Accuracy of screening for hemolysis in plasma samples using a commercial urine dipstick

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Accuracy of screening for hemolysis in plasma samples using a commercial urine dipstick

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Abstract

Objective. Identification and quantification of hemolysis in serum or plasma samples is an important requirement in laboratory diagnostics, however this is not always possible automatically and visual sample inspection is not sufficiently accurate. We have planned this study to determine whether a commercially available urine dipstick with a hemoglobin-reactive pad could be used for this purpose.

Methods: Sixty-five routine plasma samples, whose hemolysis index was previously determined on Roche Cobas 8000, were assayed with a commercial urine dipstick to obtain semi-quantitative data on plasma hemoglobin. Plasma was diluted 1:1000 in water to enter the range of hemoglobin measurement of the dipstick, and 10 µl of this dilution were applied to the hemoglobin pad. Results were visually interpreted within 60 sec by comparing the pad color with that on the dipsticks box label.

Results: The sample size consisted of 40 non-hemolyzed (hemolysis index≤0.3 g/L) and 25 hemolyzed (hemolysis index>0.3 g/L) plasma samples. Spearman’s correlation between Cobas hemolysis index and dipstick hemoglobin concentration was r=0.96 (95%CI, 0.93-0.97; p<0.001). The concordance of hemolysis detection was 95.4%, with 1.00 sensitivity, 0.93 specificity, 1.00 negative predictive value and 0.89 positive predictive value compared to the reference hemolysis index measurement on Cobas. The cumulative agreement between Cobas hemolysis index and the various plasma hemoglobin thresholds obtained with the dipstick was 75.4%.

Conclusions: We have demonstrated here that plasma hemoglobin assessment with commercially available urine dipsticks may generate semi-quantitative test results accurate enough to influence decision making regarding sample quality and its suitability for testing.

Key words: dipstick; plasma; test strip; urine; hemolysis
Introduction

Although the assessment of the so-called hemolysis index (HI), together with the icterus index (II) and the lipemia index (LI) altogether abbreviated as "HIL", has become a cornerstone of preanalytical and analytical quality in laboratory medicine because they allow the amount of these three interfering substances in the test sample to be determined and quantified accurately, rapidly and inexpensively, their routine use in clinical laboratories is unfortunately not as widespread as it should be [1].

First, not all laboratory analyzers are already equipped with these measures (e.g., they are still absent in some stand-alone immunochemical and hemostasis platforms, cannot be used for manual tests including ELISA and other similar techniques) [2]. Notably, hemolysis may seriously interfere with several immunological and clotting assays, as more comprehensively described elsewhere [3]. The interference deriving from the presence of cell-free hemoglobin may arise from three possible mechanisms [2,3]. The first and most widely recognized is attributable to the release of hemoglobin and other intracellular compounds in serum or plasma after blood cells injury, which would then cause a false increase in these analytes (i.e., hyperkalemia is a paradigmatic example). The second mechanism involves chemical interference of cell-free hemoglobin in various analytical reactions (e.g., interference with the enzymatic creatine kinase assay is a typical case). Finally, hemoglobin may also trigger significant spectrophotometric interference caused by an increase in optical absorbance or a change in blank value in various laboratory tests based on measurements at 415, 540, and 570 nm, where hemoglobin absorbs more, including immunoassays (interference in cardiac troponin assays is very common).

A large survey by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), which received 1405 responses from 37 European countries, recently showed that around 15% of facilities do not routinely monitor HIL
and, more importantly, this percentage was found to be unexpectedly high for microbiology (up to 44%), molecular biology (42%), and toxicology (40%) testing. Another interesting aspect that emerged from this study was that up to one-third of all laboratories did not use automatic HIL detection, but instead employed a visual scale for hemolysis detection, usually represented by a reference color photograph indicating different hemolysis levels in reference plasma samples [4]. Responses to the question of at what hemolysis threshold samples should be defined as hemolyzed were even more heterogeneous, as there was an almost even distribution of responses among four graded hemolysis thresholds [4]. Similar evidence emerged from other surveys conducted in various countries. For example, the College of American Pathologists (CAP) surveyed 846 participants in the Chemistry Survey [5] and reported that only a limited number (always <50%) had standardized procedures for handling hemolyzed specimens. In addition, less than half of them had taken recent corrective action to reduce hemolysis burden, using many different approaches.

