


## BRIEF COMMUNICATION

# Reliability of the Enterprise Point-of-Care (EPOC) blood analyzer's calculated arterial oxygen-hemoglobin saturation in immobilized white rhinoceroses (*Ceratotherium simum*)

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## Abstract

**Background:** Enterprise Point-of-Care (EPOC) blood analysis is used routinely in wildlife veterinary practice to monitor blood oxygenation, but the reliability of the EPOC calculated arterial oxygen-hemoglobin saturation (cSaO<sub>2</sub>) has never been validated in the white rhinoceros (*Ceratotherium simum*), despite their susceptibility to hypoxemia during chemical immobilization.

**Objectives:** We aimed to evaluate the reliability of the EPOC cSaO<sub>2</sub> by comparing it against arterial oxygen-hemoglobin saturation (SaO<sub>2</sub>) measured by a co-oximeter reference method in immobilized white rhinoceroses.

**Methods:** Male white rhinoceroses in two studies (both  $n=8$ ) were immobilized by darting with different etorphine-based drug combinations, followed by butorphanol or saline (administered intravenously). Animals in both studies received oxygen via intranasal insufflation after 60 min. Blood samples were drawn, at predetermined time points, from a catheter inserted into the auricular artery and analyzed using the EPOC and a co-oximeter. Bland-Altman (to estimate bias and precision) and area root mean squares (ARMS) plots were used to determine the reliability of the EPOC cSaO<sub>2</sub> compared with simultaneous co-oximeter SaO<sub>2</sub> readings.

**Results:** The rhinoceros were acidotic (pH of  $7.3 \pm 0.1$  [mean  $\pm$  standard deviation]), hypercapnic (PaCO<sub>2</sub> of  $73.7 \pm 10.5$  mmHg), and normothermic (body temperature of  $37.4 \pm 1.8$  °C). In total, 389 paired cSaO<sub>2</sub>-SaO<sub>2</sub> measurements were recorded (the cSaO<sub>2</sub> ranged between 13.2% and 99.0%, and the SaO<sub>2</sub> ranged between 11.8% and 99.9%). The EPOC cSaO<sub>2</sub> readings were unreliable (inaccurate, imprecise, and poor ARMS) across the entire saturation range (bias -6%, precision 5%, and ARMS 8%).

**Conclusions:** The EPOC cSaO<sub>2</sub> is unreliable and should not be used to monitor blood oxygenation in immobilized white rhinoceroses.

## KEYWORDS

blood gas analysis, hypoxemia, oxygen saturation, wildlife

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## 1 | INTRODUCTION

Enterprise Point-of-Care (EPOC) blood analyzers are used routinely in wildlife veterinary practice to monitor ventilatory, acid–base, and blood oxygenation status of chemically immobilized white rhinoceros, which are susceptible to opioid-induced cardiorespiratory derangements that can result in severe hypoxemia.<sup>1,2</sup> To assess blood oxygenation, the EPOC blood analyzer measures arterial oxygen partial pressures ( $\text{PaO}_2$ ) with a single-use test card comprised of a Clark-type oxygen sensing electrode, a reference electrode, a counter electrode, and fluid used for self-calibration.<sup>3</sup> Although the Clark-type electrode used in blood analysis reliably measures  $\text{PaO}_2$ ,<sup>3</sup> dissolved oxygen accounts only for about 2%–4% of oxygen in the total blood oxygen content.<sup>4</sup> Conversely, oxygen bound to hemoglobin ( $\text{SaO}_2$ ), which does not exert a partial pressure, accounts for the remaining 96%–98%,<sup>4</sup> making it a more informative indicator of blood oxygenation. Thus, the EPOC uses the measured  $\text{PaO}_2$  to calculate arterial oxygen-hemoglobin saturation, but it does so using an equation based on a typical human oxygen–hemoglobin dissociation curve.<sup>3</sup>

