Clin Chem Lab Med 2006;44(3):311–316 © 2006 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2006.054

Influence of hemolysis on routine clinical chemistry testing

Giuseppe Lippi*, Gian Luca Salvagno, Martina Montagnana, Giorgio Brocco and Gian Cesare Guidi

Istituto di Chimica e Microscopia Clinica, Dipartimento di Scienze Morfologico-Biomediche, Università degli Studi di Verona, Verona, Italy

Abstract

Background: Preanalytical factors are the main source of variation in clinical chemistry testing and among the major determinants of preanalytical variability, sample hemolysis can exert a strong influence on result reliability. Hemolytic samples are a rather common and unfavorable occurrence in laboratory practice, as they are often considered unsuitable for routine testing due to biological and analytical interference. However, definitive indications on the analytical and clinical management of hemolyzed specimens are currently lacking. Therefore, the present investigation evaluated the influence of in vitro blood cell lysis on routine clinical chemistry testing.

Methods: Nine aliquots, prepared by serial dilutions of homologous hemolyzed samples collected from 12 different subjects and containing a final concentration of serum hemoglobin ranging from 0 to 20.6 g/L, were tested for the most common clinical chemistry analytes. Lysis was achieved by subjecting whole blood to an overnight freeze-thaw cycle.

Results: Hemolysis interference appeared to be approximately linearly dependent on the final concentration of blood-cell lysate in the specimen. This generated a consistent trend towards overestimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, creatine kinase (CK), iron, lactate dehydrogenase (LDH), lipase, magnesium, phosphorus, potassium and urea, whereas mean values of albumin, alkaline phosphatase (ALP), chloride, γ-glutamyltransferase (GGT), glucose and sodium were substantially decreased. Clinically meaningful variations of AST, chloride, LDH, potassium and sodium were observed in specimens displaying mild or almost undetectable hemolysis by visual inspection (serum hemoglobin <0.6 g/L). The rather heterogeneous and unpredictable response to hemolysis observed for several parameters prevented the adoption of reliable statistic corrective measures for results on the basis of the degree of hemolysis.

Conclusion: If hemolysis and blood cell lysis result from an in vitro cause, we suggest that the most convenient corrective solution might be quantification of free hemoglobin, alerting the clinicians and sample recollection.

Keywords: hemolysis; laboratory testing; preanalytical variability.

*Corresponding author: Prof. Giuseppe Lippi, MD, Istituto di Chimica e Microscopia Clinica, Dipartimento di Scienze Morfologico-Biomediche, Università degli Studi di Verona, Ospedale Policlinico G.B. Rossi, Piazzale Scuro, 10, 37121 Verona, Italy
Phone: +39-045-8074517, Fax: +39-045-8201889,
E-mail: ulippi@tin.it

Introduction

Over the past decade, the increasing availability of automation and improvements in laboratory technology have contributed to diminishing the incidence of errors throughout the analytical laboratory process, increasing in parallel the reliability of laboratory data and allowing a major sense of security in the value of laboratory results. A major emphasis is currently being placed on preanalytical variables, believed to exert a significant influence on results of laboratory tests. As a consequence, the accurate standardization of each aspect of the preanalytical phase seems pivotal to achieve reliable and accurate results and to provide clinically relevant information (1).

Besides procedures immediately related to patient preparation and blood collection, such as physical activity (2), the fasting state, the blood collection technique (3–5) and the tourniquet time (6, 7), there are additional preanalytical circumstances that might influence the reliability of laboratory testing. In particular, problems arising from troublesome specimen collection or handling, such as wet alcohol transfer from the skin to the blood specimen, small-gauge needles, difficulty in locating easy venous access, small or fragile veins, unsatisfactory attempts, vein missing, partial obstruction of catheters and other collection devices, application of a negative pressure to the blood in the syringe, 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, frequently compromise the integrity of blood and vascular cells, causing leakage of intracellular components and producing significant biological and analytical interference (8, 9).