Overall, these data paint a worrying picture regarding the practice of pre-test hemolysis assessment for potentially impaired tests, which is now unavoidable and recommended by most international and national laboratory medicine organizations [6,7]. In addition, visual inspection of the hue of a sample without automated HIL monitoring has a number of disadvantages [8], such as subjective and arbitrary interpretation of color, the influence of relative ambient lighting, sample composition (i.e., high bilirubin concentration), and so on. Therefore, the availability of simple and rapid means to obtain semi-quantitative information about the hemolysis index of the test sample could help to overcome most of the limitations highlighted in the EFLM survey. To this end, we have planned an analytical study to determine whether a commercially available urine dipstick containing a hemoglobin-reactive pad could be used for this purpose.
**Materials and Methods**

We selected 65 anonymized remnant plasma samples (3.5-mL lithium-heparin blood tubes; Vacutest Kima, Padova, Italy) obtained on a morning of a regular working day in the Laboratory Medicine Service of the University Hospital of Verona, remaining after completion of routine tests. Sample identity was replaced with specific identifiers for prohibiting identifiability, but remaining suitable for analysis. Half of the selected plasma samples had hemolysis index values $\leq 30$ (i.e., $\leq 0.3$ g/L, classified as non-hemolyzed), whilst the remaining half had hemolysis index values $>30$ (i.e., $>0.3$ g/L, classified as hemolyzed), as widely recommended [9]. The hemolysis index on Roche Cobas analyzers is assayed with by-chromatic measurement at double wavelengths (570 and 600 nm, with correction for lipaemia), and is reported in arbitrary units, which can be roughly converted in hemoglobin concentration, where 1 arbitrary units of hemolysis index corresponds to 0.01 g/L of hemoglobin [10].

These 60 plasma samples were then used to assess plasma hemoglobin concentration with a commercially available urine dipstick (AUTION Sticks, Arkray, Kyoto, Japan), the technical and analytical characteristics of which have been described elsewhere [11]. Briefly, a 1:1000 plasma dilution (i.e., $10 \, \mu$l of plasma in 10 mL of water) was performed in distilled water to fit the concentration of hemoglobin within the measurement range of the urine hemoglobin pad in the dipstick, and $10 \, \mu$l of this 1:1000 dilution were then pipetted onto the “blood” pad of the dipstick. Excess plasma was carefully removed with a paper towel, and test results were interpreted visually within 60 sec by direct comparison of the color produced in the pad with that reported in the label of the dipstick box label (Figure 1). Two experienced laboratory physicians were responsible for interpreting the visual
readings, and any discrepancies were resolved by a third laboratory physician. The dipstick hemoglobin assay reagent contains 30 mg of cumene hydroperoxide and 15 mg of 3,3’,5,5’-tetramethylbenzidine with a measurement range between 0.03-1.0 mg/dL (0.0003-0.01 g/L) of hemoglobin, and stepwise semi-quantitative thresholds of 0 (negative), 0.0003 (±), 0.0006 (1+), 0.002 (2+), and 0.01 (3+) g/L of hemoglobin (Figure 1). Dipstick results were reported as hemoglobin concentration (in g/L) after adjustment for the dilution factor (i.e., visual results were multiplied by 1000).

Test results were reported as median and interquartile range (IQR). Semi-quantitative test results obtained with the urine dipstick were directly correlated with those of the same samples assayed on Roche Cobas 8000 using non-parametric Spearman’s correlation. Concordance on categories of plasma hemoglobin values between the two techniques was also assessed within the following ranges: ≤0.3 g/L (non-hemolyzed), 0.3-0.99 g/L (mildly hemolyzed), 1.0-3.0 g/L (significantly hemolyzed), and >3.0 g/L (grossly hemolyzed) [8]. Statistical analysis was performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK). The study was performed on anonymized patient samples after routine testing was completed, such that informed consent was unnecessary. The study was conducted in accordance with the Declaration of Helsinki, according to the relevant local legislation, and was approved by the Ethics Committee of the University Hospital of Verona (970CESC; July 20, 2016).

**Results**

The Cobas hemolysis index values in non-hemolyzed (n=40) and hemolyzed (n=25) samples were 0.06 (IQR, 0.03-0.08) g/L and 0.74 (IQR, 0.43-1.11) g/L, respectively. The semi-quantitative distribution of dipstick hemoglobin values was as follows: “negative” or “±”: 37 samples; “1+”: 12 samples; “2+”: 9 samples, and “3+”:
7 samples, respectively. The Spearman’s correlation between Cobas hemolysis index and dipstick hemoglobin concentration is shown in Figure 2, yielding to r=0.96 (95%CI, 0.93-0.97; p<0.001). The concordance of hemolysis detection (i.e., plasma hemoglobin >0.3 g/L) between Cobas hemolysis index and urine dipstick was 95.4% (kappa statistics, 0.90; 95%CI, 0.80-1.01; p<0.001) (Table 1). Accordingly, the urine dipstick displayed 1.00 (95%CI, 0.86-1.00) sensitivity, 0.93 (95%CI, 0.80-0.98) specificity, 1.00 (95%CI, 0.91-1.00) negative predictive value and 0.89 (95%CI, 0.74-0.96) positive predictive value for detecting plasma hemolysis compared to the reference hemolysis index measurement on Cobas. The cumulative agreement between Cobas hemolysis index and urine dipstick at the different plasma hemoglobin thresholds of ≤0.3 g/L, 0.3-0.99 g/L, 1.0-3.0 g/L, and >3.0 g/L was 75.4% (kappa statistics, 0.58; 95%CI, 0.44-0.72; p<0.001).