The relationship between the oxygen bound to hemoglobin and the partial pressure of oxygen in the blood is represented by the oxygen–hemoglobin dissociation curve, and since the characteristics of hemoglobin differ between species, especially when considered across a broad range of body mass, so too does this curve.<sup>5,6</sup> The partial pressure of oxygen at which hemoglobin is 50% saturated ( $p50$ ) is used to quantify the position of the oxygen–hemoglobin dissociation curve under a normal physiologic resting state (body temperature of 37°C and pH of 7.4).<sup>4</sup> Compared with the human curve, which typically has an average  $p50$  of 26.7 mmHg at rest,<sup>7</sup> the  $p50$  of white rhinoceroses is between 15 mmHg<sup>5</sup> and 20 mmHg,<sup>8</sup> indicating a more left-shifted oxygen–hemoglobin dissociation curve. Because the EPOC blood analyzer is calibrated specifically for human use, any differences in the oxygen–hemoglobin dissociation curve from that of humans may affect the reliability of the EPOC  $\text{cSaO}_2$  values.

Oxygen–hemoglobin dissociation curves also shift dynamically with changes in the partial pressure of carbon dioxide ( $\text{PaCO}_2$ ), pH, 2,3-diphosphoglycerate (2,3-DPG), chloride ions ( $\text{Cl}^-$ ), and temperature of the blood.<sup>4,9</sup> Immobilized white rhinoceroses often develop hypercapnia and acidosis,<sup>1,2,5</sup> both of which tend to shift their oxygen–hemoglobin dissociation curve to the right,<sup>5</sup> and therefore closer to that of a healthy human at rest. For this reason, it is important that the reliability of the EPOC  $\text{cSaO}_2$  is assessed based on the specific physiologic conditions under which the analyzer is used on different species.

The reliability of the EPOC  $\text{cSaO}_2$  readings can be assessed by comparing against the  $\text{SaO}_2$  measured by co-oximetry, which is considered a “reference method” according to the Food and Drug Administration (FDA) and International Organization for Standardization (ISO).<sup>10</sup> For example, a recent study on immobilized impala (*Aepyceros melampus*) showed that the EPOC  $\text{cSaO}_2$  readings were indeed reliable when compared against a co-oximeter  $\text{SaO}_2$  readings across the saturation range of 0%–100%.<sup>11</sup> However, there

has been no research on the reliability of the EPOC  $\text{cSaO}_2$  readings in the white rhinoceros, despite their susceptibility to hypoxemia during chemical immobilization. Therefore, in this study, we assessed the reliability of EPOC  $\text{cSaO}_2$  readings by comparing them against a co-oximeter  $\text{SaO}_2$  readings in immobilized white rhinoceroses.

## 2 | MATERIALS AND METHODS

Studies were approved by the University of Pretoria Animal Ethics Committee (V035-17 and REC246-19) and the University of Pretoria Animal Use and Care Committee (001/16 and 012/20). In total, 16 subadult male white rhinoceroses (*Ceratotherium simum*) were captured from the wild and relocated to holding facilities (bomas) at the Veterinary Wildlife Services, Kruger National Park, South Africa (23° 49' 60S, 31° 30' 0 E; ~320m altitude), where they were habituated for 4–6 weeks. Animals were fed lucerne (*Medicago sativa*) and teff hay (*Eragrostis tef*), with water provided ad libitum, and their adaptation to captivity was observed closely by trained staff.<sup>12</sup>

