Received: 24 March 2023 | Accepted: 14 July 2023

DOI: 10.1111/ijlh.14146

ORIGINAL ARTICLE

ISLH International Journal of Laboratory Hematology WILEY

Interference of bovine hemoglobin-based oxygen carrier-201 (Hemopure) on four hematology analyzers

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Abstract

Introduction: Hemoglobin-based oxygen carriers, for example HBOC-201 (Hemopure), are aimed to bridge acute anemia when blood transfusion is not available or refused by the patient. However, since HBOC-201 appears free in plasma, it interferes with laboratory tests. This study presents an overview of HBOC-201 interference on four commonly used hematology analyzers and suggests treatment monitoring possibilities.

Methods: Blood samples were spiked with therapeutic doses of HBOC-201 and nine hematology parameters were measured with the Sysmex XN-20, Siemens Advia 2120i, Abbott Alinity Hq and Abbot Cell Dyn Sapphire hematology analyzers. The results were compared to control samples and the bias was determined.

Results: Most parameters, including all cell counts, hematocrit and MCV, showed a non-significant bias compared to control. However, the standard, total hemoglobin (Hb) measurement as well as MCH and MCHC showed poor agreement with control, as HBOC-201 was included in this measurement. Yet, the flow cytometry-based Hb method quantified intracellular Hb in spiked samples, excluding HBOC-201.

Conclusion: Of all included hematology parameters, only total Hb and the associated MCH and MCHC suffered from interference. In contrast, the flow cytometry-based Hb measurement provided an accurate measure of intracellular Hb. The difference between total Hb and cellular Hb represents the HBOC-201 concentration and can be used to monitor HBOC-201 treatment.

KEYWORDS

extracellular hemoglobin, hematology analyzer, hemoglobin-based oxygen carrier, interference, intracellular hemoglobin

1 | INTRODUCTION

Hemoglobin-based oxygen carrier-201 (HBOC-201, bovine hemoglobin glutamer-250, Hemopure; HbO_2 Therapeutics, Boston, MA, USA) is an erythrocyte-free transfusion product. HBOC-201 is aimed to restore oxygen carrying capacity in severe anemia, mainly to bridge the acute anemic period, when standard of care blood transfusions are not available or are refused by the patient.[1] In the production process, bovine hemoglobin is purified and polymerized to form chains of on average 250 kDa molecular weight, thereby stabilizing the

hemoglobin, reducing tissue toxicity and increasing the circulatory half-life from 0.5 to 19 h.[1,2] Additionally, blood-borne pathogens, including bacteria, viruses and prions, as well as pro-inflammatory stimuli are removed. In contrast to erythrocytes, HBOC-201 can be stored at room temperature (2–30°C) for 3 years, making it suitable for use in remote locations without blood bank infrastructure. Because HBOC-201 contains no blood group antigens, HBOC-201 is suitable for patients with antibodies against erythrocytes for whom no regular transfusion options are available.[2] To this date, HBOC-201 has been approved for use in adults with acute anemia in South Africa (since 2001) and in Russia (since 2010). Although neither the Food and Drug Administration (FDA) nor the European Medicines Agency (EMA) has provided approval for human use, HBOC-201 has been administered to over 2000 patients in the US and Europe. Besides clinical trials, these cases of HBOC-201 therapy were on named-patient basis or part of compassionate use programs, for individual patients or patient groups, respectively, who would suffer from life-threatening anemia without HBOC-201 and for whom standard of care blood transfusions are not available or safe.

HBOC-201 is polymerized hemoglobin (Hb) that is administered intravenously in an iso-oncotic balanced modified Ringer's lactate at a concentration of 13 g/dL (8 mmol/L). Therefore, HBOC-201 will appear as free Hb in blood plasma.[2] This may pose a challenge to the clinical laboratory, as it interferes with general laboratory testing. Several publications have investigated the influence of HBOC-201 on clinical chemistry[1,3–5] and hematology tests,[4] but these publications are at least a decade old. We hypothesized that modern hematology analyzers may be able to distinguish HBOC-201 from the patient's native Hb using flow cytometry-based methods, providing a means to monitor HBOC-201 treatment. Therefore, our primary aim was to investigate patient samples spiked with HBOC-201 using four commonly used hematology analyzers.

2 | MATERIALS AND METHODS

HBOC-201 (Lot RSH11T01) was kindly provided by the manufacturer (HbO_2 Therapeutics, Boston, MA, USA). For the spiking experiments, nine residual anonymized human EDTA anticoagulated blood samples were selected, collected after completing all ordered hematology tests. In order to perform measurements in the relevant Hb range,[6] these EDTA samples (blood group O or A) were diluted to Hb 3.2–6.5 g/dL (2–4 mmol/L) using residual EDTA plasma from the transfusion laboratory (pool of 17 plasmas, with blood group A or AB to prevent erythrocyte agglutination). Subsequently, samples were spiked with each of these three options: 10% HBOC-201 (vol/vol), 5% HBOC-201 supplemented with 5% saline (0.9% NaCl; Baxter), or 10% saline (control). In this way, we approximated the conditions of up to three transfused units of HBOC-201 to an acutely anemic patient,[1,6] while keeping the amount of patient material equal for all conditions.

All samples were gently mixed and analyzed by four different hematology analyzers (located at three clinical laboratories), following the routine standard operating procedures. The analyzers included the Sysmex XN-20 (Sysmex Corporation, Kobe, Japan), Siemens Advia 2120i (Siemens Healthineers, Erlangen, Germany), Abbott Alinity Hq (Abbott Diagnostics, Santa Clara, CA, USA) and Abbott Cell Dyn Sapphire (Abbott Diagnostics, Santa Clara, CA, USA). Most hematology parameters included in this study are currently in use for routine diagnostic testing. A few parameters were not routinely used as diagnostic parameters, but as research parameters. The measurement principles of all tests reported in this study are summarized in Table 1. Proper calibration and quality control is ensured based on each laboratory's standard operating procedures. Testing was completed within 10 h of the moment of phlebotomy.

The HBOC-201-spiked samples were compared to their corresponding control samples (spiked with saline) using Analyse-it for Microsoft Excel (version 5.40, Analyse-it Software Ltd., Leeds,

TABLE 1 Summary of measurement principles of all hematology tests investigated in this study.

Test	Sysmex XN-20	Siemens Advia 2120i	Abbott Alinity Hq	Abbott Cell Dyn Sapphire
Leukocyte count	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry
Erythrocyte count	Impedance	Flow cytometry	Flow cytometry	Flow cytometry and impedance
Platelet count	Impedance	Flow cytometry	Flow cytometry	Flow cytometry
Hb total	Spectrophotometry (and sulphate-based reagent)	Spectrophotometry (cyanmethemoglobin method)	Spectrophotometry (and imidazole reagent)	Spectrophotometry (and imidazole reagent)
Hb cellular	Flow cytometry[a]	Flow cytometry	Flow cytometry[a]	N.A.
Hematocrit	Impedance (cumulative pulse height)	Calculated (MCV × ery count)	Calculated (MCV × ery count)	Calculated (MCV × ery count)
MCV	Calculated (hematocrit/ery count)	Flow cytometry (low angle light scatter)	Flow cytometry	Impedance
MCH	Calculated (Hb/ery count)	Calculated (Hb/ery count)	Calculated (Hb/ery count)	Calculated (Hb/ery count)
MCHC	Calculated (Hb/hematocrit)	Calculated (Hb/hematocrit)	Calculated (Hb/hematocrit)	Calculated (Hb/hematocrit)

[a]Designated "for research use only" and not (yet) regularly used for diagnostics.
Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

United Kingdom). The bias of 5% and 10% spiked samples versus control was obtained using Bland–Altman analysis. These data is also graphically shown in difference plots, in which the bias ($\Delta Hb = Hb_{5\% \text{ or } 10\%} - Hb_{control}$) is plotted against the Hb of the control samples. Statistical difference between spiked samples and control was defined as $p < 0.05$ following a Student's t test (Microsoft Excel, 2016, Redmond, WA, USA).

3 | RESULTS

In order to determine the interference of HBOC-201 on the Sysmex XN-20, Siemens Advia 2120i, Abbott Alinity Hq and Abbot Cell Dyn Sapphire hematology analyzers, nine hematology parameters were determined in nine blood samples, spiked with 5% or 10% HBOC-201, and compared to control samples. Most parameters, including all cell counts, hematocrit and mean corpuscular volume (MCV) show a non-significant bias compared to control (p value >0.05). However, the total Hb as well as mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) show poor agreement with control. These results are summarized in Table 2. The explanation for this poor agreement lies in the method of the total Hb measurement. All analyzers make use of cell lysis and spectrophotometry to determine the Hb concentration. For this reason, all free Hb in plasma including HBOC-201 is measured in addition to intracellular, physiologically relevant Hb. Because of the discrepancy in Hb, the associated calculated parameters that us MCH and MCHC, also show a bias and statistically significant deviation from control.