Hemolysis is classically understood as the release of hemoglobin and other intracellular components from erythrocytes to the surrounding plasma, following damage or disruption of the cell membrane. Hemolysis may occur either in vivo or in vitro, and is a most undesirable condition that influences the accuracy and reliability of laboratory testing (9). Along with preanalytical causes, in vivo blood cell lysis can originate from hereditary, acquired, and iatrogenic conditions, such as autoimmune hemolytic anemia, severe infections, intravascular disseminated coagulation and transfusion reactions; it does not depend

on the technique of the healthcare provider and is thus virtually unavoidable and cannot be resolved (10). Visible hemolysis, as a hallmark of a more generalized process of blood cell damage, is usually not apparent until the separation of serum or plasma has occurred. It is commonly defined as an extracellular hemoglobin concentration of >0.3 g/L (4.65 mol/L), resulting in a detectable pink-to-red hue of serum or plasma with a visible appearance in specimens containing as low as 0.5% hemolysate (11). Hemolyzed specimens are a rather frequent occurrence in laboratory practice, with prevalence described as being as high as 3.3% of all of routine samples presented to a clinical laboratory and accounting for nearly 60% of rejected specimens, five-fold more than the second most common cause (8, 12). Although laboratory testing of hemolyzed specimens has traditionally been discouraged to avoid unreliable results of some biochemical parameters, there are no definitive guidelines or recommendations on this topic and clinical laboratories usually follow arbitrary or individual procedures to handle results for unsuitable samples due to blood cell lysis. Therefore, the aim of the present investigation was to evaluate interference caused by in vitro hemolysis and blood cell lysis on several routine biochemical parameters and to provide tentative guidelines for the clinical management of hemolyzed specimens.

Materials and methods

Experimental design and blood sampling

In the morning of the first day of the evaluation, 3.5 mL of blood from 12 healthy volunteers, who gave explicit informed consent for the investigation, was separately collected into two 6.0-mL siliconized vacuum tubes containing no additives (Becton-Dickinson, Oxford, UK), using a 20G, 0.80×19 mm Venoject® multi-sample straight needle (Terumo Europe NV, Leuven, Belgium). Volunteers were selected on the basis of a homogeneous range of values for white blood cell count ($3.25–4.98\times10^3/\mu$L), platelet count ($153–281\times10^3/\mu$L) and hemoglobin concentration (137–146 g/L). The first specimen (sample 1) was immediately stored at –70°C, whereas the second (sample 2) was centrifuged at $1500\times g$ for 10 min at room temperature. Serum was separated and stored at –70°C.

In the morning of the second day of the evaluation, blood from each of the same 12 volunteers was collected into four additional 6.0-mL tubes of the same type using the same type of needle. Samples were pooled (sample 3) and finally divided into aliquots of 2 mL. Samples 1 and 2 were thawed; nine serial dilutions of free serum hemoglobin were prepared by mixing scalar aliquots of samples 1 and 2. Then 200 μL of each of these dilutions was added to 2-mL aliquots of blood collected on day 2 (sample 3) to achieve free serum hemoglobin concentrations in the specimens ranging from 0 to 20.6 g/L, thus being almost representative of the hemolysis degree we randomly observe in samples sent to our laboratory. Blood was then centrifuged at $1500\times g$ for 10 min at room temperature and serum was separated and immediately analyzed.

Laboratory testing

Hemolysis was assayed by measuring the concentration of free serum hemoglobin by the reference cyanmethemoglobin method on the UV-1700 spectrophotometer (Shimadzu Italia S.l.r., Milano, Italy) (13). Hemoglobin determination, white blood cell and platelet counts were performed on an ADVIA 120 system (Bayer Diagnostics, Newbury, Berkshire, UK). Serum albumin (bromocresol green colorimetric method), alkaline phosphatase (ALP) (IFCC method with aminomethyl-propanol buffer), alanine aminotransferase (ALT) (IFCC method with pyridoxal phosphate activation), aspartate aminotransferase (AST) (IFCC method with pyridoxal phosphate activation), total bilirubin (diazo method, employing 2,5-dichlorophenyl diazonium tetrafluoroborate), calcium (colorimetric method, o-cresolphthalein complexone), creatine kinase (CK) (N-acetylcysteine-activated IFCC method), creatinine (Jaffe kinetic method), γ-glutamyltransferase (GGT) (Szasz-Persijn method, using L-γ-glutamyl-3-carboxy-4-nitroanilide), glucose (enzymatic, hexokinase method), iron (FerroZine method), lactate dehydrogenase (LDH) (DGKC method), lipase (colorimetric method, using 1,2-O-dilauryl-rac-glycero-3-glutaric acid-6-methylresorufin ester), magnesium (chlorophosphonazo method), inorganic phosphorus (ammonium molybdate method), urea nitrogen (urease/glutamate dehydrogenase kinetic method), and uric acid (uricase/peroxidase enzymatic method) were assayed on a Roche/Hitachi Modular System P (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's specifications and using proprietary reagents. Sodium, chloride and potassium were measured on a Roche/Hitachi Modular System by an indirect method using ion-selective electrodes. Total imprecision, as expressed by the coefficient of variation (CV), was less than 2.5% for all analytes tested. All measurements were performed in duplicate within a single analytical session and results were finally reported as the mean of paired measurements. The significance of differences between samples was assessed by a paired Student's t-test; the level of statistical significance was set at $p<0.05$. Bland-Altman and limits-of-agreement analyses were used to compare the results of the independent measurements and differences were finally reported as the percentage of averages (14).

