Using the hemolysis index of Abbott’s Alinity c for the measurement of plasma free hemoglobin in ECMO patients

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Abstract: Highlights • The Alinity c HI allows the quantitative determination of plasma free hemoglobin. • The method is rapid and cost-effective with a measuring range from 80 to 7250 mg/L. • Bilirubin up to 710 mg/L and Intralipid® up to 5580 mg/L did not affect the measurement. • The method can be used for STAT analysis in a routine laboratory. Objectives Quantitative measurement of plasma free hemoglobin (fHb) concentrations is essential for monitoring pediatric ECMO patients, since hemolysis has a great impact on the patient’s clinical outcome. The aim of this study was to validate the hemolysis index (HI) assay on Abbott’s Alinity c system as a quantitative method to measure fHb.

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Using the hemolysis index of Abbott’s Alinity c for the measurement of plasma free hemoglobin in ECMO patients

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ABSTRACT

Objectives: Quantitative measurement of plasma free hemoglobin (fHb) concentrations is essential for monitoring pediatric ECMO patients, since hemolysis has a great impact on the patient’s clinical outcome. The aim of this study was to validate the hemolysis index (HI) assay on Abbott’s Alinity c system as a quantitative method to measure fHb.

Methods: The performance of the HI assay, based on an automated spectrophotometric method recording the absorption at four different wavelength pairs, was evaluated using the 20 × 2 design according to the CLSI-EP05-A3 guidelines. LLOQ and LLOD were calculated according to CLSI-EP17 guidelines with CVs set to 10% and 20%, respectively. Furthermore, the method was tested for interferences with bilirubin and Intralipid®.

Results: Linearity was ensured over an analytical measurement range of 30–7250 mg/L and the calculated LLOQ and LLOD were 80 mg/L and 50 mg/L, respectively. Intra-run and total imprecisions ranged from 0.9–3.4% and 1.0–3.4%, respectively. The HI assay correlated well with the Harboe method (HI (mg/L) = 0.998 * fHb (mg/L) + 28 mg/L, R = 0.998, n = 50) and interference testing showed no impact of bilirubin and Intralipid® up to 709 mg/L and 5580 mg/L, respectively.

Conclusions: The HI assay on Abbott’s Alinity c system allows a precise and accurate determination of fHb concentrations with no significant interferences in a simple, rapid and cost-effective way.

1. Introduction

The simultaneous measurement of HIL indices in clinical laboratories is a common way to monitor serum/plasma sample integrity and allows for an estimation of pre-analytical errors through hemolysis, icterus or lipemia [1]. Hemolysis is defined as the release of hemoglobin and other intracellular components from erythrocytes into the plasma, which can occur in vivo or in vitro. Intravascular (in vivo) hemolysis can be recognized by a parallel reduced haptoglobin concentration, which is a hemoglobin-binding protein. In vitro hemolysis, however, has unchanged haptoglobin concentrations [2]. After centrifugation, hemolyzed plasma/serum samples appear in a reddish color, which is visually detectable from a hemoglobin concentration of 300 mg/L [2,3].

Hemolysis can interfere with various clinical laboratory assays leading to falsely high results of the corresponding parameters. Reasons can be that hemoglobin absorbs at the same wavelength as the parameter, that other erythrocyte constituents leak into serum/plasma through the fragmentation of erythrocytes or that such constituents interfere with chemical reactions used to measure certain parameters [2,3]. Hence, the determination of the hemolysis index (HI) on clinical chemistry automated analyzers is justified. However, they are most often only approved to give qualitative or semi-quantitative results and are not intended for diagnostic purposes [4,5].

The aim of this work was to validate the performance of the HI as a quantitative diagnostic assay for plasma free hemoglobin (fHb). One of the intended diagnostic purposes is the surveillance of the onset of hemolysis in patients with extracorporeal membrane oxygenation (ECMO) therapy [6]. ECMO is a technology used in patients with severe...
cardiopulmonary dysfunction. Oxygenation of the patient’s blood outside its body is possible through a complex apparatus with circuit tubing [7]. Through mechanical stress, mainly resulting from clots in the tubes or excessive centrifugal pump speed, erythrocyte membranes can rupture whereby hemoglobin is released into the plasma [8,9]. Such hemolysis should be detected rapidly because the presence of fHb is associated with a need for blood transfusions, an increased risk of renal impairment and death [8,9]. Lou et al. classified hemolysis with respect to ECMO patients as no hemolysis (<100 mg/L), mild (100–500 mg/L), moderate (500–1000 mg/L) and severe (>1000 mg/L) [9].

