

# Lactate Measurement Interference by Hemoglobin-Based Oxygen Carriers (Oxyglobin<sup>®</sup>, Hemopure<sup>®</sup>, and Hemolink<sup>™</sup>)

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We sought to determine whether hemoglobin-based oxygen carriers (HBOCs), hemoglobin glutamer-200 [bovine] (HBOC-200, Oxyglobin<sup>®</sup>), hemoglobin glutamer-250 [bovine] (HBOC-201, Hemopure<sup>®</sup>), and hemoglobin raffimer (Hemolink<sup>™</sup>) interfere with the accuracy of lactate measurements. Combinations of concentrated L-lactate solution, HBOC, and blood or plasma with added PlasmaLyte-A<sup>™</sup> were added to sample tubes to make a linear and constant increase in lactate concentration in consecutive samples. Sample lactate concentrations ranged from 5–110 mg/dL (0.6–12 mM) (physiological reference range: 5–20 mg/dL [0.56–2.2 mM]). Comparisons were made between machine measured lactate concentrations and calculated lactate concentrations. For Hb glutamer-250, the average difference between measured and calculated lactate concentrations was  $-5.1$  mg/dL ( $-0.57$  mM) (LX-20<sup>®</sup>),

with greater underestimation at larger lactate concentrations. For Hb raffimer, the average difference was  $-2.2$  mg/dL ( $-0.24$  mM) (LX-20<sup>®</sup>). The veterinary product, Hb glutamer-200, was tested on 3 analyzers (LX-20<sup>®</sup>, YSI 1500, and YSI 2300). The YSI 1500 was the most accurate instrument with the mean difference between measured minus calculated lactate being  $+1.3$  mg/dL versus  $-2.6$  mg/dL (YSI 2300) and  $-8.4$  mg/dL (LX-20<sup>®</sup>). The clinical implications of this study are that with increasing levels of an HBOC in plasma, lactate interpretation may become inaccurate, especially at larger lactate concentrations, causing underestimation of measured lactate values and possible under-treatment of the patient. Therefore, caution must be exercised when interpreting lactate results when a HBOC is present in plasma.

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**H**emoglobin-based oxygen carriers (HBOCs) are solutions of cell-free hemoglobin (Hb) that have been developed for treatment of anemia or resuscitation from hemorrhagic shock. They combine oxygen transport with volume-expanding properties and thus may be ideal candidates for resuscitation,

superior to traditional plasma expanders (e.g., hydroxyethyl starch solutions) (1–8). The HBOCs used in this study, hemoglobin glutamer-200 [bovine] (HBOC-200, Oxyglobin<sup>®</sup>), hemoglobin glutamer-250 [bovine] (HBOC-201, Hemopure<sup>®</sup>; Biopure Corporation, Cambridge, MA), and hemoglobin raffimer (Hemolink<sup>™</sup>; Hemosol Inc., Toronto, ON), have not been tested for interference with lactate measurements (9–15). We hypothesized that these three blood substitutes may interfere with the accuracy of lactate determination. It should be noted that the University of California Los Angeles (UCLA) Clinical Laboratory reference ranges of plasma lactate are 5–20 mg/dL (0.56–2.2 mM).

We further hypothesized that one of the reasons for underestimation of accurate lactate levels in the lactate analyzers may be scavenging of hydrogen peroxide, an intermediary, by the cell-free HBOC molecules in the sample. In fact, all Hbs, including modified Hbs in

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particular, have more of a propensity to react in an enzymatic fashion with peroxide than unmodified Hbs (16-19). Because the analyzers use hydrogen peroxide as a means to detect lactate concentration, this process could be interfered with by HBOCs.

The lactate measurements were made by three clinical lactate analyzers (Synchron LX-20<sup>®</sup>, Beckman Coulter, Fullerton, CA; YSI 1500 SPORT and YSI 2300 STAT; YSI Inc., Yellow Springs, OH) in combination with three HBOCs, one approved veterinary product (Hb glutamer-200) and two experimental human therapeutics (Hb glutamer-250 and Hb raffimer).

