Review

Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories

Giuseppe Lippi1,3,10,*, Norbert Blanckaert2,9, Pierangelo Bonini3,9,10, Sol Green3,9, Steve Kitchen5,9, Vladimir Palicka6,9, Anne J. Vassault7,9 and Mario Plebani8,10

1 Clinical Chemistry Laboratory, University of Verona, Verona, Italy
2 Laboratory Medicine, University Hospital Leuven, Leuven, Belgium
3 Clinical Biochemistry, School of Medicine, University of San Raffaele, Milano, Italy
4 BD Diagnostics – Preanalytical Systems, Franklin Lakes, NJ, USA
5 Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Sheffield, UK
6 Institute of Clinical Biochemistry and Diagnostics, Faculty Hospital, Hradec Kralove, Czech Republic
7 Laboratoire de Biochimie B Hôpital Necker Enfants Malades, APHP, Paris, France
8 Department of Laboratory Medicine, University of Padova, Padova, Italy
9 EPSC – European Preanalytical Scientific Committee (www.specimencare.com)
10 International Federation of Clinical Chemistry Working and Laboratory Medicine Group on Patient’s Safety

Abstract

Prevention of medical errors is a major goal of healthcare, though healthcare workers themselves have not yet fully accepted or implemented reliable models of system error, and neither has the public. While there is widespread perception that most medical errors arise from an inappropriate or delayed clinical management, the issue of laboratory errors is receiving a great deal of attention due to their impact on the quality and efficiency of laboratory performances and patient safety. Haemolytic specimens are a frequent occurrence in clinical laboratories, and prevalence can be as high as 3.3% of all of the routine samples, accounting for up to 40%–70% of all unsuitable specimens identified, nearly five times higher than other causes, such as insufficient, incorrect and clotted samples. This article focuses on this challenging issue, providing an overview on prevalence and leading causes of in vivo and in vitro haemolysis, and tentative guidelines on identification and management of haemolytic samples in clinical laboratories. This strategy includes continuous education of healthcare personnel, systematic detection/quantification of haemolysis in any sample, immediate clinicians warning on the probability of in vivo haemolysis, registration of non-conformity, completing of tests unaffected by haemolysis and request of a second specimen for those potentially affected.


Keywords: haemolysis (hemolysis); laboratory testing; preanalytical variability; unsuitable specimens.

Introduction

The original definition of laboratory diagnostics has extended its horizons so much over the past decades, in that it does not only include diagnosing pathologies, but also identifying risk factors (prevention) and monitoring treatments (therapy). Recently, the broad introduction of the “omics”, namely genomics, pharmacogenomics, proteomics and the promising theranostics (a term denoting the fusion of therapeutics and diagnostics) (1, 2), has been a major catalyst for promoting changes in organisation and complexity of laboratory testing. Regardless of this promising perspective, laboratory diagnostics is still struggling with older problems, which endorse the potential to make the entire diagnostic reasoning vulnerable and potentially unreliable, enhancing costs and jeopardising the patients’ health. Such problems have a common background: the errors. Traditionally, errors are intended as acts, assertions or beliefs that unintentionally deviate from what is correct, right or true, and conditions of having incorrect or false knowledge. The medical error is typically referred to as any preventable event that may cause or lead to inappropriate medication use or patient harm, while the medication is in the control of the healthcare professional, patient or consumer (3). Such events may be related to professional practice, healthcare products, procedures and systems, including prescribing; order communication; product labelling, packaging and nomenclature; compounding; dispensing; distribution; administration; education; monitoring; and use. There is a widespread perception that most medical errors arise from an inappropriate or delayed clinical management. Nevertheless, mistakes associated with diagnosis, either delayed or missed, may still occur.
with frequency, varying from 26% to 78% of identified medical errors in the primary care. Moreover, they are most likely to result in major harm to the patient or precipitate hospital admission and are overall less preventable (4). Diagnostic errors are an important component of medical errors, in that they can be defined as any defect from ordering tests to reporting results and appropriately interpreting and reacting on these (5). According to the Lundberg brain-to-brain loop, the total testing process begins from the physician’s brain with formulation of a clinical hypothesis and selection of the most appropriate examinations, further develops through patient’s preparation, sampling and handling of the biological specimens (pre-analytical process), sample analysis (analytical process), results reporting to the requesting physician (postanalytical process). With this comprehensive description of the testing process, the integrity of patient test results is hence dependent on many parameters in the preanalytical workflow of the specimens and on the specific performance of the assay (6). Although some of these steps tend to be placed outside the direct jurisdiction of the laboratory, they all contribute to and influence the quality and efficiency of laboratory performances.