Results

As a result of the overnight freeze-thaw cycle, all the blood cell counts fell beyond the analytical sensitivity of the instrument (white blood cell counts $<0.2\times10^3/\mu$L, platelet count $<5\times10^3/\mu$L and erythrocytes $<0.1\times10^6/\mu$L). The results of the present investigation on hemolyzed specimens are presented in Tables 1 and 2. Hemolysis interference, expressed as mean absolute and percentage relative bias, appeared to be approximately linearly dependent on the final concentration of blood cells lysate in the specimen. As expected, the addition of blood cell lysate generated a consistent and dose-dependent trend towards overestimation of ALT, AST, creatinine, CK, iron, LDH, lipase, magnesium, phosphorus, potassium and urea, whereas mean values of albumin, ALP, chloride, GGT, glucose and sodium were substantially decreased when compared with the baseline specimens (no lysate). Statistically significant differences in samples containing 0.6 g/L of serum hemoglobin or less could

Table 1 Influence of hemolysis on routine clinical chemistry testing. Differences are given as absolute values and percentage relative bias (mean ± SD) from the baseline sample (no lysis). Values are compared with current analytical quality specifications for desirable bias (15). Statistically significant differences were evaluated by Student's paired t-test ([a]$p < 0.05$; [b]$p < 0.01$). Results with hemolysis-induced variations exceeding the relative desirable bias are marked in bold.