## Methods

To study three lactate analyzers and three HBOCs, a series of experiments were performed: 1) validating the veterinary HbOC (Hb glutamer-200) with the analyzers used for our published veterinary shock and resuscitation experiments (6-8), the YSI 1500 and YSI 2300 with bovine blood, 2) testing the HBOCs undergoing human trials (Hb glutamer-250 and Hb raffimer) with a currently used clinical lactate analyzer, LX-20<sup>®</sup>, with human plasma diluted in Plasma-Lyte A<sup>™</sup> (Baxter, Deerfield, IL), and 3) an external validation of the LX-20<sup>®</sup> without HBOCs at clinically increased concentrations of lactate. Despite performing an external validation series on the LX-20<sup>®</sup> at a wider range of lactate concentrations, all three analyzers continually self-calibrate for lactate in either blood or plasma accurately during routine use.

The correlation between machine-measured lactate and calculated lactate concentrations was studied, testing for interference by HBOCs. Because HBOCs themselves contain lactate in their initial preparation, the calculated final lactate concentration in each test sample was the sum of baseline lactate in human plasma or bovine blood, lactate contained in the HbOC solution itself, and lactate added to the test sample from a known concentrated stock solution.

This series of experiments was conducted at our laboratories at the University of California (UC) Davis using the YSI 1500 and 2300 lactate analyzers. The YSI 1500 and 2300 instruments report lactate concentrations in mM; however we converted all data to mg/dL (mg/dL is the unit clinically reported at UCLA and recorded by the LX-20<sup>®</sup>). The conversion factor between units for lactic acid is 1.00 mg/dL = 0.111 mM (MW lactic acid = 90.079 Daltons). Lithium L-lactate (Sigma, St. Louis, MO) was dissolved in Plasma-Lyte A<sup>™</sup>, a lactate-free, balanced salt solution. From this concentrated stock lactate solution, specific amounts were added to 5 identical sample sets to create a linear and constant increase in lactate concentration in consecutive samples. Final lactate concentrations ranged

from 5 to 70 mg/dL. Bovine whole blood was collected from a healthy live animal (these were remainder samples of collected bovine blood for other experiments, not requiring veterinary institutional approval at UC Davis), in gray-top tubes (Becton Dickinson, Franklin Lakes, NJ) and aliquots were added to each of the samples (therefore, a veterinary HbOC was matched with blood from an animal source). Finally, Hb glutamer-200 was added to make each sample set have a different final HbOC concentration (0.13, 1.3, 2.6, 3.6, and 5 mg/dL), with each set still having the identical range of lactate concentrations. We wanted to test to whether larger concentrations of HbOC had a greater interference effect. The final sample volume was 1.5 mL. Single measurements for each sample were performed on both YSI instruments.

IRB exemption at UCLA for the use of remainder sample human plasma for routine lactate concentration measurements was obtained. Discard human plasma samples from the clinical laboratory were pooled (human plasma was donated by the Chemistry Laboratory at UCLA from non-expired lactate samples in gray top tubes). We studied all 3 HBOCs on the LX-20<sup>®</sup>: Hb glutamer-200, Hb-250 (0.13, 1.3, 2.6, 3.9, and 6.5 g/dL), and Hb raffimer (0.1, 1, 2, 3, and 5 g/dL). Five sample sets were made for each HbOC at the above concentrations in a similar manner as for the YSI series. The difference being the use of human plasma instead of bovine blood (we wanted to match products intended for human use with human plasma) and a slightly larger lactate concentration range. Final calculated lactate concentrations ranged from 10 to 110 mg/dL. Single measurements were performed on the LX-20<sup>®</sup>.

Lithium L-lactate was dissolved in saline and added to a human plasma pool, obtained from the UCLA Medical Center Clinical Laboratory. Dilutions were made to yield target lactate concentrations of 20, 40, 60, 80, and 100 mg/dL (designed to fit high normal and increased plasma lactate concentrations, the same dilutions used by the UCLA clinical laboratory for periodic validation of their analyzers). Each sample was analyzed in triplicate on the LX<sup>®</sup> 20 analyzer (see Table 2). This external validation was only designed for the LX-20<sup>®</sup>, as this is the only clinically used analyzer at UCLA. This additional series was presented to sample wider ranges of lactate without HBOCs present to determine if simply high lactate levels would cause the analyzer to become inaccurate, as the internal automatic calibration on this analyzer does not test at such large lactate concentrations.