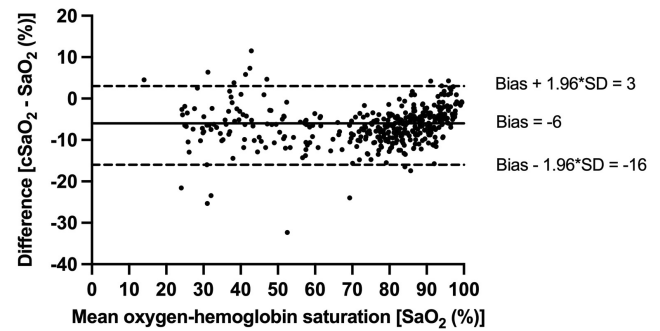
Data were collected and pooled from two separate studies on the physiologic effects of etorphine-based immobilization to maximize the sample size. Immobilizing drugs were administered intramuscularly using a 3 mL plastic dart with a 60mm uncollared needle launched by a compressed air rifle (DAN-INJECT International SA, Skukuza, South Africa). In the first study (Study 1), rhinoceroses were immobilized twice with etorphine (M99; Voluplex, Mmandi, Centurion, South Africa), and then given either butorphanol (Butonil; Wildlife Pharmaceuticals Pty Ltd, White River, South Africa), or a 0.9% sodium chloride (NaCl) saline solution (control) at 30min into recumbency. Then intranasal oxygen insufflation (flow rate of 15 L/min) was administered at 60min. In the second study (Study 2), rhinoceroses were immobilized four times using different immobilizing drugs: etorphine (M99) and a 0.9% NaCl saline solution (control); etorphine and azaperone (Stresnil; Elanco, ON, Canada); etorphine and midazolam (Dazonil; Wildlife Pharmaceuticals Pty Ltd); and etorphine and medetomidine (Metonil; Wildlife Pharmaceuticals Pty Ltd). Butorphanol was then administered at 12min into recumbency and intranasal oxygen insufflation (flow rate of 15L/min) was given at 60min to obtain a wide range of saturation values. See dose calculations based on body weight in (Tables S1 and S2).

Once an immobilized rhinoceros was recumbent, the concave (inner) surface of the ear pinna was aseptically prepared, and a 25mm, 22 Gauge over-the-needle intravenous (IV) catheter (Introcan; B Braun Medical Inc) was inserted into the medial auricular artery for blood sample collection. A digital thermometer (HI 98509 Checktemp 1; Hanna Instruments) with a protective sheath over the probe was inserted into the rectum to measure body temperature. Once instrumented, arterial blood samples were collected anaerobically into 3 mL heparinized syringes (BD Present A-Line; BD Medical) at 30, 40, 50, and 60 min into recumbency in Study 1 and at 10, 15, 20, 30, and 40 min into recumbency in Study 2. After 60 min, up to six additional arterial blood samples were collected at random times during oxygen insufflation.

Visible air bubbles were immediately removed from the collected blood samples and placed on ice. Within 15 min, samples were assessed in a laboratory, under controlled temperature, using an EPOC blood analyzer (EPOC Software version 3.35.4 with sensor configuration version 39.2; Epocal Inc) with single-use, self-calibrated test cards (BGEM3; Epocal Inc),<sup>3</sup> and a co-oximeter (AVOXimeter 4000; Surgical Innovations, Johannesburg, South Africa) with test cuvettes (ITC QV8; Surgical Innovations). The co-oximeter was calibrated daily using calibration cuvettes (Surgical Innovations).<sup>13</sup> Just prior to the measurement, the blood sample was remixed by gently rolling the syringe back and forth for approximately 20s. Ensuring no visible air bubbles were introduced, the blood was carefully injected into the test card and cuvette, which were then introduced into the EPOC and co-oximeter, respectively, for analyses. Because of limited supplies, we did not take repeated measurements per blood sample.

The EPOC measures dissolved arterial oxygen partial pressures ( $\text{PaO}_2$ ) and pH and then calculates the arterial oxygen-hemoglobin saturation ( $\text{cSaO}_2$ ) using an equation based on the human oxygen-hemoglobin dissociation curve, preprogrammed into the blood analyzer.<sup>7</sup> Conversely, the co-oximeter measures fractional arterial oxygen-hemoglobin saturation ( $\text{SaO}_2$ ), methemoglobin (MetHb), and carboxyhemoglobin (COHb) using multiple wavelengths of light (488.4, 520.1, 562.4, 585.2, 597.5, 621.7 and 671.7 nm) and was used as the reference method in this study.<sup>13</sup> Although co-oximetry has not been tested in rhinoceroses, the spectrophotometric characteristics of hemoglobin derivatives (i.e., infrared absorbance of oxy- and deoxyhemoglobin) are nearly identical among humans, white rhinoceroses, and the closely related horse (Equidae), indicating that the algorithms used in co-oximetry are likely also suitable for use in rhinoceroses.<sup>5</sup>