2. Material and methods

2.1. Materials

For the evaluation experiments, artificial plasma was prepared by using sodium chloride (0.9%) from Merck (Schaffhausen, Switzerland), Bovine Serum Albumin (7%) from Honeywell Fluka (NJ, USA) and ultrapure water from the in-house ELGA Purelab Ultra water purification system (Labtec Service AG, Wohlen, Switzerland).

For interference testing, crystalline powder bilirubin (>98%) and dimethyl sulfoxide (DMSO) from Sigma-Aldrich (Buchs, Switzerland), sodium carbonate anhydrous from Merck (Schaffhausen, Switzerland), Intralipid® emulsion (20%) from Fresenius Kabi Austria GmbH (Graz, Austria) and 0.9% saline solution from B. Braun (Sempach, Switzerland) were used. Furthermore, anonymized left over lithium heparinate samples (S-Monovette®, Sarstedt AG & Co. KG, Nümbrecht, Germany) from the routine Clinical Chemistry laboratory of the University Children’s Hospital in Zurich were used for the preparation of standard solutions and quality control samples (QCs) as well as for the method comparison. Samples were less than one month old before analysis and the hemolysis index ranged between 0 and 4+.

2.2. Instrumentation

For the measurement of the fHb concentration, the HI assay on the Abbott Alinity c system (Abbott Laboratories, IL, USA) was used. This is an automated spectrophotometric method, which records the absorption at four different wavelength pairs, namely 500/524, 572/604, 628/660 and 524/504 nm, compared to 0.9% saline solution. The resulting, mathematically calculated concentration of fHb is corrected for spectral overlap and is presented in mg/L [5,10]. As a reference method, the absorption spectrum of hemoglobin was measured with the 3-wavelength method (415/380/450 nm) according to Harboe using the Tecan Reader Infinite 200 Pro (Tecan Group Ltd., An~~\textsuperscript{d}ernorf, Switzerland).

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2.3. Preparation of standard solutions and quality control samples

For the linearity check, ten standard solutions with concentrations that cover a broad fHb measurement range (30–7250 mg/L) were produced. Therefore, a lithium heparinate blood sample (4.5 mL) was hemolyzed by two quick-freezing (liquid nitrogen) and thawing cycles according to Barbhuiya et al. [12]. The thawing process was accelerated by gently stirring the tube at 37 °C (300 rpm, 10 min). To remove the cell debris the sample was centrifuged (9600 g, 20 min) and the supernatant was collected as hemoglobin stock solution. The hemoglobin concentration was determined on a Sysmex XN-1000 analyzer (Sysmex America, IL, USA). A working solution (5000 mg/L) was produced by diluting the hemoglobin stock solution with artificial plasma.

Three QCs were produced in the same way with concentrations of 170, 500 and 1000 mg/L, respectively. All plasma samples were stored at −20 °C until analysis, according to Lippi et al. who showed that hemoglobin is stable at this temperature for at least six months [1]. All samples were only thawed once.

2.4. Linearity, lower limit of quantification and lower limit of detection

To investigate linearity, ten standard solutions with targeted concentrations of 30, 50, 100, 130, 290, 500, 1000, 1450, 2900 and 7250 mg/L were measured in duplicate and least-squares linear regression analysis was used to fit the targeted and measured concentrations of fHb. For the determination of the lower limit of quantification (LLOQ) and lower limit of detection (LLOD), these ten standard solutions were measured seven times in a row and a precision profile was created by comparing the reciprocal of the targeted concentration with the corresponding coefficient of variation (CV%). A quadratic fit allowed the calculation of the concentrations at which the CV was 10% and 20%, which corresponds to the LLOQ and LLOD, respectively (adapted from CLSI [13]). We used 10% of CV for LLOQ because we expect such a precision for a quantitative determination of free hemoglobin and we used 20% of CV for the LLOD according to the often used cut-off for the functional assay sensitivity.

