Letter to the Editor

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Values and stability of serum (or plasma) indices in uncentrifuged serum and lithium-heparin plasma

https://doi.org/10.1515/dx-2018-0021
Received May 14, 2018; accepted June 23, 2018; previously published online September 24, 2018

Keywords: errors; hemolysis; preanalytical variability; serum indices.

To the Editor,

The automatic assessment of the so-called serum (or plasma) hemolysis/icterus/lipemia (HIL) indices is now regarded as a mainstay for establishing sample quality and ruling out the presence of high concentrations of potential interfering substances such as cell-free hemoglobin, bilirubin and lipids [1]. Due to its high accuracy and reproducibility, the use of these measures has virtually replaced visual inspection of serum or plasma in most clinical laboratories [2, 3]. Therefore, this study was aimed to verify the possible difference of HIL indices in serum and lithium-heparin (LH) plasma, as well as their stability in uncentrifuged serum and LH plasma samples.

Blood was drawn by the same expert phlebotomist using 19 gauge straight needles (Kima, Padova, Italy) from the antecubital veins of 33 healthy subjects (mean age, 42 ± 12 years; 15 women and 18 men), enrolled from the laboratory staff. Three 3.5 mL LH tubes with gel separator (Ref. 12554, Vacutest Kima, Kima, Padova, Italy) and three 3.5 mL serum (S) tubes with gel separator and clot activator (Ref. 10010, Vacutest Kima, Kima, Padova, Italy) were sequentially drawn in a precise order, by shifting the order of draw by one tube on each following volunteer (i.e. volunteer 1: LH0-LH1-LH2-S1-S2-S3; volunteer 2: LH1-LH2-S1-S2-S3-LH0; volunteer 3: LH2-S1-S2-S3-LH0-LH1; volunteer 4, S1-S2-S3-LH0-LH1-LH2, etc.). The first LH0 and serum (S0) tubes were centrifuged (at 1300 × g, at room temperature and for 15 min) within 15 min after collection, and the HIL indices were then measured in both sample matrices. The second (LH1 and S1) and third (LH2 and S2) tubes were stored in a vertical position at room temperature (i.e. between 22 and 24 °C) for 1 and 2 h, respectively. One hour after LH0 and S0 were separated, LH1 and S1 were also centrifuged and the HIL indices were assayed in both sample matrices. Two hours after LH0 and S0 were separated, LH2 and S2 were also centrifuged and the HIL indices were assayed in both sample matrices.

The HIL indices were assayed using an identical clinical chemistry analyzer (Roche Cobas c702, Roche Diagnostics, Risch-Rotkreuz, Switzerland), as specified elsewhere [4]. Briefly, the hemolysis index (H-index) is measured with double combined wavelengths readings (i.e. 570/600 and 660/700 nm), the icterus index (I-index) is measured with triple combined wavelengths readings (i.e. 480/505, 570/600 and 660/700 nm), whilst the lipemic index (L-index) is measured with single combined wavelengths reading (660/700), respectively. The results of these measurements are then resolved by a specific set of equations, and finally expressed as arbitrary units, on a continuous scale. The results of our measurements were found to be normally distributed, as evaluated by the Kolmogorov-Smirnov test, and were hence expressed as mean and standard deviation. The significance of variations was analyzed with a paired Student’s t-test. Statistical significance was set at p < 0.05. All subjects signed a written informed consent for being enrolled in this study, and the research was approved by the local Ethical Committee (University Hospital of Verona, n. 970CESC, August 25, 2016).

In the baseline specimens, centrifuged within 15 after collection (i.e. LH0 and S0), the values of all HIL indices were found to be different between the two sample matrices. More specifically, the H-index was significantly higher in serum than in LH plasma (6 ± 3 vs. 4 ± 2; p = 0.001), the I-index was also slightly but significantly
higher in serum than in LH plasma (23 ± 12 vs. 22 ± 12; \( p = 0.011 \)), whilst the L-index was significantly higher in LH plasma than in serum (15 ± 10 vs. 10 ± 5; \( p < 0.001 \)).

