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Harmonization of automated hemolysis index assessment and use: Is it possible?

Alberto Dolci [a,*], Mauro Panteghini [a,b]

[a] Clinical Chemistry Laboratory, University Hospital "Luigi Sacco", Milan, Italy
[b] Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

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ABSTRACT

The major source of errors producing unreliable laboratory test results is the pre-analytical phase with hemolysis accounting for approximately half of them and being the leading cause of unsuitable blood specimens. Hemolysis may produce interference in many laboratory tests by a variety of biological and analytical mechanisms. Consequently, laboratories need to systematically detect and reliably quantify hemolysis in every collected sample by means of objective and consistent technical tools that assess sample integrity. This is currently done by automated estimation of hemolysis index (HI), available on almost all clinical chemistry platforms, making the hemolysis detection reliable and reportable patient test results more accurate. Despite these advantages, a degree of variability still affects the HI estimate and more efforts should be placed on harmonization of this index. The harmonization of HI results from different analytical systems should be the immediate goal, but the scope of harmonization should go beyond analytical steps to include other aspects, such as HI decision thresholds, criteria for result interpretation and application in clinical practice as well as report formats. With regard to this, relevant issues to overcome remain the objective definition of a maximum allowable bias for hemolysis interference based on the clinical application of the measurements and the management of unsuitable samples. Particularly, for the latter a recommended harmonized approach is required when not reporting numerical results of unsuitable samples with significantly increased HI and replacing the test result with a specific comment highlighting hemolysis of the sample.

1. Introduction

The prevention of errors is a major goal in healthcare. Toward this widely shared goal, laboratory errors have received a great deal of attention due to their impact on the quality and efficacy of laboratory performances and, in turn, on patient safety [1]. Despite the common perception that excellence in Laboratory Medicine is synonymous with analytical quality, there is an increasing body of evidence that pre-analytical problems are the major source of laboratory errors, accounting for 60–70% of total errors encountered within the total testing process [2]. It is evident that pre-analytical errors not only may lead to spurious test results, but also, more importantly, may influence patient care, even dramatically [3]. Among the pre-analytical nonconformities more repeatedly audited for inappropriate procedures of sampling, handling and shipping specimens to the laboratory, the inadequate quality of the specimen due to hemolysis is the most frequent. As an example, in our university hospital significant hemolysis occurs on average in 1.15% of all requested tests, affecting ~20,000 determinations per year, mostly from critical care clinical wards, i.e. neonatology, oncology, emergency department [4]. This paper aims to address why hemolysis still represents a major problem in clinical chemistry laboratories despite the availability of hemolysis index (HI) on almost every platform and then suggests how to move toward harmonization of both measurement of HI and criteria for the application of HI in result interpretation.

2. Hemolysis

Hemolysis is the breakdown of erythrocytes in blood that frees the hemoglobin and intracellular contents from the cells to the surrounding plasma [5]. Hemolysis is visible, after centrifugation of the tube containing the whole blood, as a pink to red coloration of the plasma or serum, depending upon the concentration of free hemoglobin. Traces of hemoglobin, i.e. <0.05 g/l of free hemoglobin spectrophotometrically determined, can be physiologically present in plasma [6]. Visible hemolysis, the hallmark of red blood cell destruction, begins to appear in both plasma and serum at hemoglobin concentrations of ~0.20 g/l, which confers a slightly detectable pink tinge to the biological matrix; however, bilirubin or lipemia/turbidity may hide even greater concentrations of hemoglobin at visual inspection of the sample. Hemolysis becomes clearly visible in samples containing as low as 0.5% pathologically lysated erythrocytes, roughly corresponding to 0.50 g/l

* Corresponding author at: Laboratorio Analisi, Ospedale Luigi Sacco, Via GB Grassi 74, 20157 Milano, Italy. Fax: +39 02 503 19835.
E-mail address: dolci.alberto@hsacco.it (A. Dolci).

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of free hemoglobin [7]. Abnormal disruption of erythrocytes may occur in vivo or in vitro, due to clinical or artifactual causes, respectively [8]. Many problems due to troublesome specimen collections or handling may affect the samples and cause in vitro hemolysis, as thoroughly reviewed by Lippi et al. [9]. In vitro hemolysis remains the leading cause of unsuitable specimens both for outpatient and inpatient samples, hemolyzed specimens accounting for 40–70% of all unsuitable specimens, nearly five times higher than the second leading cause of assay interference [4,10].

2.1. Mechanisms of hemolysis interference in laboratory testing

In clinical chemistry, 'interference' is any cause of clinically significant bias in the measured analyte concentrations due to the effect of another component or property of the sample [5]. Hemolysis can cause interference in many laboratory tests for a variety of biological and analytical mechanisms, the latter being not only optical, but also chemical. In addition to the hemoglobin, hemolysis induces the release of other intracellular components into the surrounding fluid. Some of these components, i.e. lactate dehydrogenase (LDH), aspartate aminotransferase (AST), potassium, phosphate and magnesium, are present in high concentration in the erythrocytes and their release may produce a significant positive interference when their concentrations are measured in serum. Other erythrocyte contents also may affect analytical determinations. For instance, intracellular proteases released from erythrocytes when a sample is hemolyzed may degrade cardiac troponin T released in blood after a myocardial injury, thus causing a significant decrease in marker detection [11].