Analyte	Desirable bias, %	Free serum hemoglobin								
		No lysis	0.16 g/L	0.3 g/L	0.6 g/L	1.3 g/L	2.6 g/L	5.1 g/L	10.3 g/L	20.6 g/L
Albumin, g/L	±1.3	44.9 ± 1.1	44.8 ± 1.0 (−0.1 ± 0.4%)	44.6 ± 1.3 (−0.5 ± 0.9%)	44.5 ± 1.1 (−0.8 ± 0.4%)	44.3 ± 1.0 (−1.1 ± 0.7%)	**44.3 ± 1.2** **(−1.3 ± 0.8%)**	**44.2 ± 1.1** **(−1.4 ± 0.7%)**	**43.7 ± 1.1** **(−2.5 ± 0.4%)**	**42.2 ± 1.1** **(−5.9 ± 1.0%)**
Alkaline phosphatase, U/L	±6.4	52 ± 8	52 ± 8 **(0.0 ± 1.0%)**	52 ± 7 (−0.4 ± 1.6%)	51 ± 8 (−1.4 ± 2.1%)	50 ± 7[b] (−3.0 ± 1.4%)	**47 ± 7[b]** **(−9.2 ± 2.0%)**	**43 ± 7[b]** **(−18.0 ± 2.3%)**	**36 ± 7[b]** **(−31.4 ± 3.6%)**	**24 ± 6[b]** **(−53.7 ± 5.3%)**
Alanine aminotransferase, U/L	±12.0	20 ± 13	20 ± 13 **(+1.6 ± 2.8%)**	20 ± 13 **(+2.2 ± 8.0%)**	20 ± 13 **(+2.3 ± 7.2%)**	20 ± 13 **(+2.9 ± 7.8%)**	22 ± 13[a] **(+10.2 ± 11.9%)**	**23 ± 13[b]** **(+18.2 ± 11.6%)**	**26 ± 13[b]** **(+37.1 ± 16.3%)**	**32 ± 13[b]** **(+73.8 ± 28.2%)**
Aspartate aminotransferase, U/L	±5.4	22 ± 4	23 ± 4[b] **(+4 ± 3%)**	**25 ± 4[b]** **(+12 ± 5%)**	**27 ± 4[b]** **(+24 ± 7%)**	**31 ± 4[b]** **(+42 ± 9%)**	**40 ± 5[b]** **(+84 ± 14%)**	**59 ± 7[b]** **(+169 ± 25%)**	**95 ± 11[b]** **(+336 ± 48%)**	**169 ± 22[b]** **(+677 ± 87%)**
Bilirubin (total), μmol/L	±10.0	20.8 ± 10.3	20.8 ± 10.4 (−0.1 ± 0.8%)	20.6 ± 10.4 (−1.1 ± 2.9%)	20.6 ± 10.0 (−0.3 ± 2.2%)	20.4 ± 10.8 (−4.0 ± 5.9%)	20.4 ± 10.9 (−4.2 ± 7.4%)	20.3 ± 10.2 (−2.8 ± 4.9%)	20.3 ± 9.6 (−0.6 ± 7.7%)	20.2 ± 9.3 (−1.3 ± 9.2%)
Calcium, mmol/L	±0.8	2.40 ± 0.09	2.40 ± 0.10 (+0.1 ± 0.9%)	2.40 ± 0.09 (+0.1 ± 0.5%)	2.40 ± 0.09 (+0.1 ± 0.7%)	2.41 ± 0.09 (+0.3 ± 0.5%)	2.41 ± 0.09 (+0.4 ± 0.8%)	2.42 ± 0.09 (+0.6 ± 0.7%)	2.42 ± 0.10 (+0.6 ± 0.7%)	2.40 ± 0.09 (−0.1 ± 0.7%)
Chloride, mmol/L	±0.5	105.5 ± 1.9	105.5 ± 1.9 (0.0 ± 0.0%)	**105.0 ± 1.7** **(−0.5 ± 0.5%)**	**104.8 ± 1.6** **(−0.7 ± 0.6%)**	**104.6 ± 1.5** **(−0.8 ± 0.6%)**	**104.3 ± 1.3** **(−1.2 ± 0.6%)**	**104.0 ± 1.5** **(−1.4 ± 0.8%)**	**103.6 ± 1.9** **(−1.8 ± 0.7%)**	**102.5 ± 1.6** **(−2.8 ± 0.9%)**
Creatine kinase, U/L	±11.5	118 ± 46	120 ± 47[a] **(+2 ± 2%)**	121 ± 47[b] **(+3 ± 2%)**	125 ± 47[b] **(+7 ± 4%)**	**131 ± 48[b]** **(+13 ± 4%)**	**145 ± 47[b]** **(+27 ± 10%)**	**175 ± 47[b]** **(+56 ± 22%)**	**240 ± 50[b]** **(+120 ± 46%)**	**380 ± 55[b]** **(+259 ± 102%)**
Creatinine, μmol/L	±3.4	81 ± 10	82 ± 11 **(+0.2 ± 1.8%)**	82 ± 11 **(+0.3 ± 2.3%)**	82 ± 10 **(+0.4 ± 2.0%)**	83 ± 10[a] **(+1.5 ± 1.4%)**	**85 ± 11[b]** **(+4.4 ± 2.4%)**	**86 ± 10[b]** **(+5.8 ± 2.4%)**	**86 ± 10[b]** **(+6.3 ± 2.3%)**	**87 ± 9[b]** **(+7.5 ± 3.6%)**
γ-Glutamyltransferase, U/L	±10.8	17 ± 8	16 ± 8[a] (−4.7 ± 4.6%)	16 ± 8[b] (−6.0 ± 3.9%)	16 ± 8[b] (−6.4 ± 3.7%)	16 ± 8[b] (−7.8 ± 3.1%)	**15 ± 8[b]** **(−11.1 ± 5.3%)**	**15 ± 8[b]** **(−14.5 ± 5.7%)**	**14 ± 7[b]** **(−18.3 ± 5.4%)**	**12 ± 7[b]** **(−35.5 ± 9.7%)**