Three out of these four analyzers (Sysmex XN-20, Siemens Advia 2120i and Abbott Alinity Hq) also provide an alternative method to determine Hb: a method based on the scattering properties of Hb-filled erythrocytes in flow cytometry mode, exclusively quantifying intracellular Hb. In contrast to the total Hb measurement, the flow cytometry-based Hb provides an accurate representation of the native, intracellular Hb and does not show a deviation from the control (p value >0.05). In Figure 1 the cellular Hb measurements are displayed alongside the total Hb measurements in difference plots. From these graphs it becomes clear that the cellular Hb method only measures intracellular Hb (as the difference with control approaches 0 g/dL). On the other hand, the total Hb method includes HBOC-201: the observed biases for total Hb are approximately 0.65 g/dL (0.4 mmol/L) at 5% HBOC-201 and 1.3 g/dL (0.8 mmol/L) at 10% HBOC-201, which corresponds to the expected HBOC-201 concentration based on a Hb content of approximately 13 g/dL (8 mmol/L) for HBOC-201.[2] By subtracting the cellular Hb fraction from the total Hb, the cell-free Hb-fraction remains, representing the HBOC-201 concentration.

TABLE 2 Summary of the relevant hematology parameter results of the four hematology.

		Measurement range (control)			5% HBOC-201 (vs. control)			10% HBOC-201 (vs. control)		
Test	Unit	Mean	Min	Max	Bias	p value	n	Bias	p value	n
Sysmex XN-20										
Hb total	g/dL	3.4	2.1	4.7	0.680	0.00	9	1.271	0.00	9
Hb cellular	g/dL	3.4	2.3	4.7	0.040	0.71	8	0.021	0.60	8
MCH	pg	29.0	23.8	31.0	6.0	0.00	9	11.6	0.00	9
MCHC	g/dL	32.9	30.1	36.3	6.6	0.00	9	12.9	0.00	9
Siemens Advia 2120i										
Hb total	g/dL	3.7	2.3	5.0	0.59	0.00	9	1.181	0.00	9
Hb cellular	g/dL	3.7	2.4	5.0	−0.018	0.59	9	0.000	1.00	9
MCH	pg	29.0	24.2	30.6	5.3	0.00	9	10.4	0.00	9
MCHC	g/dL	32.2	30.0	34.5	5.8	0.00	9	11.5	0.00	9
Abbott Alinity Hq										
Hb total	g/dL	3.7	2.4	5.0	0.625	0.00	9	1.215	0.00	9
Hb cellular	g/dL	3.5	2.3	5.2	0.018	0.38	9	0.034	0.38	9
MCH	pg	30.6	25.8	33.8	5.4	0.00	9	10.8	0.00	9
MCHC	g/dL	34.0	32.7	35.1	6.0	0.00	9	11.9	0.00	9
Abbott Cell Dyn Sapphire										
Hb total	g/dL	3.7	2.4	5.2	0.612	0.00	9	1.246	0.00	9
Hb cellular	g/dL	–	–	–	–	–	–	–	–	–
MCH	pg	29.0	25.8	32.2	5.0	0.00	9	10.5	0.00	9
MCHC	g/dL	32.7	31.6	34.0	5.7	0.00	9	11.7	0.00	9

Note: The mean and range of measured hematology parameters for the control (spiked with saline) as well as the bias and p value of 5% and 10% HBOC-201-spiked samples are displayed. Hemoglobin and the associated parameters (MCH and MCHC) are influenced by the presence of HBOC-201 ($p < 0.05$).
Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