Discussion

The identification and management of hemolyzed specimens represents one of the most critical steps in laboratory diagnostics, since the presence of cell-free hemoglobin in serum or plasma can trigger a variety of biological, chemical, and analytical interferences that can ultimately compromise the quality of test results for many analytes [9]. The clear interference of hemolysis with various clinical chemistry [12] and hemostasis [13] parameters has led most manufacturers of laboratory analyzers to equip their instrumentation with the so-called HIL check, i.e., an automatic, accurate, and nearly inexpensive means of detecting and quantifying cell-free hemoglobin in the test sample that compensates for the inherent limitations of visual sample control. With the exception of analyzers that do not have this important technological advance but are integrated into a system of partial or total laboratory automation where HIL control can be performed by other preanalytical or analytical
modules, the unique means of identifying sample hemolysis for a wide range of laboratory tests (i.e., those performed using a stand-alone immunochemical platform, semi-automated or even manual ELISA, and so forth) [14,15], remains visual identification, which is fraught with a number of well-known drawbacks (imprecision, inaccuracy, inter-user variability, need to identify and adopt a validated benchmark, impossibility to store the extent of inference in the LIS, etc.). Therefore, any objective means that can support the identification and quantification of hemolysis in routine practice (even if a semi-quantitative approach is used) should be welcomed.

This is precisely why we planned this proof-of-concept study, i.e., to determine whether the widely available and largely inexpensive urine test strips can be a potentially valuable tool for detecting and quantifying hemolysis in plasma or serum samples. The results of our study are consistent with this premise, as we have shown that semi-quantitative hemolysis assessment by this approach provides data that are consistent with those obtained from the hemolysis index performed with a fully automated clinical chemistry analyzer. In particular, the urine dipstick at the routine plasma dilution of 1:1000 showed a very high correlation ($r=0.96$), associated with an accuracy of 95%, a sensitivity of 1.00, and a specificity of 0.93 for identifying hemolyzed samples. Satisfactory performance was also observed in categorizing samples according to their degree of hemolysis, resulting in a cumulative accuracy of 75%. Notably, we decided to dilute the plasma in water rather than in a specific buffer because the use of a buffer can be potentially misleading and impractical for two main reasons. First, different analyzers and even assays use their own buffers, so that diluting the plasma in a particular buffer may generate some types of interference with other assays when other tests are requested. Second, the use of water is cheap, requires no preparation, and is widely available.
Conclusions

The results of our study suggest that the assessment of plasma hemoglobin using a commercially available urine dipstick can yield semi-quantitative test results that are accurate enough to inform the decision-making regarding sample quality and its eventual suitability for testing. This approach could also be used to obtain information about the potential extent of in vivo hemolysis in patients with a variety of hemolytic anemias, when more accurate practices for measuring cell-free hemoglobin are unavailable [16].

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

References


Table 1. Concordance of hemolysis index measured on Roche Cobas 8000 and plasma hemoglobin assayed with a commercially available urine dipstick.

<table>
<thead>
<tr>
<th>Cobas hemolysis index</th>
<th>Dipstick ≤0.3 g/L</th>
<th>Dipstick &gt;0.3 g/L</th>
<th>Total</th>
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<tbody>
<tr>
<td>≤0.3 g/L</td>
<td>37</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>&gt;0.3 g/L</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>28</td>
<td>65</td>
</tr>
</tbody>
</table>
**Figure 1.** Picture of the commercial urine dipstick used for assaying plasma hemoglobin concentration.

<table>
<thead>
<tr>
<th>Item</th>
<th>Time</th>
<th>Test results interpretation</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Pro.</td>
<td>60 sec</td>
<td><img src="image" alt="Pro. Test Results" /></td>
</tr>
<tr>
<td>Uro.</td>
<td>60 sec</td>
<td><img src="image" alt="Uro. Test Results" /></td>
</tr>
<tr>
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<td>60 sec</td>
<td><img src="image" alt="Bil. Test Results" /></td>
</tr>
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<tr>
<td>Cal. Pad</td>
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</tr>
</tbody>
</table>

10 µL of 1:1000 plasma dilution
Figure 2. Spearman’s correlation of plasma quantified by a commercially available urine dipstick and the hemolysis index (HI) measured with Roche Cobas 8000.