Glucose, mmol/L	±2.2	5.02 ± 0.66	5.02 ± 0.68 (−0.2 ± 0.6%)	5.02 ± 0.66 (−0.1 ± 0.4%)	5.02 ± 0.64 (0.0 ± 0.8%)	5.02 ± 0.64 (0.0 ± 0.5%)	5.02 ± 0.64 (−0.1 ± 0.3%)	5.01 ± 0.64 (−0.2 ± 0.9%)	4.97 ± 0.65[b] (−1.0 ± 0.4%)	4.95 ± 0.65[b] (−1.4 ± 1.0%)
Iron, μmol/L	±8.8	18.4 ± 5.8	18.4 ± 5.8 **(+0.1 ± 0.9%)**	18.6 ± 5.6 **(+1.5 ± 2.1%)**	18.6 ± 5.7[b] **(+1.5 ± 1.3%)**	18.8 ± 5.8[b] **(+2.1 ± 0.6%)**	19.2 ± 5.7[b] **(+4.7 ± 2.0%)**	**20.1 ± 5.8[b]** **(+10.6 ± 3.9%)**	**22.2 ± 5.9[b]** **(+23.1 ± 8.3%)**	**27.3 ± 6.1[b]** **(+53.1 ± 16.0%)**
Lactate dehydrogenase, U/L	±4.3	338 ± 37	**369 ± 36[b]** **(+9 ± 2%)**	**397 ± 38[b]** **(+17 ± 2%)**	**451 ± 39[b]** **(+34 ± 4%)**	**550 ± 42[b]** **(+63 ± 7%)**	**746 ± 52[b]** **(+122 ± 13%)**	**1203 ± 69[b]** **(+259 ± 34%)**	**1906 ± 116[b]** **(+470 ± 69%)**	**3804 ± 140[b]** **(+1039 ± 132%)**
Lipase, U/L	±10.1	28 ± 6	28 ± 6 **(0.0 ± 0.0%)**	29 ± 6 **(+0.5 ± 1.3%)**	29 ± 5 **(+2.2 ± 4.0%)**	31 ± 5[a] **(+8.2 ± 7.8%)**	**35 ± 6[b]** **(+23.1 ± 7.7%)**	**39 ± 5[b]** **(+38.0 ± 9.9%)**	**43 ± 5[b]** **(+53.1 ± 12.7%)**	**45 ± 5[b]** **(+60.7 ± 15.9%)**
Magnesium, mmol/L	±1.8	0.92 ± 0.04	0.92 ± 0.04 **(0.0 ± 0.4%)**	0.92 ± 0.04 **(0.0 ± 0.8%)**	0.92 ± 0.03 **(+0.4 ± 2.1%)**	0.93 ± 0.03[b] **(+1.5 ± 2.1%)**	**0.94 ± 0.04[b]** **(+2.3 ± 0.7%)**	**0.95 ± 0.03[b]** **(+3.5 ± 1.0%)**	**0.98 ± 0.04[b]** **(+6.8 ± 1.6%)**	**1.02 ± 0.03[b]** **(+11.6 ± 2.3%)**
Phosphorus, mmol/L	±3.2	1.17 ± 0.12	1.17 ± 0.11 **(+0.1 ± 1.3%)**	1.17 ± 0.11 **(+0.6 ± 1.3%)**	1.18 ± 0.12 **(+0.8 ± 1.0%)**	1.18 ± 0.11 **(+1.4 ± 1.6%)**	1.20 ± 0.11[b] **(+2.9 ± 1.7%)**	**1.23 ± 0.11[b]** **(+5.5 ± 2.0%)**	**1.29 ± 0.11[b]** **(+10.9 ± 3.4%)**	**1.42 ± 0.11[b]** **(+22.1 ± 7.4%)**
Potassium, mmol/L	±1.8	4.12 ± 0.22	4.15 ± 0.22[a] **(+0.7 ± 0.7%)**	4.18 ± 0.23[b] **(+1.5 ± 0.8%)**	**4.25 ± 0.21[b]** **(+3.2 ± 1.2%)**	**4.37 ± 0.22[b]** **(+6.1 ± 1.1%)**	**4.65 ± 0.24[b]** **(+12.9 ± 1.4%)**	**5.22 ± 0.25[b]** **(+26.7 ± 1.6%)**	**6.36 ± 0.30[b]** **(+54.4 ± 3.1%)**	**8.78 ± 0.42[b]** **(+113.2 ± 5.6%)**
Sodium, mmol/L	±0.3	140.1 ± 1.5	**139.6 ± 1.1[a]** **(−0.4 ± 0.4%)**	**139.3 ± 1.0[b]** **(−0.6 ± 0.4%)**	**138.9 ± 0.8[b]** **(−0.9 ± 0.6%)**	**138.6 ± 0.9[b]** **(−1.1 ± 0.5%)**	**138.3 ± 1.2[b]** **(−1.3 ± 0.5%)**	**137.9 ± 1.4[b]** **(−1.6 ± 0.5%)**	**136.5 ± 0.9[b]** **(−2.6 ± 0.5%)**	**133.5 ± 1.0[b]** **(−4.7 ± 0.8%)**
Urea nitrogen, mmol/L	±5.5	6.3 ± 1.2	6.3 ± 1.1 (−0.4 ± 2.3%)	6.3 ± 1.0 (−0.4 ± 2.6%)	6.3 ± 1.1 (−0.6 ± 2.3%)	6.3 ± 1.1 (−0.5 ± 1.5%)	6.4 ± 1.1[a] (+1.4 ± 1.6%)	6.5 ± 1.1[a] (+3.0 ± 2.4%)	6.6 ± 1.2[b] (+3.9 ± 2.2%)	**6.8 ± 1.1[b]** **(+8.9 ± 2.6%)**
Uric acid, μmol/L	±4.8	305 ± 89	303 ± 89 (−0.6 ± 1.0%)	303 ± 89 (−0.8 ± 1.1%)	303 ± 88 (−0.9 ± 1.1%)	302 ± 89 (−1.0 ± 1.1%)	301 ± 89 (−1.3 ± 1.1%)	296 ± 89 (−3.2 ± 1.4%)	**290 ± 87** **(−5.1 ± 2.0%)**	**275 ± 86** **(−10.3 ± 2.4%)**