While enormous advances in technology (analytical techniques, automation, computer science) have greatly contributed to limit the probability and the adverse clinical outcome of errors in the analytical phase of testing, a large degree of variability still occurs for individual or system design defects in extra-analytical phases of the total testing process, especially in the preanalytical setting, which is incidentally one of the most labour-intensive activities (7). Preanalytical problems, which still account for nearly 70% of total errors encountered within the testing process, may generate doubtful or spurious results and they consistently appear as cross-cutting issues which may affect all the stakeholders of laboratory testing, consuming healthcare resources and influencing the patient care. The most common preanalytical problems are traditionally identified as inadequate procedures for collection, including inappropriate quality of the specimen (haemolysis, clotting, contamination), insufficient volume, inappropriate containers and misidentification. Haemolytic specimens are a frequent occurrence in laboratory practice, and prevalence is described as being as high as 3.3% of all of the routine samples afferent to a clinical laboratory and they account for 40%–70% of all unsuitable specimens, nearly five times higher than the second cause. In vitro haemolysis remains the leading cause of unsuitable specimens for both outpatient and inpatient samples, for routine and stat specimens (7–10). Through their work on specimencare.com, the European Preanalytical Scientific Committee (EPSC) realised that there was a need for a further review of haemolysis, which is a challenging cause of specimen rejection in clinical laboratories.

**Causes and nature of haemolytic specimens**

Haemolysis (or hemolysis), from the Latin hemo (blood) and lysis (to break open), is the release of haemoglobin and other intracellular components from erythrocytes to the surrounding plasma, following damage or disruption of the cell membrane. The upper reference limit for free haemoglobin in plasma is 20 mg/L and 50 mg/L for serum, respectively (11). Visually, haemolysis is defined for free haemoglobin concentrations above 0.3 g/L (18.8 μmol/L), which confers a detectable pink to red hue to serum or plasma and becomes clearly visible in specimens containing as low as 0.5% lysated erythrocytes (11, 12). Haemolysis may occur both in vivo and in vitro, and

**Figure 1** Major causes of haemolytic specimens in clinical laboratories (European Preanalytical Scientific Committee, EPSC. Available at: www.specimencare.com).
is a most undesirable condition that influences the accuracy and reliability of laboratory testing (Figure 1). Problems due to troublesome specimen collection or handling, such as wet alcohol transfer from the skin into the blood specimen, small gauge needles (usually smaller than 21 G), difficulty to locate easy venous access, small or fragile veins (alternative sites to the antecubital area, such as hand veins are fragile and easily traumatised), unsatisfactory attempts, vein missing, partial obstruction of catheters and other collection devices, application of excessive negative pressure to the blood in the syringe, underfilling of the tube (excessive concentrations of additives, especially EDTA, can cause rupture of the erythrocytes cell membrane), excessive shaking or mixing of the blood after collection, exposure to excessively hot or cold temperature, centrifugation at a too high speed for a prolonged period of time and centrifugation of partially coagulated specimens from patients on anticoagulants, frequently compromise the integrity of blood and vascular cells, causing leakage of intracellular components and producing significant biological and analytical interference (11–17). Additional causes of in vitro haemolysis are delayed separation of the specimens, incomplete formation of the separator barrier integrity (some erythrocytes can move into serum or plasma) and re-centrifugation (re-spin) of tubes with gel separators (the gel barrier may open and allow any supernatant, which has been in contact with erythrocytes, to mix with the supernatant previously above the separator) (14). When collecting capillary blood, the use of a manual lancet, where the depth of the incision is not controlled, and the excessive squeezing, which induce extreme hydraulic pressure in the capillaries, could also cause haemolysis (14). Finally, in vitro haemolysis has been reported by the use of some innovative pneumatic tube systems (plain serum samples without a gel barrier might be more susceptible to haemolysis than the other sample types) (18).