The differences between measured (Y)-minus-calculated (X) lactate were quantified by the mean difference and by the root mean square error (RMSE) defined as  $RMSE = \sqrt{[\text{mean of calculated minus measured difference}]^2 + [\text{standard deviation of the}]}$

**Table 1.** Physical and chemical properties of hemoglobin glutamer-200, hemoglobin glutamer-250, and hemoglobin raffimer

Variable	Hemoglobin glutamer-200	Hemoglobin glutamer-250	Hemoglobin raffimer
Source	bovine	bovine	human
Molecular modification	glutaraldehyde polymerization	glutaraldehyde polymerization	o-raffinose cross-linked polyhemoglobin
Hemoglobin concentration (g/dL)	13	13	10
p50 (mm Hg)	34	36-38	39
pH	7.8	7.6-7.9	7.5
Osmolality (mOsm/kg)	300	290-310	N/A
Average Molecular weight (kD)	approximately 50% between 65 and 130	average 250	64-500
Unpolymerized hemoglobin	<5%	<5%	N/A
Methemoglobin	<15%	<10%	<10%
Colloid oncotic pressure (mm Hg) <sup>a</sup>	42	25	26
Endotoxin	≤0.05 EU/mL	N/A	N/A
Baseline Lactate (mg/dL) <sup>b</sup>	116	120	139

Modified from Jahr et al. (Reference #1).

<sup>a</sup> Values listed are provided by the manufacturer, except the colloid osmotic pressure, which was measured in our laboratories by using the Colloid Osmometer 4420 (Wescor, Logan, UT).

<sup>b</sup> Measured at UC Davis on YSI 1500 STAT lactate analyzer.

N/A = data not available.

**Table 2.** Accuracy of Synchron LX-20® analyzer when testing human plasma samples containing added lactate but no hemoglobin-based oxygen carrier

External Validation Experiment			
Calculated lactate concentration mg/dL (mM)	Measured lactate concentration mg/dL (mM)		
19 (2.1)	20 (2.2)	20 (2.2)	20 (2.2)
38 (4.2)	40 (4.4)	40 (4.4)	41 (4.6)
58 (6.4)	58 (6.4)	59 (6.5)	58 (6.4)
77 (8.5)	79 (8.8)	78 (8.7)	77 (8.5)
96 (10.7)	95 (10.5)	95 (10.5)	97 (10.8)