Besides the leakage of other components abundant in erythrocytes, hemoglobin itself may have a direct effect on several tests through multiple mechanisms which may at any one time combine to cause interferences causing spuriously increased or decreased test results. The spectral properties of hemoglobin, with the major absorbance peak at approximately 420 nm and significantly absorbing even between 340 and 440 nm and between 540 and 580 nm (Fig. 1), may cause a spectrophotometric interference particularly for those assays based on measurements at these critical wavelengths [5,7]. Furthermore, chemical reactions produced by heme or its iron atoms with reagent components or the analyte itself can introduce a bias, either positive or negative, in assays. The peroxidase activity of iron atoms causes interference in reactions utilizing hydrogen peroxide and their oxidation–reduction chemical reactivity may affect other assays, e.g. the Malloy–Evelyn method for total bilirubin determination [12]. Mechanisms induced by hemolysis may interfere not only in spectrophotometric tests, but also in immunoassays [13] and coagulation tests [14]. In addition, released hemoglobin can directly inhibit chemical reactions in polymerase chain reaction-based assays of viral and bacterial DNA and RNA [5].

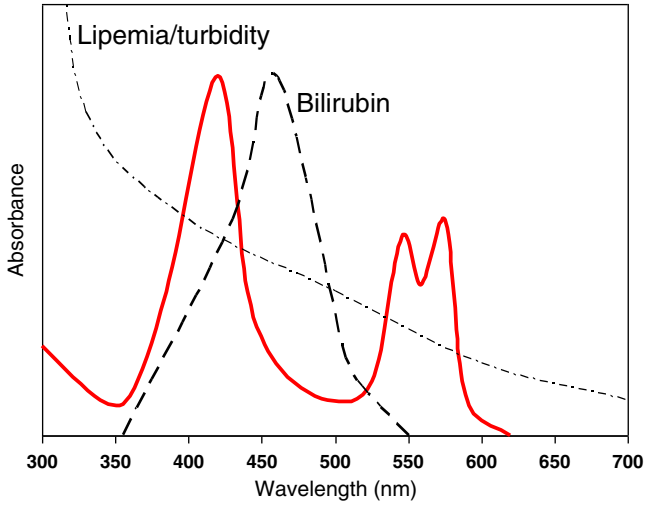

Fig. 1. Absorbance spectrum of hemoglobin [red]. Bilirubin and lipemia/turbidity spectra are also depicted [dotted lines] to show overlapping of the spectra that may affect the serum index testing.

2.2. Detection of hemolysed specimens in the clinical laboratory

As a consequence of these issues, the correct identification of any hemolysed specimen plays a central role in clinical laboratories increasing the quality of the laboratory service by providing clinically relevant information. Traditionally, hemolysis has been detected by visual scrutiny of the specimens after centrifugation and the help of pictures showing the color of specimens with increasing concentration of free hemoglobin to make a direct comparison, but nowadays the inherent limits of this procedure are fully described [15–17]. Visual inspection is not sensitive enough to detect the low level of hemolysis affecting determination of analytes more prone to the interference, such as LDH, AST and potassium. Furthermore, it shows high inaccuracy, being difficult (if not impossible) to standardize, and poor reproducibility, being subject to high inter-operator variability. Some studies of the correlation between visually reported hemolysis and calculated indices report that laboratory staff tend to be conservative and err on the side of over-reporting hemolysis [16].

The clinical laboratory therefore needs a systematic process for detection and reliable quantification of hemolysis in every received sample. HI is the automated detection system for hemolysis in specimens that offers an objective and consistent technical tool to assess sample integrity. HI is a calculation, based on absorbance measurements performed on serum or plasma at different wavelengths, which provides semi-quantitative estimate of hemolysis detected in the sample [5]. Nowadays, almost all clinical chemistry platforms provide HI, together with lipemia/turbidity and icteric indices, in a single test usually described as "serum indices" which is included in the test menu. Serum indices may gather useful information on sample quality before analysis by capturing the presence of a major interfering substance or combinations of these substances [5].

3. Hemolysis index: strengths and limitations

The use of automated HI estimation overcomes the inherent limitations of classical visual estimation by providing a more objective and accurate estimate of hemolysis. HI can be transmitted to the laboratory information system (LIS) through middleware, where the HI cut-offs indicating clinically significant interference for each test are recorded, together with decision rules and actions to be taken in handling test results. When HI is above the cut-off for an analyte indicating significant interference, the test report is automatically managed according to the criteria established by the laboratory manager, i.e. a comment to alert the clinician, flagging the result and/or rejecting the sample [18]. In that way, detection of clinically significant hemolysis interference, HI values and corrective actions taken are directly archived into the LIS for further evaluations. Automated monitoring of serum indices on every tested specimen is the only suitable approach to assess sample quality in high-volume clinical laboratories, which use continuous-flow automation with integration of pre-analytical and analytical workstations, and to detect unsuitable specimens in "real time" before results are released from conveyor belts [19]. Thus, automation of the specimen inspection process has made hemolysis detection more reliable, improving the efficiency of laboratory workflow and enhancing the quality of reportable test results.

Despite these advantages, some differences exist in the way HI is assessed and reported on different automated analytical platforms with a degree of variability still affecting the HI estimate [20]. Several parameters account for this variability: sample size, diluent used and its volume, read wavelengths, calculations and estimate report, where HI may be expressed in ordinal values (e.g. +1,+2,+3), actual mass