Although rarely, usually less than 2% of all the specimens with detectable haemolysis, haemolytic specimens may also be due to in vivo haemolysis (19). This clinical condition may have at more than 50 causes, including hereditary, acquired and iatrogenic conditions, such as autoimmune haemolytic anaemia and other haemoglobinopathies, drugs, severe infections, intravascular disseminated coagulation, transfusion reactions, heart valves (20) and HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome (21). Typically, in vivo haemolysis does not depend on the technique of the healthcare provider and it is thus virtually unavoidable and potentially insurmountable (20).

Visible haemolysis, as a hallmark of a more generalised process of blood cell damage, is usually not apparent until the separation of serum or plasma has occurred. Although laboratory testing on haemolytic specimens has been traditionally discouraged to avoid unreliable results of some biochemical parameters, most clinical laboratories follow arbitrary procedures to manage this challenging problem.

Consequences of haemolysis on laboratory testing

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) provides the following definition: “analytical interference is the systematic error of measurement caused by a sample component, which does not, by itself, produce a signal in the measuring system” (11). An additional reliable definition is that provided by Selby: “the effect of a substance present in an analytical system, which causes deviation of the measured value from the true value” (22). Haemolysis and/or blood cell lysis is a challenging problem in laboratory diagnostics, as it may not be evident until whole blood specimen centrifugation has been performed, exposing the serum or plasma to scrutiny. Moreover, it might significantly influence the reliability of laboratory tests for a variety of biological and analytical reasons. Basically, the interference from haemolysis, not solely from free haemoglobin, on laboratory testing might be caused by leakage of haemoglobin and other intracellular components into the surrounding fluid, which induces false elevations of some analytes or dilution effects, chemical interference of free haemoglobin in a variety of analytic reactions and method- and analyte concentration-dependent spectrophotometric interference, due to an increase of the optical absorbance or a change in the blank value, especially for laboratory tests employing measurements at 415, 540 and 570 nm, where haemoglobin absorbs more strongly (11, 13, 23–28). Besides haemoglobin, erythrocytes contain several structural proteins, enzymes, lipids and carbohydrates, and many of these may also interact or compete with the assay reagents (13). Clearly, at high levels of serum haemoglobin, such interferences might coexist, producing variations that do not necessarily go in the same direction and making the situation rather difficult to handle. Interference from haemolysis is approximately linearly dependent on the final concentration of free haemoglobin in the specimen, generating a consistent trend towards overestimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, creatine kinase (CK), iron, lactate dehydrogenase (LDH), lipase, magnesium, phosphorus, potassium and urea, whereas the values of albumin, alkaline phosphatase (ALP), bilirubin, chloride, γ-glutamyltransferase (GGT), glucose and sodium might be substantially decreased by dilutional effects. The relative increase of AST, LDH, magnesium, phosphorus and potassium has been attributed to large differences between intracellular and extracellular concentrations for these analytes (13, 23, 24, 27). Increased concentrations of CK are most likely attributable to analytical interference, due to release of intracellular adenylate kinase, which is not completely inhibited under operating conditions (24–27), the interference on bilirubin, iron, lipase and GGT measurement is most likely due to spectral overlap and by a chemical reaction between haemolysate and reaction components, whereas that
on ALP is mostly attributable to denaturation of haemoglobin during the reaction in alkaline medium (24–27). A positive bias at normal neonatal bilirubin concentrations and a negative one at intermediate and high neonatal bilirubin concentrations have been described in capillary heel-stick samples (29). The reliability of serum protein electrophoresis might also be influenced by haemolysis, as haemoglobin-haptoglobin complexes move between the α2- and β-globulin fractions and free haemoglobin migrates as a diffuse reddish band in the β-globulin fraction (11).

The first step to eliminate interference from an immunoassay is recognising its existence. How the laboratory deals with possible interference depends on the type of interference suspected. Haemolysis interferences in immunoassays are reportedly less frequent than in photometric methods (22). However, a generalised problem of blood cell lysis, rather than the presence of free haemoglobin in the plasma or serum, may have a substantial impact through release of cellular enzymes, detrites and other materials (30). Interference may also occur if the reagent antibodies used in immunoassays are less specific and cross-react with some of the compounds released from the erythrocyte. Alternatively, some of the released compounds may bind to the analyte and inhibit antibody binding sites. Haemolysis may also interfere by contributing to, or suppressing, the reactions used in the detection method employed (31). So far, interference has been detected in specific circumstances (32), including falsely decreased levels of troponin T due to haemoglobin per se and/or proteolysis (33, 34), elevated troponin I and prostate specific antigen levels due to interference by free haemoglobin in certain assays (31, 35). Vitamin B12, testosterone and cortisol might also exhibit a negative bias with increasing amounts of haemolysate; the effect was greatest on the vitamin B12 assay, but all three analytes had approximately a 20% decrease in signal when gross haemolysis was present (31). Even small degrees of haemolysis may be unacceptable for certain immunoassays, principally due to the release of proteolytic enzymes that destroy small peptides, such as insulin, glucagon, calcitonin, parathyroid hormone (PTH), adrenocorticotropic hormone (ACTH) and gastrin. Folate is also present in erythrocytes at concentrations approximately 30 times greater than in serum, hence it should not be assayed in samples with any degree of haemolysis (36). Finally, haemolysis has been reported to exert a negative bias in a fluorescence polarisation immunoassay (for homocysteine) (37) and some immunonephelometric methods, especially when assaying haptoglobin (38). Plasma haptoglobin depletion is universally considered a reliable marker for the instant diagnosis of accelerated in vivo red cell destruction, irrespective of the site of haemolysis. However, in contrast to alternative markers, haptoglobin levels are not influenced by in vitro haemolysis, as the haptoglobin-haemoglobin complexes which are formed upon erythrocytes destruction can be rapidly cleared from circulation only by monocytes and tissue macrophages via CD163 receptors.

The presence of haemolysis in the specimen might strongly influence several coagulation tests (39, 40). The interference is not exclusively attributable to free haemoglobin in plasma, as many other substances are released from the blood cells after lysis. It is hence more likely that the major interference arises from the release of intracellular and thromboplastic substances form either leukocytes or platelets (39). Prolongations in prothrombin time and dimerised plasmin fragment D (D-dimer) have been observed in samples containing final lysate concentrations of 0.5% and 2.7%, respectively, whereas samples containing a final lysate concentration of 0.9% might show significant shortenings of activated partial thromboplastin time and decreases in fibrinogen (39). A significant decrease of antithrombin assayed by a chromogenic method has also been reported in haemolytic specimens (41) (all these data are synthesised in Table 1).

How to prevent spurious results of laboratory testing on haemolytic specimens

The issue of haemolytic specimens has traditionally plagued clinical laboratories, calling for operative guidelines and recommendations (Figure 2). At variance with biological causes, in vitro haemolysis is preventable in most circumstances, as it is usually caused by inappropriate collection, handling and processing of the specimen. Unfortunately, the incidence of haemolytic specimens has increased with the decentralisation of phlebotomy practices to non-laboratory personnel. Because a constellation of manual factors, including practice of blood sample collection and proficiency of the nursing staff in phlebotomy, influences the quality of the specimen, guidelines or recommendations for collecting samples and for evaluating submitted specimens are essential, as acceptance of improper specimens for analysis may lead to erroneous information that could affect patient care. A comprehensive knowledge on the type of interference exerted by haemolysis on laboratory testing, along with an appropriate training of phlebotomists, are essential prerequisites to minimise errors at any step of the laboratory activity (17). This can be achieved by dissemination of operative guidelines and recommendations of laboratory best practice for collection and handling of the specimens, implementing standardised collection procedures, such as the use of conventional straight needles greater than 22 G (15) and evacuated tube systems. In particular, practices and procedures should be carefully reviewed when collecting blood samples from catheterised patients (such as those in emergency, intensive care and maternity departments) (14) or in subjects with small and fragile veins (neonates, paediatric, geriatric and oncology patients). Collection from a haematoma site and prolonged tourniquet time should be avoided, along with equipments and connections that may lead to turbulent blood path (butterfly devices or syringes). Vigorous mixing of the specimens after collection should be prevented and...
### Table 1  Laboratory parameters affected by haemolysis and/or blood cell lysis in the specimen.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bias</th>
<th>Cause</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenocorticotropic hormone</td>
<td>Negative</td>
<td>Proteolysis</td>
<td>36</td>
</tr>
<tr>
<td>Activated partial thromboplastin</td>
<td>Negative</td>
<td>Release of thromboplastic substances</td>
<td>39</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>41</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>Positive</td>
<td>Cellular release</td>
<td>27</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>Positive</td>
<td>Cellular release</td>
<td>27</td>
</tr>
<tr>
<td>Albumin</td>
<td>Negative</td>
<td>Dilution</td>
<td>27</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>27</td>
</tr>
<tr>
<td>Bilirubin (neonatal)</td>
<td>Variable</td>
<td>Analytical interference</td>
<td>29</td>
</tr>
<tr>
<td>Bilirubin (total)</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>23</td>
</tr>
<tr>
<td>Calcitonine</td>
<td>Positive</td>
<td>Proteolysis</td>
<td>36</td>
</tr>
<tr>
<td>Chloride</td>
<td>Negative</td>
<td>Dilution</td>
<td>27</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>31</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>Positive</td>
<td>Analytical interference</td>
<td>27</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Positive</td>
<td>Analytical interference</td>
<td>27</td>
</tr>
<tr>
<td>D-dimer</td>
<td>Positive</td>
<td>Release of thromboplastic substances</td>
<td>39</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Negative</td>
<td>Release of thromboplastic substances</td>
<td>39</td>
</tr>
<tr>
<td>Folate</td>
<td>Positive</td>
<td>Cellular release</td>
<td>36</td>
</tr>
<tr>
<td>γ-Glutamyltransferase</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>27</td>
</tr>
<tr>
<td>Gastrin</td>
<td>Negative</td>
<td>Proteolysis</td>
<td>36</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Negative</td>
<td>Proteolysis</td>
<td>36</td>
</tr>
<tr>
<td>Glucose</td>
<td>Negative</td>
<td>Dilution</td>
<td>27</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>38</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>37</td>
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<tr>
<td>Insulin</td>
<td>Negative</td>
<td>Proteolysis</td>
<td>36</td>
</tr>
<tr>
<td>Iron</td>
<td>Positive</td>
<td>Analytical interference</td>
<td>27</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Positive</td>
<td>Cellular release</td>
<td>27</td>
</tr>
<tr>
<td>Lipase</td>
<td>Positive</td>
<td>Analytical interference</td>
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</tr>
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<td>Magnesium</td>
<td>Positive</td>
<td>Cellular release</td>
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</tr>
<tr>
<td>Parathormon</td>
<td>Negative</td>
<td>Proteolysis</td>
<td>36</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Positive</td>
<td>Cellular release</td>
<td>27</td>
</tr>
<tr>
<td>Potassium</td>
<td>Positive</td>
<td>Cellular release</td>
<td>27</td>
</tr>
<tr>
<td>Prostate specific antigen</td>
<td>Positive</td>
<td>Analytical interference</td>
<td>31</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>Positive</td>
<td>Release of thromboplastic substances</td>
<td>39</td>
</tr>
<tr>
<td>Sodium</td>
<td>Negative</td>
<td>Dilution</td>
<td>27</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>31</td>
</tr>
<tr>
<td>Troponin I</td>
<td>Positive</td>
<td>Analytical interference</td>
<td>31</td>
</tr>
<tr>
<td>Troponin T</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>33</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Negative</td>
<td>Analytical interference</td>
<td>31</td>
</tr>
</tbody>
</table>

**Figure 2** Suggested procedure for identification and management of haemolytic specimens in clinical laboratories.
appropriate conditions of temperature and humidity should be warranted. Moreover, defined and standardized practices for sample transportation and storage should be observed. The blood specimens should be centrifuged within a suitable time of collection, with appropriate conditions of centrifugation (force, spin time and temperature), the supernatant (serum or plasma) timely separated from the blood cells unless the primary tube is provided with a (gel) separator (42).