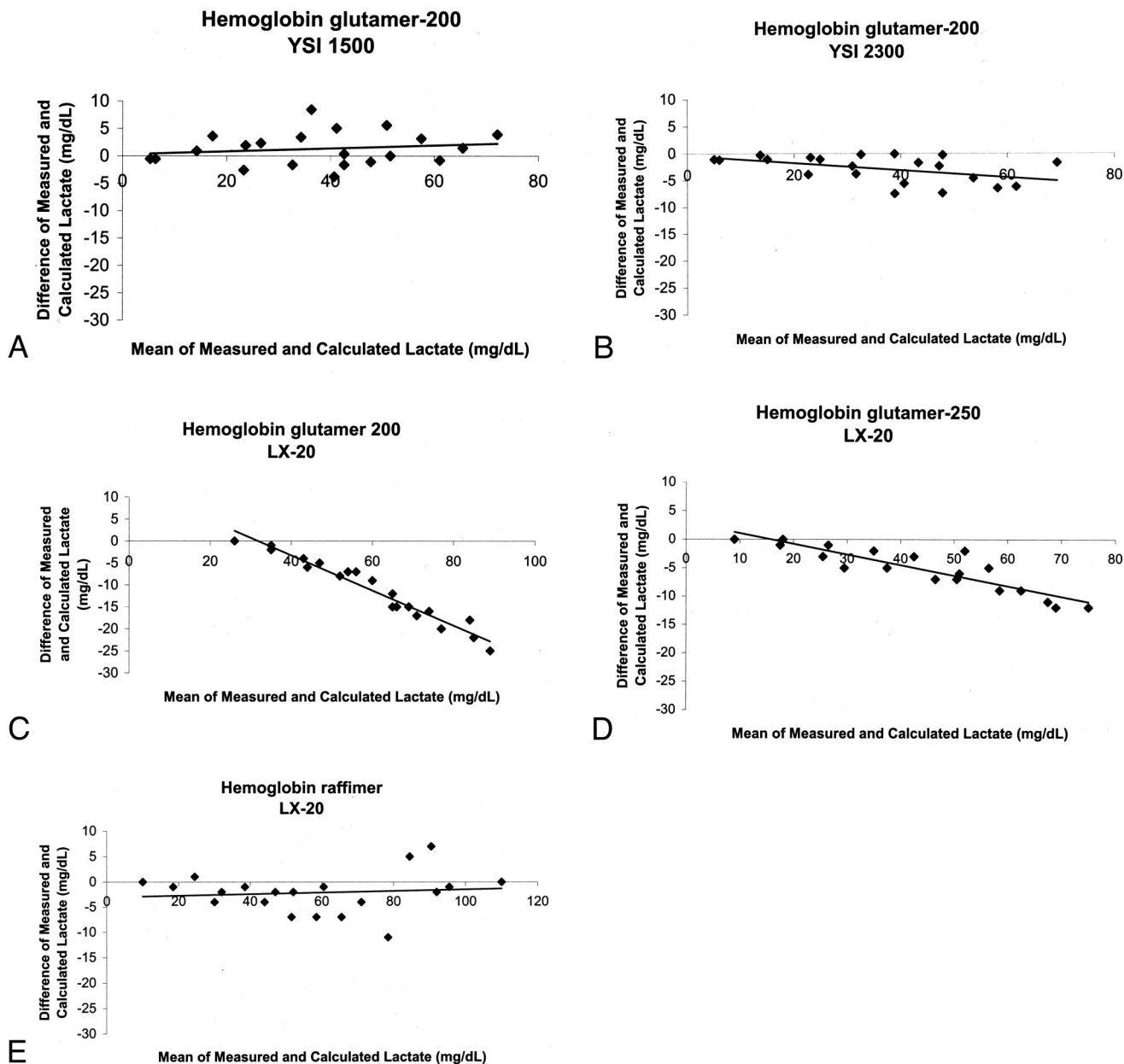
Calculated values are derived from the baseline lactate concentration in human plasma (9 mg/dL [1 mM]) (as measured by LX-20®) and the amount of concentrated lactate stock added to each test sample. Measured values represent lactate concentrations as measured by the LX-20® analyzer. Five test samples were analyzed in triplicate over a similar range of lactate concentrations as tested for in the hemoglobin-based oxygen carriers containing samples.

differences]<sup>2</sup>). The RMSE incorporates both the bias and the variability about the mean difference and therefore indicates the total variability about zero (20). Bland-Altman plots of measured-minus-calculated differences (Y-X, vertical axis) versus the mean of the measured-and-calculated value ((Y+X)/2, horizontal axis) are provided (21,22) with a linear regression analysis added to the plot highlight measurement bias. Correlations between Y versus X (corresponding to the linear regression of X on Y) and the correlations between (Y-X) versus (Y+X)/2 (Bland-Altman correlation) are also reported. If the latter correlation is zero, then there is no trend in the Y-X difference (the bias) with increasing or decreasing lactate magnitude.

## Results

The mean difference between measured-minus-calculated lactate for Hb glutamer-200 was +1.3 mg/dL (YSI 1500) (3.4% of the overall mean of measured-and-calculated lactate) and -2.6 mg/dL (YSI 2300) (7.2% of overall mean) (Fig. 1A&B and Table 3). The data points are not differentiated by HBOC concentration, as we did not observe any HBOC-concentration-dependent effects on interference. The RMSE for Hb glutamer-200 is 3.3 mg/dL (YSI 1500) (8.7% of the overall mean) and 3.8 mg/dL (YSI 2300) (10.6% of the overall mean). The Bland-Altman correlation (r) between the measured-minus-calculated difference versus the average of the measured-plus-calculated is  $r = 0.165$  ( $P = 0.49$ ) (YSI 1500) and  $r = -0.424$  ( $P = 0.07$ ) (YSI 2300). Adding Hb glutamer-200 resulted in a systematic trend in the difference between measured-minus-calculated lactate for the YSI 2300 but not for the YSI 1500. If there were perfect agreement between measured-and-calculated lactate values, the Bland-Altman correlation would be zero.