Regarding the stability of the indices in uncentrifuged blood tubes, no significant variations were found for both the H-index and I-index after up to 2 h of storage at room temperature (Table 1). Unlike these two indices, the L-index displayed a significant increase in both LH plasma (mean increase: +16% after 2 h) and in serum (mean increase: +11% and +19% after 1 and 2 h, respectively). Interestingly, the increase of the L-index was found to be already significant after 1 h of storage in uncentrifuged serum tubes, whilst it only become statistically significant after 2 h of storage in uncentrifuged LH plasma tubes (Table 1).

Taken together, the results of this study show that not only the values of the H-index were higher in serum than in LH plasma (mean increase: 39%), as reported in a previous study [5], but also those of the other two indices were significantly different between serum and LH plasma specimens centrifuged after 15 min from collection. More specifically, the I-index displayed a mean 3% increase in serum compared to LH plasma, whilst the L-index was found to be 33% lower in serum than in LH plasma. Notably, a previous study showed that both high-density lipoprotein cholesterol and triglycerides were higher in plasma than in serum when measured with Roche analyzers, thus possibly explaining the difference of the L-index between the two samples matrices which was also observed in our study [6]. This has been at least in part attributed to a volume displacement effect and to the impact of the different sample matrix on the enzymatic tests used for measuring both triglycerides and cholesterol.

Albeit no quality specifications have been set for the HIL indices so far, we can reasonably speculate that the difference observed for the L-index is overall modest and probably meaningless, whilst that observed for the H-index and L-index (both >30%) may have important consequences for defining cut-offs of interference, according to which test results obtained in hemolyzed and lipemic (turbid) samples should be released, flagged or even suppressed. Therefore, the decisional thresholds of these two indices should be probably defined taking into account the sample matrix used in the laboratory (i.e. serum or plasma). Moreover, whenever clinical laboratories are engaged in defining local cut-offs of hemolysis- and lipemia-sensitive assays, as currently recommended [7, 8], they should probably test the impact of hemolysis and lipemia by using either serum or LH plasma samples depending on the sample matrix they are currently using.

Another important aspect emerged from our study is that room temperature storage for up to 2 h of uncentrifuged serum and LH plasma samples does not generate a substantial bias in both H-index and L-index. Unlike these measures, however, room temperature storage of uncentrifuged serum and LH plasma samples caused a gradual increase of the L-index, which became especially evident after 2 h of storage (i.e. +16% in LH plasma and +19% in serum, respectively). Albeit the results of the L-index may be rarely influenced by molecules other than lipids (i.e. paraproteins or contrast dyes) [2], previous evidence has been provided that lipids, especially cholesterol, are

| Table 1: Values (mean and standard deviation; SD) of serum (plasma) indices in uncentrifuged serum and lithium-heparin blood tubes stored for 1 and 2 h at room temperature. |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | Baseline Values | 1-h Storage Values | 2-h Storage Values |
|                                 | Values p-Value\(^{a}\) | Values p-Value\(^{a}\) | Values p-Value\(^{b}\) |
| H-index                          |                 |                 |                 |
| Lithium-heparin plasma          | 4 ± 2           | 4 ± 2           | 4 ± 2           |
| Serum                           | 6 ± 3           | 6 ± 3           | 6 ± 3           |
| I-index                          |                 |                 |                 |
| Lithium-heparin plasma          | 22 ± 12         | 22 ± 12         | 22 ± 12         |
| Serum                           | 23 ± 12         | 23 ± 12         | 23 ± 12         |
| L-index                          |                 |                 |                 |
| Lithium-heparin plasma          | 13 ± 6          | 13 ± 6          | 15 ± 10         |
| Serum                           | 9 ± 5           | 11 ± 5          | 13 ± 5          |

\(^{a}\)Compared to baseline, \(^{b}\)compared to 1 h. H-index, hemolysis index; I-index, icteric index; L-index, lipemic index.
continuously removed from blood cells during storage of uncentrifuged serum or LH plasma [9], and this would hence parallel our data showing increasing L-index values over time in both serum and LH plasma sample which cannot be timely centrifuged. Caution should hence be observed when using the L-index for defining sample quality when blood tubes are not centrifuged in due time after collection.

Indeed, these results would only apply to our study condition, and our findings cannot be straightforwardly translated to samples with higher levels of hemolysis, icterus or lipemia [10]. A follow-up multicenter study would hence be necessary or recommended for assessing the stability of HIL indices using different types of blood tubes and/or analytical instrumentation, as well as samples with higher values of HIL indices.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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