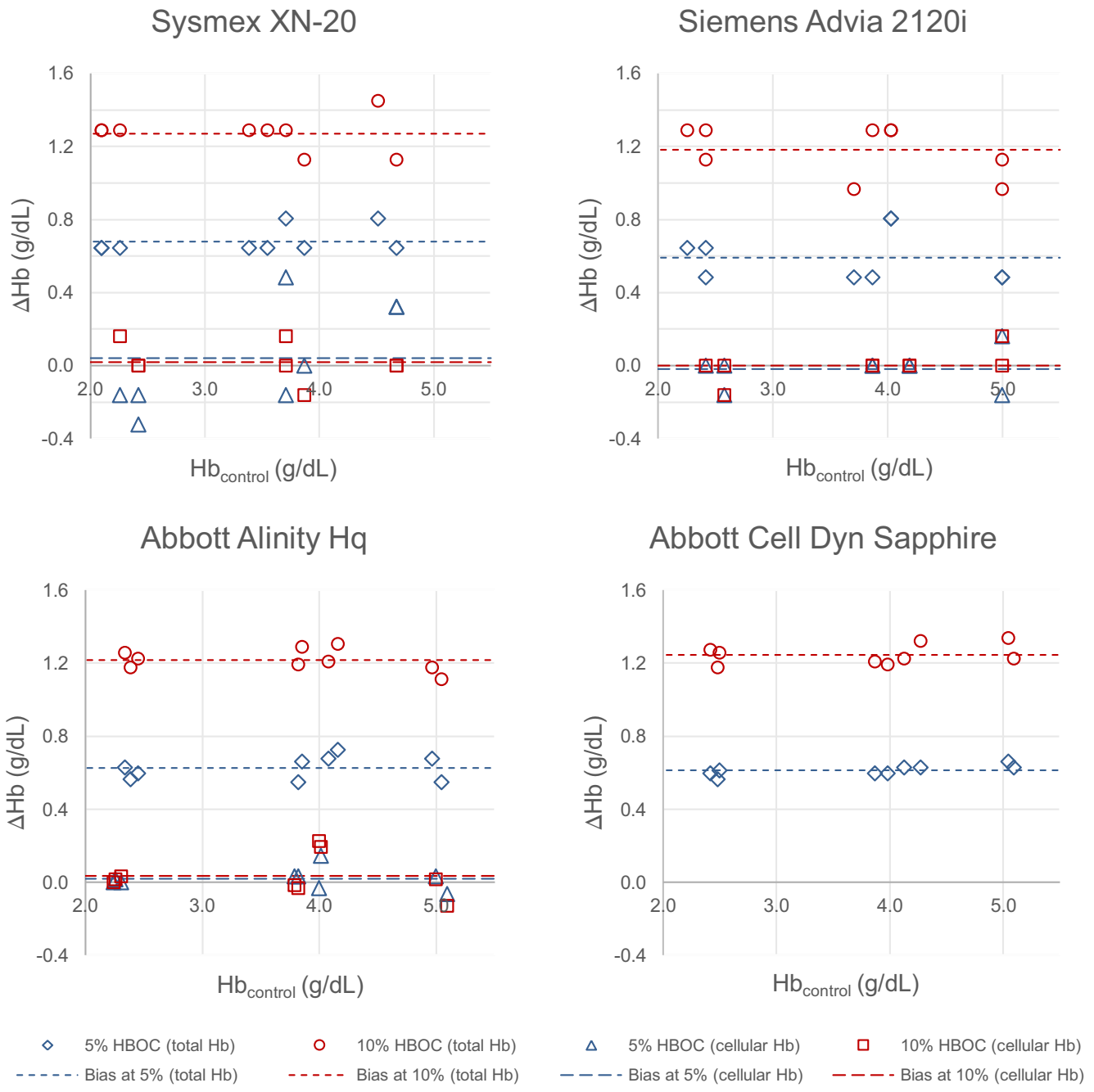

FIGURE 1 Difference plots for hemoglobin measurements. The difference (ΔHb) between either 5% or 10% HBOC-201-spiked samples and their corresponding control samples are plotted against their corresponding control Hb ($Hb_{control}$). Both total Hb results (blue) and cellular Hb results (red) are displayed, if available. The dashed lines denote the average bias for each condition. The presence of HBOC-201 consistently results in an increase in Hb of approximately 0.65 g/dL (5% HBOC-201) or 1.3 g/dL (10% HBOC-201). In contrast, the optical Hb accurately represents the control Hb (leading to $\Delta Hb \approx 0$) and is not influenced by the presence of HBOC-201.

4 | DISCUSSION

For all four evaluated hematology analyzers the hematology results in the presence of HBOC-201 are consistent: most parameters are not influenced by the presence of HBOC-201, while the affected parameters are the same for each analyzer. Notably, the total Hb measurement on all four analyzers is influenced by the presence of HBOC-201, as are the associated calculated parameters MCH and MCHC. Three out of four analyzers offer an alternative Hb measurement based on flow cytometry to quantify intracellular Hb. While this cellular Hb measurement is designated for research use only on the Sysmex XN-20 and Abbott Alinity Hq – and cleared for diagnostic use in the Siemens Advia 2120i—it is advised to use this method instead of the total Hb measurement. That is to say, we have demonstrated

that this cellular Hb is not affected by the presence of HBOC-201. Additionally, these analyzers offer intracellular Hb-based MCH and MCHC, providing valid results for these parameters as well (data not shown). The other tests evaluated in this study—that is all cell counts, hematocrit and MCV—are not influenced at all by the presence of HBOC-201. This is as expected, as the underlying methods are based on either flow cytometry or impedance, which are not influenced by free Hb or discolored plasma.