Table 2 Influence of hemolysis on routine clinical chemistry testing: mean coefficient of variation (CV) (n = 12) determined from the reference specimen (no lysis) and each one of the homologous eight samples containing scalar dilutions of free serum hemoglobin.

Analyte	Desirable bias, %	Free serum hemoglobin							
		0.16 g/L	0.3 g/L	0.6 g/L	1.3 g/L	2.6 g/L	5.1 g/L	10.3 g/L	20.6 g/L
Albumin, g/L	±1.3	0.2%	0.4%	0.4%	0.6%	0.7%	0.7%	1.3%	3.1%
Alkaline phosphatase, U/L	±6.4	0.2%	0.5%	1.0%	1.6%	4.8%	9.9%	18.7%	36.9%
Alanine aminotransferase, U/L	±12.0	0.8%	3.2%	2.6%	3.0%	5.9%	8.1%	15.2%	26.1%
Aspartate aminotransferase, U/L	±5.4	2.1%	5.6%	10.5%	17.3%	29.4%	45.5%	62.3%	77.0%
Bilirubin (total), μmol/L	±10.0	0.3%	1.2%	1.0%	2.9%	3.0%	1.8%	3.3%	3.9%
Calcium, mmol/L	±0.8	0.3%	0.2%	0.3%	0.3%	0.4%	0.3%	0.3%	0.3%
Chloride, mmol/L	±0.5	0.0%	0.2%	0.4%	0.4%	0.6%	0.7%	0.9%	1.4%
Creatine kinase, U/L	±11.5	1.1%	1.6%	3.4%	6.0%	11.6%	21.3%	36.2%	54.3%
Creatinine, μmol/L	±3.4	0.8%	0.8%	0.9%	0.7%	2.1%	2.8%	3.0%	3.6%
γ-Glutamyltransferase, U/L	±10.8	2.5%	3.2%	3.4%	4.1%	6.0%	7.9%	10.2%	22.0%
Glucose, mmol/L	±2.2	0.2%	0.1%	0.3%	0.1%	0.1%	0.3%	0.5%	0.7%
Iron, μmol/L	±8.8	0.3%	0.7%	0.7%	1.0%	2.3%	5.0%	10.2%	20.7%
Lactate dehydrogenase, U/L	±4.3	4.4%	8.0%	14.4%	24.0%	37.7%	56.2%	69.8%	83.7%
Lipase, U/L	±10.1	0.0%	0.3%	1.0%	3.8%	10.2%	15.8%	20.8%	23.0%
Magnesium, mmol/L	±1.8	0.2%	0.3%	0.7%	0.9%	1.1%	1.7%	3.3%	5.5%
Phosphorus, mmol/L	±3.2	0.4%	0.6%	0.5%	0.9%	1.4%	2.7%	5.1%	9.9%
Potassium, mmol/L	±1.8	0.4%	0.7%	1.6%	3.0%	6.1%	11.8%	21.4%	36.1%
Sodium, mmol/L	±0.3	0.2%	0.3%	0.4%	0.5%	0.7%	0.8%	1.3%	2.4%
Urea nitrogen, mmol/L	±5.5	1.0%	1.1%	0.9%	0.7%	0.7%	1.6%	1.9%	4.3%
Uric acid, μmol/L	±4.8	0.3%	0.4%	0.4%	0.5%	0.7%	1.6%	2.6%	5.4%

Values are compared with current analytical quality specifications for desirable bias (15).

already be observed for AST, LDH, potassium and sodium. Albumin, total bilirubin, calcium, chloride and uric acid measurements were not significantly different, even in specimens containing up to 20.6 g/L of serum hemoglobin. Clinically meaningful variations, as reflected by deviation from the current analytical quality specifications for desirable bias (15), were observed for AST, chloride, LDH, potassium and sodium in samples with mild or almost undetectable hemolysis by visual inspection (0.6 g/L of serum hemoglobin) (Table 1). Of the analytes evaluated, the bias values recorded for total bilirubin, calcium and glucose were the only ones that did not achieve a clinically significant variation, even at the highest concentration of serum hemoglobin (20.6 g/L). Despite the approximately linear relationship of variations to free hemoglobin concentration in serum, an unpredictable, heterogeneous and sample-specific response was observed for several parameters, as shown by the amplitude of the mean CVs calculated from each of the 12 independent patient samples tested (Table 2).