2.5. Precision and accuracy

Precision (CV%) was evaluated using the 20 × 2 × 2 design according to the CLSI-EPO5-A3 guidelines [14]. Therefore, three levels of QCs were measured on 20 testing days with two runs per day and two replicas per run. Total accuracy (%) was determined by comparing the calculated overall average with the targeted concentration of each QC level from the precision evaluation experiment. The method comparison was carried out with patient samples of varying grades of hemolysis (n = 50) and using least-squares linear regression analysis against the reference method.

2.6. Interferences

To test for interferences by icteric and lipemic plasmas, interference test solutions were prepared using bilirubin and Intralipid®. The bilirubin solution (2.0 g/L) was prepared by dissolving bilirubin in DMSO / 1 M sodium carbonate (ratio 2:3) (adapted from Barbhuiya et al. [12]). Three bilirubin working solutions B1, B2, and B3 with concentrations of 400, 800 and 1200 mg/L were prepared by dilution of the stock solution with artificial plasma. The Intralipid® stock solution (20.0 g/L) was prepared by diluting Intralipid® (20%) in 0.9% saline solution. Three working solutions L1, L2, L3 with concentrations of 4000, 8000, and 12,000 mg/L Intralipid® were prepared by dilution with artificial plasma.

2.7. Data analysis

Data analysis was performed using Microsoft Excel for the analysis of variance (ANOVA) according to CLSI-EPO5-A3 [14] and MedCalc® v14.8.1 (MedCalc Software Ltd, Ostend, Belgium) for least-squares linear regression analysis.

3. Results

3.1. Linearity, LLOQ and LLOD

Linear regression analysis showed good linear relationship between the targeted and measured fHb concentrations with a coefficient of regression (R) of 0.999, a slope of 1.09 and an intercept of −63.4 mg/L, as shown in Fig. 1. The calculated LLOQ and LLOD were 80 mg/L and 50 mg/L, respectively. The quadratic fit resulted in a coefficient of regression (R) of 0.981.

3.2. Precision and accuracy

Results from the 20 × 2 × 2 precision evaluation experiment showed low intra-run imprecision (0.9–3.4%) and even lower inter-run (0%) and
Total accuracy ranged from 96.9 to 101%.

Comparing the fHb measurements on the Abbott Alinity c to the reference method for fHb according to Harboe showed good correlation from 96.9 to 101%.

### 3.3. Interferences

Bilirubin concentrations of 277, 470 and 709 mg/L (corresponds to 479, 804, 1214 µmol/L) were spiked to four different levels of fHb in plasma (600, 1000, 1500 and 3000 mg/L) and the difference in fHb measurements between the bilirubin spiked and non-spiked plasma was calculated, which ranged from −11.8 to 2.0% (Table 2). Similarly, Intralipid® concentrations of 1430, 3163 and 5580 mg/L were spiked to the four different levels of fHb in plasma and the difference in fHb measurements between spiked and non-spiked samples was assessed, which ranged from −1.2 to 1.0%.

To corroborate these findings in real human plasma samples, one lipemic and one icteric patient sample were each mixed with two different hemolytic patient samples (1340 and 1890 mg/L, respectively) and the mean differences in fHb measurement between the spiked and non-spiked samples were calculated. Again, there was only a minor influence of lipemia and icterus on the fHb measurements in human samples (Table 2).

### 4. Discussion

Quantitative measurement of plasma free hemoglobin concentrations is essential for monitoring pediatric ECMO patients, since hemolysis has a great impact on the patient’s clinical outcome [6,9]. Omar et al. stated that fHb concentrations should be checked within the first inter-day (0.3–0.4%) imprecisions (Table 1). Total accuracy ranged from 96.9 to 101%.

In addition, the HI method has a very high precision and accuracy, with a total imprecision of 0.9–3.4% and an inaccuracy of –3.1 to 0.6% in the measuring range of 80–7250 mg/L. This allows to classify hemolysis in pediatric ECMO patients, including newborns with a high fraction of fetal hemoglobin (HbF) [9].