Our results have important implications. Presently, a consensus guideline from clinical experts advises to monitor plasma Hb (or "free Hb") as well as total Hb levels during HBOC-201 therapy in order to evaluate the effect of HBOC-201 and accordingly redose or stop the supplementation.[6] However, it is not mentioned how the plasma Hb is best measured. We have demonstrated that the contribution of HBOC-201 can be determined with flow cytometry-based intracellular Hb measurement besides the total Hb measurement. Subtraction yields the plasma Hb and thereby corresponds to the HBOC-201 concentration. Therefore, the following formula can guide the physician in the dosing: HBOC-201 concentration = total Hb − cellular Hb.

A limitation of this study is that we have not included blood samples from patients with an underlying hemolytic anemia in this study. Hemolytic anemia can also result in an endogenous free Hb-fraction and may therefore be detected in the total Hb measurement. In cases of severe hemolysis, which may for example occur in sickle cell crises, this endogenous free Hb will add to the polymerized Hb from HBOC-201. This results in an overestimation of the oxygen carrying capacity of the free Hb fraction, because endogenous free Hb is quickly cleared from circulation. It may be possible to distinguish the contributions of HBOC-201 and endogenous Hb to the measured free Hb using size-based separation methods. To this end, the use of filters has been described,[1,4] as well as electrophoresis.[7] Unfortunately, spectrophotometry is unsuitable for this purpose, as the absorption spectra of bovine and human Hb are virtually identical.[8] We did not further explore the size-based separation methods to quantify endogenous free Hb as we hypothesized that its clinical significance is limited. Clinical symptoms and frequent monitoring of both total and free Hb remain of importance in patients with known or suspected hemolysis. In clinical practice, the contribution of endogenous free Hb could be estimated in a blood sample drawn prior to the first HBOC-201 infusion.

Additionally, our results shine new light on the measurement of hematocrit, which may also be used to assess the level of anemia during HBOC-201 therapy. While hematocrit is defined as the ratio of the erythrocyte volume and the total blood volume, in some analyzers—including blood gas analyzers—hematocrit is calculated based on measured Hb and a standard value for the intracellular Hb concentration (MCHC).[6] Such calculated hematocrit does not represent the actual erythrocyte volume in the presence of HBOC-201 as the total Hb includes more than only intracellular Hb. While hematocrit can be accurately determined using manual, centrifugation-based methods, fortunately all evaluated hematology analyzers also provide accurate hematocrit measurements by summing the measured erythrocyte volumes. Nevertheless, in our opinion it is advisable to use the total Hb measurement in combination with cellular Hb rather than hematocrit for therapy monitoring purposes.

5 | CONCLUSION

This work investigated the interference of HBOC-201 on hematology results. To the best of our knowledge, this is the first comprehensive overview using multiple modern hematology analyzers.

In the presence of HBOC-201, most hematology measurements provide reliable results on the Sysmex XN-20, Siemens Advia 2120i, Abbott Alinity Hq and Abbott Cell Dyn Sapphire analyzers. However, care has to be taken when measuring Hb: the standard method measures total Hb, including any Hb or HBOC-201 free in plasma. Fortunately, three of these analyzers (Sysmex XN, Siemens Advia and Abbott Alinity Hq) provide an alternative method quantifying the intracellular Hb. Subtracting this cellular Hb from the total Hb allows direct quantification of the HBOC-201 concentration, thereby providing a means for monitoring HBOC-201 therapy. Based on these parameters clinicians can check whether the patient's own erythrocyte production has returned to adequate levels and decide when to redose or safely stop HBOC-201 infusion. Whether in clinical trials or for compassionate use, we hope that our results aid the monitoring of acutely and severely anemic patients who are treated with HBOC-201.

ACKNOWLEDGMENTS

We acknowledge Leonie van der Heul (Van Weel-Bethesda Hospital, Dirksland, the Netherlands) for her experimental efforts. HbO_2 Therapeutics provided HBOC-201 for this study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Bronkhorst-van der Helm MW, Weerkamp F, Huisman A, Huisman EJ, Russcher H. Interference of bovine hemoglobin-based oxygen carrier-201 (Hemopure) on four hematology analyzers. *Int J Lab Hematol*. 2023;1-6. doi:10.1111/ijlh.14146