The mean difference between measured-minus-calculated lactate for Hb glutamer-200 was -8.4 mg/dL (LX-20®) (13.4% of the overall mean); for Hb glutamer-250 it was -5.1 mg/dL (LX-20®) (11.8% of the overall mean), and for Hb raffimer it was -2.2 mg/dL (LX-20®) (3.7% of overall mean) (Fig. 1C-E and Table 3). Note that data points are not differentiated by HBOC concentration, as there were no significant differences among the different groups. Hb glutamer-200 and Hb glutamer-250 have greater bias than Hb raffimer with measured values systematically less than calculated values, especially at larger lactate concentrations. The RMSE for Hb glutamer-200 was 11.0 mg/dL (LX-20®) (17.6% of the overall mean); for Hb glutamer-250 it was 6.4 mg/dL



**Figure 1.** Lactate interference by hemoglobin-based oxygen carriers. Bland-Altman plots with linear regression lines for samples containing added lactate, Plasma-Lyte A™, and hemoglobin glutamer-200, hemoglobin glutamer-250, or hemoglobin raffimer oxygen carriers tested on YSI 1500 SPORT, YSI 2300 STAT, and Synchron LX-20® lactate analyzers. A. YSI 1500 with hemoglobin glutamer-200. B. YSI 2300 with hemoglobin glutamer-200. C. LX-20® with hemoglobin glutamer-200. D. LX-20® with hemoglobin glutamer-250. E. LX-20® with hemoglobin raffimer.

(LX-20®) (14.9% of the overall mean), and for Hb raffimer it was 4.6 mg/dL (LX-20®) (8.0% of the overall mean). These findings show that Hb glutamer-200 and Hb glutamer-250 cause the LX-20® to consistently underestimate calculated lactates with a larger error at larger concentrations. The difference between measured-minus-calculated lactate varies systematically, not randomly, for Hb glutamer-200 and Hb glutamer-250 but not for Hb raffimer. For Hb raffimer, the Bland-Altman correlation was  $r = 0.106$  ( $P = 0.67$ ) (LX-20®), indicating

no significant trend in the differences. However, for Hb glutamer-200 and Hb glutamer-250, the Bland-Altman correlation was  $r = -0.742$  ( $P < 0.001$ ) (LX-20®) and  $r = -0.891$  ( $P < 0.001$ ) (LX-20®), respectively.

For the LX® 20 analyzer measuring the control (no HBOC) samples, the Bland-Altman concordance coefficient ( $r$ ) was nearly zero, and the mean difference in the Bland-Altman analysis was  $<1$  mg/dL lactate, indicating that the LX-20® was accurate in the absence of HBOC.

**Table 3.** Summary Statistics for Measured (Y) Versus Calculated (X) Lactate

Type of Blood Substitute	Hb glutamer-200	Hb glutamer-200	Hb glutamer-200	Hb glutamer-250	Hb raffimer
Type of Lactate Analyzer	YSI 1500	YSI 2300	LX-20	LX-20	LX-20
Mean Difference (mg/dL) (Measured - Calculated)	1.31	-2.59	-8.38	-5.10	-2.15
Difference Standard Deviation (mg/dL)	3.02	2.78	7.06	3.92	4.09
Minimum Difference (mg/dL)	-3.78	-7.48	-27.00	-12.00	-11.00
Maximum Difference (mg/dL)	8.47	1.71	3.00	0.00	7.00
RMSE of Differences (mg/dL)	3.30	3.80	10.96	6.43	4.62
Sample size (n)	21	21	20	20	20
Overall Average Lactate (mg/dL)	37.78	35.83	62.41	43.25	57.73
RMSE/Overall Average	8.72%	10.60%	17.55%	14.87%	8.01%
Measured vs Calculated correlation (Y vs. X)	0.987	0.990	0.977	0.995	0.989
Bland-Altman correlation (r): (Y - X) vs. (Y + X)/2	0.165	-0.424	-0.742*	-0.891*	0.106

RMSE = root mean square error; Hb = hemoglobin.

\*Significantly different from zero indicating trend in the bias.