The second step is the identification of haemolytic specimens in the laboratory and, in particular, the definition of a haemolysis degree which is most likely to influence results of testing. Modern laboratory technology takes advantage of preanalytical modules and analysers provided with a software capable of automatically testing and eventually correcting for a broad series of analytical interferences, including haemolysis (haemolysis index). The implementation of this technology is recommendable for several reasons, in that it would help overcome the limits of the visual inspection, improve the recognition of mild haemolytic specimens (<0.6 g/L of serum haemoglobin) which are almost undetectable by visual inspection but still unsuitable for some measurements (AST, LDH, potassium and sodium) (27), standardise and harmonise behaviours among operators in the same laboratory and among different facilities. As already mentioned, the interference of haemolysis is rather heterogeneous, depending on the nature of the analyte, the analytical technique and the instrument. Because certain analytes typically suffer from a biological and non-analytical interference (e.g., potassium, AST, LDH), in such cases, universal recommendations might be drafted. Different is the case when the interference is reportedly analytical. Because methods and/or instruments are variably affected by such interference, detailed knowledge of the analytical technique is pivotal and the individual laboratory should clearly specify within its operative procedures (e.g., quality manuals) the type of analyses that might be influenced and the relative degree of unsuitable haemolysis (a threshold established on the basis of the concentration of free haemoglobin in the specimen). The instrument/reagent manufacturer should always provide the user with the level of haemoglobin interference for each assay. However, since the information is often vague, additional experiments might also be performed locally, if needed. Either the total allowable error or the analytical quality specifications for desirable bias, which are both derived from intra- and inter-individual variations (43), can be used to identify the acceptable bias from results obtained in reference, non-haemolytic specimens (44). Once the haemolytic specimen has been identified, three potential approaches can be suggested to deal with: (a) data correction for haemolysis, (b) report of test results with a standard indication (interpretative comment) of the potential interference arising from haemolysis, and (c) clinicians warning and specimen recollection.

Data correction for haemolysis
First of all, analytical methods, which are reportedly unaffected by haemolysis should be preferred. Although elimination of the analytical interference is always problematic, it is ideally possible to influence spectral interferences by multi-wavelength analysis and blank measurement or preparation before testing (24). Although some degree of analytical interference might be subtracted, this solution would not apply for reporting results of those analytes whose concentration is affected by intracellular leakage or dilution effects. Because corrective factors for factitious hyperkalaemia in a clinically relevant range have been identified, results adjustment for haemolysis has also been recommended in reasonable faith to provide clinically useful information on unsuitable specimens (45). This approach is based on specific equations, where the haemoglobin concentration is multiplied by the slope obtained from a linear regression analysis between the bias observed for each analyte at the relative free haemoglobin concentration in serum or plasma. Hypothetically, when the lower bound of the predicted delta potassium results in a corrected value within the reference range, a second blood draw might be unnecessary (25, 45–47). Several clinical chemistry instruments and a few coagulation analysers report ability to identify haemolytic specimens and eventually correct results for the entity of haemolysis in plasma or serum (48). Methods may vary among different manufacturers, but they are generally based on the haemolysis index (HI), which is reported in haemolysis units corresponding to haemoglobin concentration in mg/dL. These units are linear and semiquantitative and a HI of 500 is equivalent to a known haemoglobin concentration of approximately 500 mg/dL. However, such a strategy might be inaccurate and potentially misleading due to the broad and heterogeneous bias induced in the measurement of several parameters by haemolysis and/or blood cell lysis (27), and it is virtually unsuitable until intravascular haemolysis is safely ruled out (47). In fact, although the main error might be approximately linearly dependent on the free haemoglobin concentration in the specimen and independent from the initial concentration of the analyte, the mean variations observed for several parameters (namely AST and LDH) exceed the quality specifications for desirable bias already in mild haemolytic specimens. Moreover, in the range of 1.3–10.3 g/L free haemoglobin, which includes the majority of specimens which are usually categorised as haemolytic by visual inspection, the mean variation of most clinical chemistry and coagulation parameters also exceeds the quality specifications for desirable bias, thus preventing the identification of suitable ratios when correcting the values for the degree of haemolysis (27).

Results reporting with interpretative comments
Rather than reporting data corrected for haemolysis interference (when possible and suitable), it has been
suggested that a qualitative comment may accompany results on the laboratory report, expressing the potential range of the analyte concentration as predicted according to the haemolysis degree. When results lay within a critical range, they would be timely reported to the requesting clinician (49). It has also been suggested to provide results on haemolytic specimens including some sort of alerts or flags [e.g., “overestimation of K concentration: exclude in vivo haemolysis or repeat sampling” (50)]. Although there are some practical advantages to implement this strategy (immediate notification of the problem upon identification, no need to waste time to reach the clinician by phone), there are also some shortcomings that should be highlighted (51). Because haemolytic specimens are mostly due, up to 98% of the cases, to in vitro haemolysis (19), laboratory data obtained on such samples and notified to the healthcare staff would be in the large majority of cases imprecise or even “erroneous”. Conversely, it is commonly acknowledged that results of testing obtained on unsuitable specimens should never be reported. This is in agreement with the available recommendations (44) and with the current indications issued by the World Health Organization (52). Moreover, a comment accompanying test results on the report might go overlooked, especially in emergency departments or intensive care units, where activities are crowded and frenetical. On the contrary, a direct notification would probably be more effective in alerting the healthcare staff. Finally, data obtained on unsuitable specimens and included within the laboratory report would be permanently stored in the database of the Laboratory Information System (LIS), providing misleading information when comparing patients’ data longitudinally (51).

