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Reference range of hemolysis index in serum and lithium-heparin plasma measured with two analytical platforms in a population of unselected outpatients

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ABSTRACT

Background: The hemolysis index (HI) is now available in several laboratory analyzers, but doubts remain about the thresholds for suppressing test results, the degree of standardization among different instrumentations and the use of different reference ranges in different biological matrices. This study was hence planned to establish the reference ranges of HI in serum and lithium-heparin plasma in a population of unselected outpatients, using two analytical platforms.
Materials and methods: We analyzed the HI in serum and lithium-heparin samples collected from 135 unselected outpatients, and we also defined the relative reference ranges according to Clinical and Laboratory Standards Institute (CLSI) recommendations. Samples were collected in the morning by expert nurses, using straight needle venipuncture. The HI in serum and lithium-heparin plasma was assessed with Roche Cobas c501 and Siemens Dimension Vista 1500.
Results: The median concentration of cell-free hemoglobin was significantly higher in serum than in lithium-heparin plasma when measured with Cobas c501, but not with Dimension Vista 1500. After categorizing values according to cell-free hemoglobin thresholds, the agreement between instruments was 0.75 ($p < 0.01$) for serum and 0.95 ($p < 0.01$) for lithium-heparin plasma. The upper limits calculated according to CLSI document C28-A3 were 0.22 g/L for Roche Cobas c501 and 0.25 g/L for Siemens Dimension Vista 1500 in serum, whereas they were 0.13 g/L for Cobas c501 and 0.10 g/L for Dimension Vista 1500 in lithium-heparin plasma.
Conclusions: According to our data, different thresholds of cell-free hemoglobin should be used between serum and lithium-heparin plasma for monitoring phlebotomy practice.

1. Introduction

Analytical and/or biological interferences play a pivotal role in decreasing quality throughout the total testing process [1]. Among the various sources of bias, spurious hemolysis is the prevailing source of problems in diagnostic samples, due to the higher prevalence as compared with other potential preanalytical and analytical pitfalls, and for the multifaceted biological and analytical interferences that may bias a large number of tests. It has hence become clear that hemolysis-sensitive laboratory data obtained in spuriously hemolyzed samples should not be reported in order to safeguard patient safety and prevent inappropriate clinical actions guided by unreliable laboratory data [2].

In general, the cut-off for considering a sample as hemolyzed has been fixed at 0.5 g/L of cell-free hemoglobin, which is a value that can also be identified by visual inspection of the specimen. Several lines of evidence attest, however, that visual inspection is an unreliable approach for assessing sample quality, since it is arbitrary, scarcely reproducible, not traceable and also plagued by poor sensitivity [3]. Recently, automatic spectrophotometric detection of cell-free hemoglobin, along with other interfering substances such as bilirubin and lipids, has become available in a large number of laboratory analyzers. As specifically regards hemolysis, cell-free hemoglobin is automatically quantified by absorbance measurements on serum or plasma at different wavelengths, and the concentration of cell-free hemoglobin is finally reported as "hemolysis index" (HI) [4]. This approach has now become virtually unavoidable for identifying unsuitable specimens in clinical laboratories equipped with continuous-flow automation, where the physical link between preanalytical and analytical modules hides the samples from scrutiny [5]. Automatic assessment of HI is also a reliable means for establishing sample quality, as attested by the equivalence with the

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reference cyanmethemoglobin method [6–8], and its quantification has a negligible impact on the turnaround time [9].

All that said, several areas of uncertainty remain. These mostly involve the heterogeneous definition of the threshold of cell-free hemoglobin that should be used for suppressing test results across different laboratories, and the still poor standardization of HI among different instrumentations [4,5]. Another important aspect, that has recently been emphasized when the HI has been used for monitoring phlebotomy practices, is the lack of definitive evidence about the potential difference of cell-free hemoglobin concentration and relative reference range between serum and plasma. Although some data have been published about this last issue [6,10–12], there is a broad heterogeneity of results, no definitive information is available about the difference between serum and plasma and, especially, data were obtained with manual spectrophotometric techniques and not using automatic assessment of HI on the new generation of routine laboratory instrumentation. Therefore, the aim of this study was to establish the reference ranges of HI in serum and lithium-heparin plasma samples obtained from a population of unselected outpatients, using two different analytical platforms, i.e., the Roche Cobas c501 chemistry module (Roche Diagnostics, Basel, Switzerland) and the Siemens Dimension Vista 1500 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

2. Materials and methods

The study population consisted in a series of consecutive and unselected outpatients, referred to the Laboratory of Clinical Chemistry and Hematology of the Academic Hospital of Verona for routine testing, and for whom at least one evacuated primary serum tube (Venosafe; Terumo Europe, Leuven, Belgium) and one evacuated primary lithium-heparin tube (Venosafe) ought to be collected according to the medical prescription. Blood was drawn in the morning (i.e., from 7:30 to 9:00 AM) of a single working day by two expert nurses, always using straight needle venipuncture. All phases of sample collection were standardized, thus including identical resting time of patients (i.e., between 5 and 10 min), time of tourniquet placing always <2 min, transportation of samples to the core lab within 1 h and separation of serum and lithium-heparin plasma by centrifugation at 1300 g for 10 min at room temperature. In patients with odd order numbers the serum tube was collected before the lithium-heparin tube, whereas the sequence was inverted in those with even order numbers. After routine analysis of serum and plasma samples was completed (i.e., always within 3 h from collection), the remaining amount of serum or lithium-heparin plasma was divided in two aliquots and refrigerated at −70 °C. After one week of storage, the aliquots were thawed. The HI was then assessed in one aliquot in the reference center (i.e., Verona) with Roche Cobas c501 chemistry module, whereas the second aliquot was shipped in the second center (i.e., Vicenza), where the HI was assessed with Siemens Dimension Vista 1500. All measurements were completed within 2 h after thawing.