## Discussion

HBOCs are purified solutions of highly polymerized bovine or human Hb. Hb glutamer-200 is Food and Drug Administration (FDA) approved for clinical use in canine anemia (1) and is closely related to Hb glutamer-250 (Table 1), which is approved for human use in South Africa and is currently under FDA review in the United States (US). Hb raffimer has also received FDA clearance for preliminary testing in the US.

When we compared the performance of the LX-20<sup>®</sup> lactate analyzer in samples containing no HBOC (external validation series), the following was notable: the control data (Table 2) demonstrate that the LX-20<sup>®</sup> instrument, *per se*, provides very accurate measurements over a wide range of lactate concentrations.

In samples analyzed on the LX-20<sup>®</sup> containing the human HBOC, Hb glutamer-250, in concentrations comparable to plasma Hb levels likely to be achieved under clinical conditions of HBOC infusion (approximately 1-2 g/dL), the average difference between measured and calculated lactate concentrations was -5.1 mg/dL (-0.57 mM), with greater underestimation at larger lactate concentrations. In fact, the difference between measured and calculated lactate at a lactate concentration of 75 mg/dL was -12 mg/dL (-1.3 mM). It is important to stress that this discrepancy is clinically important, given the normal reference range for plasma lactate. The average measured-minus-calculated lactate discrepancy represents 33% of the normal physiological lactate reference range. When Hb raffimer is present in plasma being analyzed by the LX-20<sup>®</sup>, the average difference is -2.2 mg/dL [-0.24 mM] or 14% of the reference range.

The veterinary product, Hb glutamer-200, was tested on all three analyzers. The YSI 1500 proved to be the most accurate instrument, with the mean difference between measured-minus-calculated lactate

being +1.3 mg/dL (the only series in which lactate was overestimated) versus -2.8 mg/dL (YSI 2300) and -8.4 mg/dL (LX-20<sup>®</sup>). Therefore, the YSI 1500 instrument was used to obtain the most accurate measurement of baseline lactate levels in the three HBOC stock preparations used in this study (Table 1).

In both the YSI and LX-20<sup>®</sup> analyzers, lactate is bound by a substrate specific enzyme, lactate oxidase, which oxidizes lactate producing hydrogen peroxide. In the YSI analyzers, the hydrogen peroxide passes through a cellulose acetate layer to a platinum electrode where the hydrogen peroxide is oxidized. The resulting current is proportional to the concentration of the lactate. In the LX-20<sup>®</sup> analyzer, hydrogen peroxide subsequently reacts with dichlorobenzene-sulfonic acid and 4-aminoantipyrine to form a colored chromophore that absorbs light at 520 nm; the change in absorbance is proportional to the concentration of lactate in a given sample.

Hydrogen peroxide reacts rapidly with the ferrous (HbFe<sup>2+</sup>) and ferric heme (HbFe<sup>3+</sup>) moieties of HBOCs (16). In fact, in the presence of excess H<sub>2</sub>O<sub>2</sub>, hypervalent ferryl heme (HbFe<sup>4+</sup>) can be formed. HBOCs contain Hb outside its protective environment created by the red blood cell membrane; hence, unhindered oxidation of its iron center occurs (16). If enough H<sub>2</sub>O<sub>2</sub> is being consumed by this oxidation process, these lactate analyzers will underestimate the actual lactate concentration of the sample. At higher lactate levels we observed greater underestimation of calculated lactate (Fig. 1), presumably as a result of increased generation and consequently increased scavenging of hydrogen peroxide by HBOC molecules. We did not observe HBOC concentration-dependent interference; thus, it is likely that there was adequate HBOC present,

even at the smallest concentrations we tested, to cause interference.

Other possible mechanisms of interference in spectrophotometric analyzers by HBOCs have been proposed, including light scattering by HBOC aggregates or particles and altered optical absorbance by the molecular alterations to Hb in HBOCs (13).

Based on the samples tested in this study, our results indicate that calculated lactate levels in the presence of HBOCs are underestimated most of the time when measured by a lactate analyzer. The clinical implications of this study are that with increasing levels of a HBOC in plasma, lactate interpretation may become inaccurate, especially at larger lactate concentrations, causing underestimation of measured lactate values and possible under-treatment of the patient. Therefore, caution must be exercised when interpreting lactate results when a HBOC is present in plasma.

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