Using the method-comparison approach of Jensen et al (2006), data were plotted using simple linear regression and Deming (Model II) linear regression analysis.<sup>14</sup> Acceptability was assessed based on the inherent imprecision of the two methods.<sup>14</sup> Bland-Altman analysis for multiple observations was used to determine the agreement between the EPOC  $\text{cSaO}_2$  and the co-oximeter  $\text{SaO}_2$  readings. The Bland-Altman analysis determined the accuracy (bias), precision (standard deviation [SD]), and the 95% limits of agreement (LOA) between the paired  $\text{cSaO}_2$  and  $\text{SaO}_2$  measurements.<sup>15</sup> Bias is the mean difference between the paired measurements, precision is a measure of the variability (SD) between the paired measurements, and the 95% LOA (bias  $\pm 1.96 \times \text{SD}$ ) is an estimate of the interval within which 95% of the differences between measurements lie. An area root mean squares (ARMS), which is a combined estimate of accuracy (bias) and precision (SD), was also calculated to determine the overall agreement between  $\text{cSaO}_2$  and  $\text{SaO}_2$  measurements.<sup>10</sup> To perform the Bland-Altman method at an  $\alpha = 0.05$  and  $\beta = 0.10$ , a minimum of 17 paired  $\text{cSaO}_2$ - $\text{SaO}_2$  measurements (taken from eight individuals) is required to obtain sufficient statistical power when the expected bias is 3%, precision is 3%, and the LOA is  $\pm 10\%$ . Guidelines for clinical acceptability are not available for  $\text{cSaO}_2$  reported by blood analyzers, and so, for this study, we followed the ISO performance requirements for pulse oximetry, which states that



**FIGURE 1** Bland-Altman plot showing poor agreement between the EPOC calculated arterial oxygen-hemoglobin saturation ( $\text{cSaO}_2$ ) and the co-oximeter arterial oxygen-hemoglobin saturation ( $\text{SaO}_2$ ) in 16 immobilized white rhinoceroses. The difference between  $\text{cSaO}_2$  and  $\text{SaO}_2$  is plotted against the mean arterial oxygen-hemoglobin saturation measurements obtained from the EPOC and co-oximeter readings ( $\text{cSaO}_2$  and  $\text{SaO}_2$ ) along the entire saturation range. The bias is represented by the solid line, and the limits of agreement (bias  $\pm 1.96 \times \text{SD}$ ) are represented by the dashed lines. Each data point represents the difference in the paired  $\text{cSaO}_2$ - $\text{SaO}_2$  measurements (389 paired measurements) taken from the 16 white rhinoceroses in both studies.

the device is clinically reliable (acceptable) only when the comparisons with co-oximetry are  $\pm 3\%$  for accuracy (bias),  $\leq 3\%$  for precision (SD), and  $\leq 4\%$  for ARMS.<sup>10</sup>