The Roche Cobas c501 chemistry module estimates the HI by bichromatic wavelength paired measurement at 570 and 600 nm, providing final results as absolute numbers (range: 1–1000), where one unit corresponds to 0.010 g/L. The Siemens Dimension Vista 1500 quantifies the HI by bichromatic wavelength paired measurement at 405 and 700 nm, providing semi-quantitative data, as follows: ≤1 equals to ≤0.1 g/L of cell-free hemoglobin, 2 to 0.1 and ≤0.25 g/L of cell-free hemoglobin, 3 to 0.25 and ≤0.50 g/L of cell-free hemoglobin and 4 to 0.50 and ≤2.00 g/L of cell-free hemoglobin. For final analysis of data, numerical values provided by both instruments were converted into the corresponding concentrations of cell-free hemoglobin, as previously specified.

Results were analyzed with nonparametric statistics, entailing Wilcoxon Signed-Rank Test, Spearman's rank correlation and Bland & Altman plots. The reference range was defined according to the Clinical and Laboratory Standards Institute (CLSI) document C28-A3 [13].

Table 1
Serum and plasma values (median and 5–95th percentile) of cell-free hemoglobin of 135 consecutive outpatients measured with Roche Cobas c501 and Siemens Dimension Vista 1500.

	Serum (g/L)	Plasma (g/L)
Roche Cobas c501	0.08 [0.03–0.22]	0.04 [0.0–0.012][a]
Siemens Dimension Vista 1500	0.10 [0.10–0.25][b]	0.10 [0.10–0.10][c]

[a] Roche Cobas c501, serum versus plasma, $p < 0.01$.
[b] Serum, Roche Cobas c501 versus Siemens Dimension Vista 1500, $p = 0.019$.
[c] Plasma, Roche Cobas c501 versus Siemens Dimension Vista 1500, $p < 0.01$.

Statistical analysis was performed using Analyse-it (Analyse-it Software Ltd., Leeds, UK). The study was based on pre-existing samples obtained after routine analysis was completed and thereby no informed consent or ethics committee approval was necessary. The investigation was however performed in accord with the Declaration of Helsinki and under the terms of all relevant local legislation.

3. Results

The final study population consisted in 135 consecutive outpatients (mean age 53 y, range 22–78 y; 70 males and 65 females). The mean results of this study are shown in Table 1. The median concentration of cell-free hemoglobin in serum was significantly higher than in lithium-heparin plasma when measured with Roche Cobas c501, but not when measured with Siemens Dimension Vista 1500. The concentrations of cell-free hemoglobin measured with Siemens Dimension Vista 1500 in both serum and lithium-heparin plasma were slightly but significantly higher than those measured with Roche Cobas c501 ($p = 0.02$ for serum and $p < 0.01$ for plasma, respectively) (Fig. 1). The corresponding values (median and 5–95th percentile range)

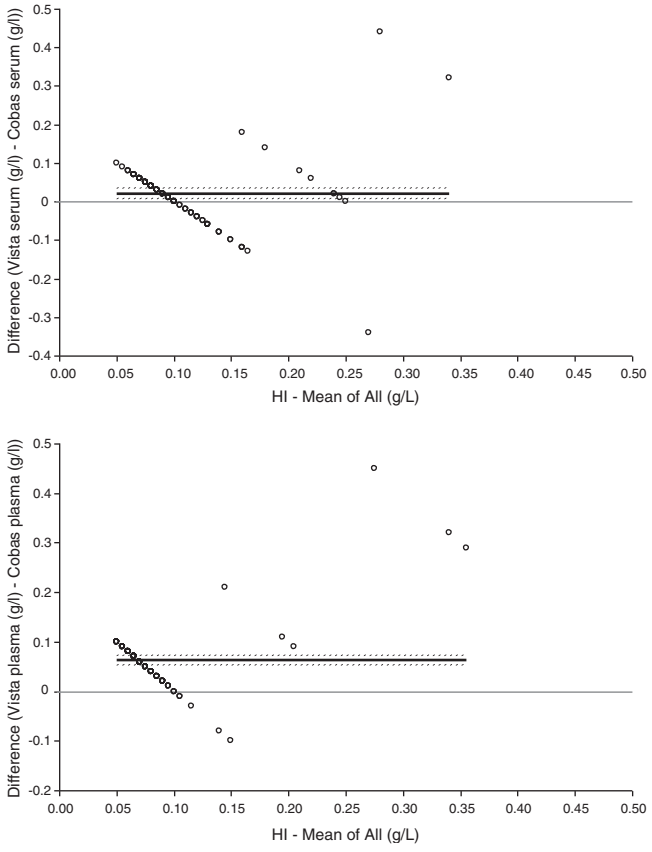

Fig. 1. Bland & Altman plots (mean bias and 95% CI) of the hemolysis index (HI) values in serum and plasma, as measured with Roche Cobas c501 and Siemens Dimension Vista 1500.