Discussion

There are many opportunities for errors in the preanalytical phase. Phlebotomists must be aware of the possibility of complications and interference in results of laboratory testing, should they fail to follow an accurate and standardized blood collection technique (16). Hemolysis, reflecting more generalized blood cell damage, is a rather common and unfavorable occurrence in laboratory practice. It is the most frequent reason for specimen rejection, as indicated by the College of American Pathologists (CAP) Chemistry Specimen Acceptance Q-Probes study, as it influences the reliability of laboratory tests for both biological and analytical reasons (12). Although the interference seems to be related to the degree of lysis and the specificity of the method being used, it has been reported that several laboratory results can be seriously influenced, especially those for potassium, sodium, calcium, magnesium, bilirubin, haptoglobin, total protein, aldolase, amylase, LDH, AST, ALT, phosphorus, alkaline phosphatase, acid phosphatase, GGT, folate, and iron (17–19). However, there are no definitive indications on the degree of lysis responsible for such interference, nor are definitive guidelines available on the most suitable procedure for reporting and handling results of biochemical testing on hemolyzed specimens. Results of the present investigation raise two critical issues: the influence of mild or almost undetectable hemolysis by visual inspection (<0.6 g/L of serum hemoglobin) on some analytes, such as AST, LDH, potassium and sodium; and the management of laboratory results for hemolytic specimens.

The interference of in vitro hemolysis on laboratory testing might be caused by: (i) leakage of hemoglobin and other intracellular components into the surrounding fluid, which induces false elevations of some analytes or dilution effects; (ii) chemical interference by free hemoglobin in the analytical reaction; and (iii) method- and analyte concentration-dependent spectrophotometric interference due to an increase in the optical absorbance or a change in the blank value, especially for laboratory measurements at 415, 540 and 570 nm, where hemoglobin shows strong absorbance (8, 20, 21). Clearly, at high levels of serum hemoglobin, such interferences might coincide, causing variations that are not necessarily in the same direction and making the situation rather difficult to

understand. As for ALT, AST, LDH, magnesium, phosphorus and potassium, the interference mechanism has previously been characterized and is attributed to large differences between intracellular and extracellular concentrations for these analytes (21). Lower values for glucose, sodium and chloride are likely caused by dilutional effects, whereas hemolysis interference in the CK assay has been attributed to intracellular adenylate kinase, which is not wholly inhibited under operating conditions (20, 21). For ALP, iron, lipase and GGT, hemolysis interference is likely caused by spectral overlap and by a chemical reaction between hemolysate and reaction components (21).

At variance with biological causes, in vitro blood cell lysis is preventable, as it is usually caused by inappropriate collection, handling and processing of the specimen. Hemolysis and blood cell lysis are almost undetectable by visual inspection until whole blood has been centrifuged, exposing the serum or plasma to scrutiny. Although it is unlikely that hemolyzed and unsuitable samples can be observed in problem-free phlebotomy activities, clinical laboratories should accurately identify and document each procedure that appears to be more sensitive to in vitro hemolysis and possibly to what extent it is affected. Three potential approaches can be identified to deal with hemolyzed specimens: hemolysis correction; result reporting with a clear indication of the potential interference arising from blood cell lysis; and sample recollection. Correction factors for factitious hyperkalemia in a clinically relevant range were identified and result correction for hemolysis was further recommended as a reliable alternative to provide clinically useful information for unsuitable specimens. This was achieved by multiplying the hemoglobin concentration by the slope obtained from a linear regression analysis between the bias observed for each analyte at the relative free serum hemoglobin concentration. Hypothetically, when the lower bound of the predicted change in potassium results in a corrected value within the reference range, a second blood draw might be unnecessary (20, 22). Accordingly, some instruments already report an ability to identify hemolytic specimens and eventually correct results for the degree of hemolysis. The results of the present investigation testify that such an approach might be inaccurate and potentially misleading. In fact, given the wide and heterogeneous interference observed in hemolytic specimens obtained from 12 different patients (Table 2), we do not recommend hemolysis correction in clinical practice. Although the main error is approximately linearly dependent on hemoglobin concentration and independent of the initial analyte concentration, the mean variations observed for AST and LDH exceeded the desirable bias in even mild hemolyzed specimens. Moreover, for specimens containing 1.3–10.3 g/L free hemoglobin, which includes the majority of visibly hemolyzed specimens sent to our laboratory, the mean CV for 13 out of the 20 analytes tested in 12 separate patient samples exceeded the desirable bias (Table 2), thus preventing the identification of a ratio for correcting values for hemolysis, as tentatively suggested for potassium (22, 23).