Clinicians warning and specimen recollection

Due to the drawbacks afflicting the previously mentioned actions in the management of haemolytic specimens, the appropriate corrective measure might be the systematic quantification of haemolysis in any serum or plasma specimen, especially in those where haemolysis might be masked by excess of other interfering substances, such as bilirubin and lipids (44, 51). Whenever this approach cannot be implemented, use of delta checks, lack of ‘fit’ with clinical details, implausible results, different results for the same analyte from different methods and non-linearity on dilution are useful indicators for the presence of potential interfering substances, including free haemoglobin. When the degree of haemolysis reasonably exceeds the threshold of biological or analytical interference, the non-conformity should be systematically recorded, allowing to store and review data and identify pre-analytical steps and clinical departments more susceptible to this type of error, thus providing the ideal basis for an efficient feedback and enabling considerations on specific responsibilities. Any further corrective action undertaken by the laboratory staff must also be recorded (44). Whenever in vivo haemolysis is reasonably suspected, good liaison between the laboratory staff and clinicians is of paramount importance for the rapid notification and the reliable identification of the underlying cause, which is frequently a life-threatening disorder. Therefore, clinicians should be immediately warned, so that any in vivo cause of haemolysis can be safely ruled out and the laboratory staff should also ask to recollect the specimen when needed (50, 51). The patient’s history should be carefully reviewed for potential causes.

The problem of synthetic blood substitutes

A new challenge for clinical laboratories is the introduction of synthetic blood substitutes, which are based on polymeric haemoglobin analogues. Because they are intensely coloured and used in high concentrations therapeutically, it is likely that such products would generate a significant analytical interference with the current technology in use in most laboratories, and a large amount of testing will be unable to be reliably performed on patients receiving these products (53, 54). While available studies in the scientific literature will be useful guidelines, individual laboratories should assess these interferences in light of the myriad testing methods in use, differences in the various haemoglobin-based blood substitute products and institutional-specific opinions as to what constitutes significant interference (55, 56).

Conclusions

The clinical usefulness of laboratory test results depends on trueness and precision (3, 5). The presence of endogenous or exogenous substances in body fluids can adversely affect the determination of many analytes in laboratory practice. Although interference-related clinical errors are relatively rare, they can still impact on either the patient care, producing wrong management with adverse clinical outcomes, or the laboratory’s reputation through their effect on test results (time, additional testing, reduced doctor confidence) (7). Haemolytic specimens have always been a major concern for clinical laboratories worldwide, in that they are the leading cause of preanalytical variability, multiple testing and they can also have a negative impact on medical care (7–10). Although multiple medical states can cause haemolysis in vivo, a variety of factors affecting haemolysis in vitro occur, beginning at the patient’s bedside and continuing through analysis and storage, depending upon the patient’s condition, the skill of the person collecting the sample and local environment. A great deal of knowledge, skill and experience is required to collect a quality specimen that yields desired results, and gathering data in local healthcare organisations will help identify the areas where haemolysis occurs most frequently and the correct use of technical and
informatics resources would also allow the correct assessment and management of this problem. Because the interference depends on the exact assay conditions and the susceptibility of each individual laboratory’s tests, practical strategies might vary on a local basis, provided that the problem is acknowledged and rationally managed, developing new models of risk management that would lead to a healthcare environment that encourages truth and disclosure rather than fear and reprimand. Once all elements are known and consensus between the medical laboratory and clinicians is reached, practices and procedures can be implemented to dramatically reduce haemolysis and avoid erroneous laboratory results affecting patient care and increasing laboratory expenditures.

References
