Editorial

Giuseppe Lippi and Janne Cadamuro

Visual assessment of sample quality: *quo usque tandem*?

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Sample hemolysis is conventionally defined as the presence of a variable amount of cell-free hemoglobin in serum or plasma. The reference (i.e. "normal") concentration of free and measurable hemoglobin conventionally ranges between 0.22 and 0.25 g/L in serum and between 0.10 and 0.13 g/L in plasma, respectively [1]. Although no definitive evidence exists about the threshold of "pathological" hemolysis in blood samples, universal consensus has been reached that clinically significant interference for the most hemolysis-vulnerable tests (i.e. potassium, lactate dehydrogenase, aspartate aminotransferase) may start with concentrations of cell-free hemoglobin ≥0.5 g/L [2, 3]. Notably, this cut-off is also conventionally used for monitoring phlebotomy practice [4].

Although the very first studies about the impact of sample hemolysis on the quality of laboratory testing have been published more than 40 years ago [5], the frequency of hemolyzed samples remains high and generates remarkable challenges in clinical laboratory practice [6–8]. The first important issue is distinguishing between *in vitro* (i.e. spurious) and *in vivo* (i.e. hemolytic anemia) hemolysis. The differentiation between these conditions is not meaningless because the former case reflects a kaleidoscope of problems emerging throughout preanalytical sample management, thus including blood drawing, handling, transportation, storage and preparation for testing, whilst the latter mirrors a life-threatening condition, which must be timely communicated to the clinicians for adopting the most appropriate care options. According to this perspective, the second and almost consequential aspect is the need of systematically monitoring sample quality for accurate and rapid identification of hemolysis in serum or plasma, as also currently endorsed by many international accreditation standards such as the International Standards Organization (ISO) 15189:2012 and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [9]. The most obvious consequence is that all laboratories should adopt a reliable strategy for systematic monitoring of sample quality and, moreover, for identifying the presence of, and quantifying, cell-free hemoglobin in either serum or plasma.

In this issue of the journal, we publish an interesting study aimed to assess whether visual inspection of sample hemolysis by comparison with a color chart, rather than automatic assessment of serum or plasma indices, may have an impact on patient safety [10]. Interestingly, the authors concluded that nearly one-third of test results generated with manual handling of hemolyzed specimens were incorrectly managed, mainly for incorrect release of hemolysis-sensitive tests results or for unnecessary suppression of data in specimens with non-clinically significant degrees of hemolysis. In both cases, these circumstances may seriously jeopardize the managed care because release of data biased for the presence of hemolysis may then trigger inappropriate therapeutic options (i.e. administration of potassium-lowering therapy in patients with "spurious" hyperkalemia), whereas unjustified test suppression may delay both diagnosis and treatment of potentially fatal diseases (i.e. delayed or inappropriate management of arrhythmias due to underdiagnosis of *in vivo* hyperkalemia).

Color vision is conventionally defined as the capability of distinguishing colors according to the wavelengths of light reflected, emitted or transmitted. The individual ability to identify colors and perceiving their intensity depends on multiple factors, such as the local environment and its relative light exposure, but may also relay on some genetic (i.e. *Opsin* gene polymorphisms), anatomic and pathological aspects, which ultimately make the eyes of one subject more capable than others (color blindness, also known as "daltonism", is a paradigmatic example) (Figure 1) [11, 12]. All these factors make color recognition rather heterogeneous in different environments and among different individuals, thus supporting the hypothesis that visual assessment of sample quality may be highly inaccurate. Previous and highly reliable evidence has been provided that visual detection of hemolyzed samples is more inaccurate compared with automatic detection [13, 14], thus paving the way to adopting more precise and standardized approaches. The current generation of preanalytical platforms and

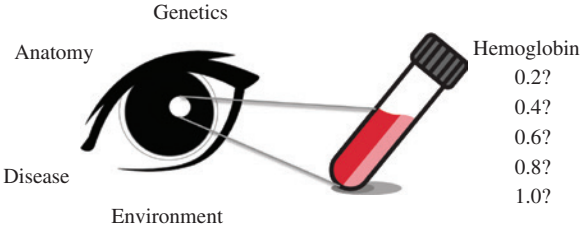

Figure 1: Sources of interindividual variability in visual assessment of sample quality.

laboratory instrumentation for either clinical chemistry or coagulation testing is increasingly equipped with the so-called serum (or plasma) indices. Briefly, the extent of hemolysis, turbidity and icterus is roughly estimated by using multichromatic wavelength readings and calculation formulas integrating adjustments for compensating spectral overlap. In most analyzers, the automatic estimation of these indices does not entail the use of specific reagents and is fully automated and rapid, so that objective interference data can be added to, and hence complement, test results. The technical and clinical advantages of this technique are unquestionable because it provides a standardized means of assessing sample quality and can be easily incorporated into automatic decision-making algorithms, thus alleviating routine activity and preventing disputes with clinicians when suppressing data [15].

Yet, some problems remain even after widespread implementation of serum or plasma indices. Beside the lack of standardized day-to-day quality control material for serum or plasma indices measurement, the use of specific cutoffs for suppressing unreliable test results is a virtually unmanageable issue because each reagent has particular vulnerability to the presence of cell-free hemoglobin or other interfering substances that may be present in the test sample. Hence, parameter-specific cutoffs should be provided by instrument or assay manufacturers based on clinical acceptability criteria [16]. Similarly, the technical approach used for estimating interference from hemoglobin, lipids and bilirubin in serum or plasma are often dissimilar among the various manufacturers, thus making data (either quantitative or semi-quantitative) poorly comparable [17]. Thereby, harmonization of both measurement and interference thresholds seems a rather unreachable target at present. Then, although it has been clearly demonstrated that the turnaround time would not be affected [18], many laboratory professionals are still persuaded that routine assessment of serum or plasma indices may wreck an optimal workflow. Another potential drawback comes from the obvious consequence that routine assessment of sample quality will generate larger suppression of test results, which may then cause adjunctive relational issues with laboratory stakeholders.

Although all these aspects cannot be ignored, the many described advantages, combined with the evidence provided by Luksic et al. [10] that visual inspection may seriously jeopardize patient safety, would finally tip the balance towards replacing manual management with routine use of serum or plasma indices for assessing sample quality. With an adaptation of the foremost Marco Tullio Cicerone's speech, we can provokingly conclude… "*visual assessment of sample quality: quo usque tandem?*"

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Corresponding author: Prof. Giuseppe Lippi, Section of Clinical Biochemistry, University Hospital of Verona, Piazzale LA Scuro, 37100 Verona, Italy, E-mail: giuseppe.lippi@univr.it; ulippi@tin.it. http://orcid.org/0000-0001-9523-9054.
Janne Cadamuro: Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria