19. Tarbert, D.K.; Behling-Kelly, E.; Priest, H.; Childs-Sanford, S. Evaluation of the i-STAT portable clinical analyzer for measurement of ionized calcium and selected blood chemistry values in Asian elephants (*Elephas maximus*). *J. Zoo Wildl. Med.* **2017**, *48*, 319–327. [CrossRef]

20. Trivedi, S.; Burnham, C.M.; Capobianco, C.M.; Boshoff, C.; Zheng, Y.; Wood-Pettiglio, J.; Ange-van Heugten, K.; Bissell, H.D.; Minter, L.J. Analysis of blood biochemistry of free ranging and human-managed southern white rhinoceros (*Ceratotherium simum simum*) using the i-STAT Alinity v. *Vet. Med. Int.* **2021**, *2021*, 2665956. [CrossRef] [PubMed]

21. Braun, J.-P.; Bourgès-Abella, N.; Geffré, A.; Concordet, D.; Trumel, C. The preanalytic phase in veterinary clinical pathology. *Vet. Clin. Pathol.* **2014**, *44*, 8–25. [CrossRef]

22. Camus, M.S.; Flatland, B.; Freeman, K.P.; Cardona, J.A.C. ASVCP quality assurance guidelines: External quality assessment and comparative testing for reference and in-clinic laboratories. *Vet. Clin. Pathol.* **2015**, *44*, 477–492. [CrossRef]

23. Gancz, A.Y.; Eshar, D.; Beaufrère, H. Paired biochemical analysis of pigmented plasma samples from zoo-kept American flamingos (*Phoenicopterus ruber*) using a point-of-care analyzer and a standard wet chemistry analyzer. *J. Zoo Wildl. Med.* **2019**, *50*, 619–626. [CrossRef]

24. Anderson, N.L.; De La Cruz, S.E.W.; Brenn-White, M.; Frankfurter, G.; Ziccardi, M.H.; Martínez-López, B. Reference values and comparison of blood chemistry and plasma protein values between gold standard analyzers and four point-of-care devices in free-ranging canvasbacks (*Aythya valisineria*). *J. Zoo Wildl. Med.* **2022**, *53*, 302–318. [CrossRef]

25. Hopper, K.J.; Cray, C. Evaluation of a portable clinical analyzer in cynomolgus macaques (*Macaca fasicularis*). *J. Am. Assoc. Lab. Anim. Sci.* **2007**, *46*, 53–57.

26. Atkins, A.; Jacobson, E.; Hernandez, J.; Bolten, A.B.; Lu, X. Use of a portable point-of-care (Vetscan Vs2) biochemical analyzer for measuring plasma biochemical levels in free-living loggerhead sea turtles (*Caretta caretta*). *J. Zoo Wildl. Med.* **2010**, *41*, 585–593. [CrossRef] [PubMed]

27. Larsen, R.S.; Haulena, M.; Grindem, C.B.; Gulland, F.M.D. Blood values of juvenile northern elephant seals (*Mirounga angustirostris*) obtained using a portable clinical analyzer. *Vet. Clin. Pathol.* **2002**, *31*, 106–110. [CrossRef] [PubMed]

28. Harrenstien, L.A.; Tornquist, S.J.; Miller-Morgan, T.J.; Fodness, B.G.; Clifford, K.E. Evaluation of a point-of-care blood analyzer and determination of reference ranges for blood paramters in rockfish. *J. Am. Vet. Med. Assoc.* **2005**, *226*, 255–265. [CrossRef] [PubMed]

29. DiMaggio, M.A.; Ohs, C.L.; Petty, B.D. Evaluation of a point-of-care blood analyzer for use in determination of select hematological indices in the Seminole killfish. *N. Am. J. Aquac.* **2010**, *72*, 261–268. [CrossRef]

30. McCain, S.L.; Flatland, B.; Schumacher, J.P.; Clarke, E.O., III; Fry, M.M. Comparison of chemistry analytes between 2 portable, commercially available analyzers and a conventional laboratory analyzer in reptiles. *Vet. Clin. Pathol.* **2010**, *39*, 474–479. [CrossRef]

31. Stoot, L.J.; Cairns, N.A.; Cull, F.; Taylor, J.J.; Jeffrey, J.D.; Morin, F.; Mandelman, J.W.; Clark, T.D.; Cooke, S.J. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: A review. *Conserv. Physiol.* **2014**, *2*, cou011. [CrossRef]

32. Element POC. Heska Corporation. Available online: https://www.heska.com/product/element-poc/ (accessed on 3 August 2022).