### 3 | RESULTS

The mean pH of the rhinoceroses was  $7.3 \pm 0.1$  (mean  $\pm$  standard deviation), indicating acidosis; the  $\text{PaCO}_2$  was  $73.7 \pm 10.5$  mmHg, indicating hypercapnia; and the body temperature was  $37.4 \pm 1.8^\circ\text{C}$ , indicating normothermia. In total, 389 paired  $\text{cSaO}_2$ - $\text{SaO}_2$  measurements were used for comparisons. The EPOC  $\text{cSaO}_2$  readings ranged from 13.2% to 99.0%, and the co-oximeter  $\text{SaO}_2$  readings ranged from 11.8% to 99.9%. There was poor agreement between the EPOC and co-oximeter  $\text{SaO}_2$  readings across the entire saturation range (Figure 1) and within other narrower ranges analyzed in both studies (Table 1). When Study 1 and Study 2 were analyzed separately (Figures S1 and S2), a similar lack of agreement was found (Study 1 [bias  $-5\%$  precision 4% ARMS 7%] and Study 2 [bias  $-7\%$  precision 5% ARMS 8%]), justifying combining the data. Nonetheless, there was good correlation between the EPOC  $\text{cSaO}_2$  and co-oximeter  $\text{SaO}_2$  readings (Table S2) in Study 1 (slope = 0.969 with 95% CI = 0.931 to 1.008,  $p < .0001$ ) and Study 2 (slope = 1.035 with 95% CI = 1.035 to 1.029,  $p < .0001$ ). Within the saturation range of 70%–79%, the EPOC  $\text{cSaO}_2$  readings were unreliable (inaccurate, precise, and poor ARMS). Below 70% and at the other saturation ranges (70%–100%, 80%–89%, and 90%–100%), the EPOC  $\text{cSaO}_2$  readings were overall unreliable (inaccurate, imprecise, and poor ARMS). Across the entire saturation range of 0%–100%, the EPOC  $\text{cSaO}_2$  readings underestimated the  $\text{SaO}_2$  by a mean difference of 6% and with a poor precision of 5%.

**TABLE 1** Bland–Altman and area root mean squares (ARMS) analysis showing poor agreement within a number of different saturation ranges between the calculated arterial oxygen-hemoglobin saturation (cSaO<sub>2</sub>) from the EPOC blood analyzer and the measured arterial oxygen-hemoglobin saturation (SaO<sub>2</sub>) by the AVOXimeter 4000 co-oximeter.

Saturation range (%)	n	Bias (%)	Precision (%)	LOA (%)	ARMS (%)
0–100	389	–6*	5*	3 to –16	8*
<70	100	–7*	6*	6 to –19	9*
70–100	289	–6*	4*	1 to –14	8*
70–79	40	–8*	3	–2 to –14	8*
80–89	112	–7*	4*	1 to –15	8*
90–100	137	–5*	4*	3 to –13	7*

Note: Guidelines for clinical acceptability are not available for cSaO<sub>2</sub> reported by blood analyzers, and so for this study, we followed the ISO performance requirements for pulse oximetry, which states that the device is clinically reliable (acceptable) only when the comparisons with co-oximetry are  $\leq \pm 3\%$  for accuracy (bias),  $\leq 3\%$  for precision (SD), and  $\leq 4\%$  for ARMS.<sup>11</sup>

Abbreviations: ARMS, area root mean squares; LOA, limits of agreement (maximum and minimum); n, sample size.

\*Result that are considered unreliable according to these (ISO) guidelines.

Each rhinoceros was immobilized successfully according to the different drug combinations, and all made full recoveries at the end of each trial.

## 4 | DISCUSSION

In immobilized white rhinoceroses, the EPOC blood analyzer, which uses the human oxygen–hemoglobin dissociation curve to calculate arterial oxygen–hemoglobin saturation, provided an unreliable (inaccurate, imprecise, and poor ARMS) estimate of arterial oxygen–hemoglobin saturation across the entire saturation range. The EPOC cSaO<sub>2</sub> readings underestimated the co-oximeter SaO<sub>2</sub> readings by an average of 6% and were associated with discernably poor precision, indicated by the SD of 5%, across the entire saturation range.

The EPOC blood analyzer and the AVOXimeter co-oximeter use two different methodologies to calculate or measure arterial oxygen–hemoglobin saturation. The EPOC measures the PaO<sub>2</sub>, PaCO<sub>2</sub>, pH, and together with body temperature and the input of the human oxygen–hemoglobin dissociation curve to calculate functional arterial oxygen–hemoglobin saturation.<sup>3</sup> By comparison, the co-oximeter uses six different wavelengths of light to estimate “fractional” arterial oxygen–hemoglobin saturation by measuring the light absorbance of oxyhemoglobin present in the blood.<sup>13</sup> Functional arterial oxygen–hemoglobin saturation refers to the percentage of hemoglobin capable of carrying oxygen, whereas fractional arterial oxygen–hemoglobin saturation is the proportion of total hemoglobin that is oxygenated, with other

fractions such as MetHb and COHb. In this study, low levels of MetHb (1.1 ± 0.5%) and COHb (<1%) were reported, meaning that functional and fractional arterial oxygen–hemoglobin saturations are effectively equivalent.