The HI method shows excellent linearity down to the LLOQ of 80 mg/L, with a coefficient of regression of 0.999, a slope of 1.09 and an intercept of 63.4 mg/L determined over the entire experimental range 30–7250 mg/L. The slightly positive slope can be attributed to the imprecision during the dilution of the stock solutions to the target concentrations and to the large inaccuracy in measuring the lowest two standards (up to 33%). Although, the determined LLOQ will not allow to measure fHb concentrations in the reference range of healthy individuals (<50 mg/L), it is sufficient to determine the fHb in patients under ECMO therapy, for whom fHb concentrations below 100 mg/L are clinically not relevant [9].

In addition, the HI method has a very high precision and accuracy, with a total imprecision of 0.9–3.4% and an inaccuracy of –3.1 to 0.6% in the measuring range of 80–7250 mg/L. Intriguingly, the intra-run imprecision, i.e. repeatability, accounts for the main part of the total imprecision (0.9–3.4%), which suggests that the method is stable and precise over time. These performance characteristics of the HI on the Alinity c are in line with the evaluation of the HI on five different clinical chemistry analyzers, namely the Modular System P (Roche, Mannheim, Germany).

### Table 1
Mean, imprecisions and accuracy of fHb measurements on the Abbott Alinity c.

| Unit                  | Level I | Level II | Level III |
|-----------------------|---------|----------|-----------|
| Mean (mg/L)           | 169     | 485      | 1006      |
| Intra-Run CV<sub>rel</sub> (%) | 3.4     | 1.5      | 0.9       |
| Inter-Run CV<sub>rel</sub> (%) | 0.0     | 0.0      | 0.0       |
| Inter-Day CV<sub>rel</sub> (%) | 0.4     | 0.3      | 0.3       |
| Total CV<sub>rel</sub> (%) | 3.4     | 1.5      | 1.0       |
| Total Accuracy A<sub>T</sub> (%) | 99.3    | 96.9     | 101       |
Germany), Integra 400 Plus (Roche, Mannheim, Germany), Architect c8000 (Abbott, IL, USA), ADVIA 2400 (Siemens, IL, USA) and ADVIA 1800 (Siemens, IL, USA). For these analyzers mean intra-run imprecisions of 0.1–2.7% have been reported, which compare very well to the intra-run precision of the Alinity c [3,4]. However, a recent comparison of several methods to measure fHb in plasma found heterogeneity in performance between different methods arguing for a thorough investigation of the performance characteristics for a particular instrument [15].

We extended the validation of the HI on the Alinity c to cover potential interferences of icterus and lipemia in the determination of fHb. These interference experiments showed that the HI is not affected by icteric and lipemic plasma samples up to bilirubin concentrations of 710 mg/L (1214 μmol/L) and Intralipid® concentrations of 5580 mg/L. Similarly, mixing lipemic and icteric human plasma samples to human samples with fHb did not interfere with the fHb measurement up to bilirubin concentrations of 125 mg/L and lipemia concentrations of 4285 mg/L. This leads to the conclusion that the measurement of moderate to severe hemolysis will not be interfered by high bilirubin or Intralipid® concentrations in the plasma sample. Similarly to our interference experiments, Gabaj et al. showed no interferences of bilirubin and Intralipid® with HI up to concentrations of 175 mg/L and 1100 mg/L, respectively, on an Abbott’s Architect c8000 [4].

By validation of Abbott’s automated qualitative HI assay as a quantitative parameter it is possible to detect hemolysis in ECMO patients in a simple way with a turn-around time necessary for STAT-analysis. This is even possible when considering that the samples need to be centrifuged at reduced speed to prevent in-vitro hemolysis. Inclusion of an internal and enrollment in an external QC program for the measurement of plasma free hemoglobin allows the necessary quality assurance. The implementation of this new method to measure fHb in our laboratory helps to optimize the therapeutic care of our pediatric ECMO patients.

5. Conclusion

The HI method can be used as a quantitative parameter on the Abbotts Alinity c system with no significant interferences and a broad measurement range. The HI allows a simple, rapid way to determine fHb concentrations, with no reagent costs and at any time during day or night.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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