33. i-STAT Alinity v. Zoetis Services LLC. Available online: https://www2.zoetisus.com/products/diagnostics/instruments/i-stat-alinity-v (accessed on 3 August 2022).

34. Prihirunkit, K.; Lekcharoensuk, C.; Pisetpaisan, K. Comparison between manual and automated methods for determination of canine and feline hematocrit and hemoglobin concentration. *Kasetsart J. (Nat. Sci.)* **2008**, *42*, 655–659.

35. Rettenmund, C.L.; Heatley, J.J.; Russell, K.E. Comparison of two analyzers to determine selected venous blood analytes of quaker parrots (*Myiopsitta monachus*). *J. Zoo Wildl. Med.* **2014**, *45*, 256–262. [CrossRef]

36. Ferrando, A.; Bobadilla, I.G.; Bobadilla, M.S.; Palou, A.; Alemany, M. Comparative estimation of hematocrit and trapped plasma in the packed cell volume in man, rabbit and chicken blood. *Comp. Biochem. Physiol. Part A Physiol.* **1981**, *70*, 611–613. [CrossRef]

37. van der Kolk, J.H.; van Leeuwen, J.P.T.M.; van den Belt, A.J.M.; van Schaik, R.H.N.; Schaftenaar, W. Subclinical hypocalcemia in captive Asian elephants (*Elephas maximus*). *Vet. Rec.* **2008**, *162*, 475–479. [CrossRef]

38. van Sonsbeek, G.R.; van der Kolk, H.J.; van Leeuwen, J.P.T.M.; Everts, H.; Marais, J.; Schaftenaar, W. Effect of calcium and cholecalciferol supplementation on several parameters of calcium status in plasma and urine of captive Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*). *J. Zoo Wildl. Med.* **2013**, *44*, 529–540. [CrossRef] [PubMed]

39. Emmanuelson, K. Neonatal care and hand rearing. In *Biology, Medicine, and Surgery of Elephants*, 1st ed.; Fowler, M.E., Mikota, S.K., Eds.; John Wiley & Sons, Incorporated: Hoboken, NJ, USA, 2006; pp. 233–242. [CrossRef]

40. Hermes, R.; Saragust, J.; Schaftenaar, W.; Göritz, F.; Schmitt, D.L.; Hildebrandt, T.B. Obstetrics in elephants. *Theriogenology* **2008**, *70*, 131–144. [CrossRef] [PubMed]

41. Allen, J.L.; Jacobson, E.R.; Harvey, J.W.; Boyce, W. Hematology and serum chemical values for young African elephants (*Loxodonta africana*) with variations for sex and age. *J. Zoo. Anim. Med.* **1985**, *16*, 98–101. [CrossRef]

42. Brown, I.R.F.; White, P.T. Elephant blood hematology and chemistry. *Comp. Biochem. Physiol.* **1980**, *65*, 1–12. [CrossRef]

43. Steyrer, C.; Miller, M.; Hewlett, J.; Buss, P.; Hooijberg, E.H. Reference intervals for hematology and clinical chemistry for the African elephant (*Loxodonta africana*). *Front. Vet. Sci.* **2021**, *8*, 599387. [CrossRef]

44. Wood, J.; Koutsos, E.; Kendall, C.J.; Minter, L.J.; Tollefson, T.; Ivor, E.; Ange-van Heugten, K. Circulating nutrients and hematological parameters in managed African elephants (*Loxodonta africana*) over a 1-year period. *Zoo Biol.* **2020**, *39*, 345–354. [CrossRef]

45. AU5800 Series Clinical Chemistry Analyzers. Beckman Coulter, Inc. Available online: https://www.beckmancoulter.com/products/chemistry/au5800#/overview (accessed on 3 August 2022).

46. Linnet, K. Necessary sample size for method comparison studies based on regression analysis. *Clin. Chem.* **1999**, *45*, 882–894. [CrossRef]

47. Bruns, D.E.; Knowler, W.C. Stabilization of glucose in blood samples: Why it matters. *Clin. Chem.* **2009**, *55*, 850–852. [CrossRef]

48. Hawkins, R. Measurement of whole-blood potassium—Is it clinically safe? *Clin. Chem.* **2003**, *49*, 2105–2106. [CrossRef]