Using the ISO guidelines for reliability (i.e.,  $\pm 3\%$  for bias,  $\leq 3\%$  for precision, and  $\leq 4\%$  for ARMS),<sup>10</sup> we found that the EPOC blood analyzer performed poorly in immobilized white rhinoceroses, with the cSaO<sub>2</sub> underestimating the co-oximeter SaO<sub>2</sub> reading across the entire saturation range (bias –6%, precision 5%, ARMS 8%). These findings differ from those reported in immobilized impalas, where the EPOC cSaO<sub>2</sub> readings were reliable compared with the co-oximeter SaO<sub>2</sub> readings measured across the full saturation range (bias –1%, precision 3%, ARMS 3%).<sup>11</sup> This finding suggests that the EPOC is likely only reliable in species, like impala, with presumably similar oxygen–hemoglobin dissociation curves to that of humans, and less reliable in species with markedly different curves, like rhinoceroses.<sup>5,6</sup>

Oxygen–hemoglobin affinity varies not only among different mammalian species under normal physiologic conditions, especially as a function of body size,<sup>6</sup> but it also varies dynamically, responding to acute changes in pH, PaCO<sub>2</sub>, temperature, 2,3-diphosphoglycerate (2,3-DPG), and chloride ions (Cl<sup>–</sup>).<sup>8,9</sup> These acute changes invoke dynamic and reversible shifts in the oxygen–hemoglobin dissociation curve, and the magnitude of these shifts could also vary depending on sensitivity among species.<sup>8,9</sup> For example, the position of the white rhinoceros' oxygen–hemoglobin dissociation curve is apparently unaffected by the presence of 2,3-DPG but instead responds to changes in Cl<sup>–</sup> concentration.<sup>8</sup> The negligible effect of 2,3-DPG on the oxygen–hemoglobin affinity is attributed to the unique structure of the rhinoceros hemoglobin, which contains a glutamic acid (GLU), instead of histidine, at the  $\beta_2$  position, a position responsible for binding 2,3-DPG.<sup>8</sup> In this study, the acidosis (pH 7.3) and hypercapnia (73.7 mmHg) of the immobilized rhinoceroses appear to have right-shifted both the EPOC cSaO<sub>2</sub> and co-oximeter SaO<sub>2</sub> readings (Figure S3). Nonetheless, the systematic bias of the EPOC remains because it uses the human oxygen–hemoglobin dissociation curve (and its specific sensitivity to pH and PaCO<sub>2</sub>), which is too dissimilar to the rhinoceros' curve, which results in the observed bias even under the acidotic and hypercapnic conditions elicited during immobilization.

The immobilization of white rhinoceroses is associated with substantial morbidity and mortality risks associated with severe hypoxemia.<sup>2,5</sup> Therefore, there is an urgent clinical need to understand the reliability of tools used to monitor blood oxygen levels in this species. We speculate that the poor reliability of the EPOC is primarily explained by its use of the human oxygen–hemoglobin dissociation curve, which is significantly right-shifted compared with that of the rhinoceros. The bias persists even under the acidotic and hypercapnic conditions elicited during immobilization. Therefore, a co-oximeter should rather be used to determine arterial oxygen–hemoglobin saturation in immobilized white rhinoceroses.

## AUTHORS CONTRIBUTIONS

Thembeke K. Mtetwa: Preparation of equipment, data collection, statistical analysis, data interpretation and preparation of article. Edward P. Snelling: Data collection, statistical analysis, data interpretation and editing of the article. Peter E. Buss: Data collection and editing of the article. Ashleigh C. Donaldson: Data collection and editing of the article. Leith C. R. Meyer: Conceived the study, data collection, data interpretation and editing of the article.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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