ORIGINAL ARTICLE

Hemolysis indexes for biochemical tests and immunoassays on Roche analyzers: Determination of allowable interference limits according to different calculation methods

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Abstract

Objectives. To determine the hemolysis interference on biochemical tests and immunoassays performed on Roche Diagnostics analyzers, according to different maximum allowable limits. Design and methods. Heparinized plasma and serum pools, free of interferences, were overloaded by increasing amounts of a hemoglobin-titrated hemolysate. This interference was evaluated for 45 analytes using Modular® and Cobas® analyzers. For each parameter, the hemolysis index (HI) corresponding to the traditional \(10\%\) change of concentrations from baseline \(\Delta \) was determined, as well as those corresponding to the analytical change limit (ACL), and to the reference change value (RCV). Then, the relative frequencies distribution (% RFD) of hemolyzed tests performed in a hospital laboratory over a 25-day period were established for each HI as allowable limit. Results. Considering the \(10\%\)Δ, the analyte concentrations enhanced by hemolysis were: Lactate dehydrogenase (LDH), aspartate aminotransferase (AST), folate, potassium, creatine kinase, phosphorus, iron, alanine aminotransferase, lipase, magnesium and triglycerides, decreasingly. The analyte concentrations decreased by hemolysis were: Haptoglobin, high-sensitive troponin T and alkaline phosphatase. Over the 25-day period, the % RFD of tests impacted more than \(10\%\) by hemolysis were \(7\%\) for LDH; \(5\%\) for AST, folates and iron; and \(1\%\) for the other analytes. Considering the ACL, HI were lower, giving % RFD substantially increased for many analytes, whereas only four analytes remain sensitive to hemolysis when considering RCV. Conclusion. This study proposes new HI based on different allowable limits, and can therefore serve as a starting point for future harmonization of hemolysis interference evaluation needed in routine laboratory practice.

Key Words: Hemolysis indexes, hemolysis interference, hemoglobin, maximum allowable limits

Introduction

Among total errors arising in laboratory medicine, nearly two-thirds occur during the pre-analytical phase [1,2]. Hemolysis is one of the most frequent pre-analytical nonconformities noticed in laboratories; it may lead to erroneous results, potentially affects the interpretation of laboratory test results, and then patient care [3]. It has been reported that 3.3% of blood samples sent to biochemistry laboratories presented a hemolysis [4], of which more than 96% were due to in vitro mechanical hemolysis, mainly linked to the blood sampling material [5] and shaking during pneumatic transport [6]. The in vitro hemolysis leads to a release of hemoglobin (Hb) of which direct spectral interference on spectrophotometric measurement can be minimized by a bichromatic reading. Moreover, other intrarerythrocytic components are released during in vitro hemolysis like potassium or lactate dehydrogenase, consequently resulting in a false increase of their plasma or serum concentrations.
In order to provide reliable results to clinicians, most of the current chemical automated systems can detect and estimate the hemolysis interference by measuring a hemolysis index (HI). The HI published or recommended as maximum allowable bias often differ, depending on the parameters, the analyzers, the protocols used for their determination, and/or the retained significant %change of concentration from non-hemolyzed baseline pool (±%Δ) [7,8]. As recently underlined, these variabilities make the harmonization necessary for the HI values, and consequently their protocols of determination, their interpretations and their applications in routine laboratory and clinical practice [9]. Given the lack of studies on hemolysis interference using Roche analyzers and the discordances in published results, we aimed to reach the following objectives: (1) To verify HI quality specifications on Roche Diagnostics analyzers for immunochemistry testing, according to the traditional ±10% change of concentrations from baseline (±10%Δ) as acceptance limit, as well as to the analytical change limit (ACL) and the reference change value (RCV), and (2) to determine the percentages of tests impacted by hemolysis, according to such allowable limits.

**Design and methods**

**Hemolyze preparation**

The hemolysate was prepared according to the classical osmotic shock procedure, adapted from the Clinical and Laboratory Standards Institute guidelines (CLSI EP7-A2) for interference testing in clinical chemistry [10]. Briefly, after removing plasma from a whole blood EDTA pool (n = 10 samples), the red blood cells were washed three times in a 0.9% NaCl solution, and then lysed in distilled water and one freeze-thaw cycle (−20°C; overnight). Then, the hemolysate was adjusted for an Hb concentration at 50 g/L (3.1 mmol/L Hb, in distilled water) measured by spectrophotometry (Beckman™ DU 640B spectrophotometer, USA), using the second-derivative method [11].