Instead of reporting the post-hemolysis corrected potassium result, Dimeski et al. recently suggested the provision of a qualitative comment indicating the likely range of the potassium concentration and recommended that if results lie in a critical range, they should be communicated promptly to the requesting clinician (24). A similar option is to report results of hemolyzed specimens that include messages or flags. However, these solutions appear rather questionable from both the analytical and clinical perspective, irrespective of the correction formulas calculated from linear regression of absolute error vs. hemoglobin concentration. In fact, we believe that whenever results of laboratory testing are reasonably thought to be analytically or clinically unreliable, they should not be reported to prevent misleading information that could finally compromise the whole clinical reasoning. Although the elimination of some types of interference, such as that caused by cell lysis, is problematic and often impossible, it is nevertheless conceivable to influence spectral interference by multiwavelength analysis and blank measurement or preparation (21). Obviously, this solution would not work for interferences due to leakage of intracellular analytes or dilution effects (i.e., AST, ALT and LDH). Therefore, if hemolysis and blood cell lysis result from an in vitro cause, we support previous indications that the most suitable corrective measure might be free hemoglobin quantification, warning of the clinicians so that in vivo hemolysis can be ruled out and, eventually, sample recollection (8).

A further issue is the clinical management of laboratory testing in patients with hemolytic disorders. In vivo hemolysis because of either a hematological disease or a medical procedure, including extracorporeal circulation, mechanical devices, irradiative treatments and drugs, generally becomes apparent when the red blood cell lifespan is shortened by more than 50%. Plasma haptoglobin is capable of binding free hemoglobin, thus allowing its effective clearance by the reticulo-endothelial system (10). Less is known about the in vivo clearance of other elements and substances released during hemolysis. When an in vivo hemolytic state is suspected, should the sample or the results of the biochemical assays, known to be influenced by hemolysis, be analyzed and/or taken into account and reported, or discarded? A recent article, reporting a critical case with hyperkalemia induces caution, and suggests that the correct laboratory procedure and reporting should be guided by clinical rather than by analytical considerations (25). Furthermore, the article raises concern about the non-critical use of automatic potassium suppression when a suspected hemolyzed sample is encountered. A different case is the occurrence of elevated enzyme activities, such as AST and CK, in samples from patients with suspected in vivo hemolysis. Correct use of the analytical resources currently available should allow the correct assessment of every uncertain condition.

We are aware that our investigation has two limitations. First, we have reproduced in vitro blood cell

lysis by freezing blood specimens at −70°C for up to 24 h. Besides lysate erythrocytes, hemolyzed specimens encountered in laboratory practice might also contain destroyed or fragmented platelets, leukocytes and vascular cells. Therefore, our study design appears to be a suitable surrogate, although it is not representative of all the possible events that induce whole blood lysis in laboratory practice, especially those represented by troublesome blood collection. We evaluated such interference on single reagents and a defined analyzer, and thus our results might not be universally reproducible with other testing systems, especially those capable of identifying hemolytic specimens.

Remarkable improvements in analytical quality guarantee that the actual performance of clinical laboratories is suitable for improving the patient's health. However, the large percentage of laboratory errors still occurring in the pre- and postanalytical phases are a source of major concern (1). The issue of hemolysis and blood cell lysis has traditionally plagued clinical laboratories, pointing to a need for operative guidelines and recommendations. Collection of specimens of consistent quality, based on proper training and knowledge of the factors that can influence laboratory results, is essential to minimize errors at any step of the laboratory work-up, to optimize resource utilization and quality, and to improve the whole patient management procedure. The frequency of in vitro hemolysis and additional preanalytical interference might be lowered by implementing standardized collection procedures, such as conventional straight needles and evacuated tube systems, instead of butterfly devices or syringes (5). Laboratory guidelines on this topic should then encompass procedures for identification of specimens with clinically significant hemolysis, such as automated hemolysis/turbidity detection-quantification, measurement and immediate transmission of results of laboratory testing, which can provide the clinician with essential information for clinical situations requiring immediate intervention (9).

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Received October 31, 2005, accepted December 5, 2005