**Pools preparation**

According to the French Society of Clinical Biology (SFBC) protocol [12], two extended ranges (14 tubes for each) from a pool of heparinized plasma and a pool of serum, both considered as free of interferences, were overloaded by increasing amounts of the Hb-titrated hemolyze, under a total constant volume of 2 mL per tube. Blood collection tubes used for the pool preparation were lithium heparin tubes (Greiner Bio-one™, 18 U/mL lithium heparin, separator gel, ref#474080) and serum tubes (Becton Dickinson Vacutainer™, Serum tube, CAT, ref#369032).

**Hemolysis measurement**

For preparation of the baseline pools, heparinized plasma or serum were selected from samples with the lowest indexes of hemolysis, icterus and lipemia, measured on Modular® analysers and selected using the MPL® middleware (Roche Diagnostics). For the two ranges of overloaded pools, the HI were measured in duplicate on two Modular P800®, by bichromatic spectrophotometry at 570 nm and 600 nm wavelength pair. The HI were measured using the following formula: HI = 1/scaling factor for Hb * (A570−600 nm – correcting factor for Hb measurement for lipemia * A660−700 nm) [13], in order to compensate the spectral overlap due to lipemia.

**Chemical assays, immunoassays and analyzers**

Interference of hemolysis was investigated for the following parameters. The principle of the methods and the references of reagents are provided in Supplementary Table A (available online at http://informahealthcare.com/doi/abs/10.3109/00365513.2014.993691):

- Twenty-seven chemistry analytes measured on ModularP800® (Roche Diagnostics, Germany): α2-macroglobulin (A2M), plasma albumin (ALB-pl; by colorimetry), alkaline phosphatase (ALP), alanine aminotransferase (ALT, with pyridoxal 5’-phosphate), pancreatic amylase (AMY), apolipoprotein A1 (APOA1), aspartate aminotransferase (AST, with pyridoxal 5’-phosphate), calcium (Ca), total cholesterol (CHOL), creatine kinase (CK), chloride (Cl), bicarbonate (CO2), creatinine (CREA), C-reactive protein (CRP), γ-glutamyltransferase (GGT), glucose (GLU), haptoglobin (HPT), potassium (K), lactate dehydrogenase (LDH), lipase (LIP), magnesium (Mg), sodium (Na), phosphorus (PHOS), triglycerides (TG), plasma total protein (TP-pl), urea (UR), and uric acid (UA).
- Two cardiac biomarkers and one hormone measured by electrochemiluminescence immunoassay (ECLIA) on ModularE170®: high-sensitive troponin T (hsTnT), N-terminal pro-B-type natriuretic peptide (NTPBNP) and cortisol (CORT).
- Seven hormones and vitamins measured by ECLIA on a single Cobas e411®: luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), progesterone (PROG), folates (FOL), vitamin B12 (B12), and α-fetoprotein (AFP).
- Seven proteins and one metal ion measured on a single Cobas c501®: α1-acid glycoprotein (A1AGP), serum albumin (ALB-se; by turbidimetry), immunoglobulins A (IGA), G (IGG),
M (IGM), pre-albumin (PALB), transferrin (TRF), and iron Fe^{2+} (IRON).

All these parameters were performed in duplicate, using Roche Diagnostics reagent kits, except for A2M and ALB-se using Diagam reagents. The chemical analytes were performed on two ModularP800® and cardiac biomarkers on two ModularE170® analyzers, except for A2M, APOA1 and CORT, which were performed on a single one. For each parameter, the plasma or serum matrix is specified in Table I.

### Calculations and statistics

**Determination of hemolysis indexes giving significant change of concentration**

For each parameter, mean positive or negative % change of concentration from baseline pool (± %Δ) has been determined for all the overloaded pools, using the formula: %Δ = 100* (measured value – baseline value)/baseline value, wherein baseline value was the plasma or serum analyte concentration free within-subject variation; (D), Decreased; HI, hemolysis index; (I), Increased; Min, minimum; Max, maximum; nd, not determined; ns, not significant; Pl, plasma; Se, serum.

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**Table I. Hemolysis interference on biochemical tests and immunoassays performed on Roche analyzers.**

| Analyte | Unit | Pl/Se | ACL (%) | RCV (%) | %Δ from BPC | HI |
|---------|------|-------|---------|---------|------------|----|
| A1AGP   | g/L  | Se    | 6.40    | 32.0    | 0.90       |    |
| A2M     | g/L  |       | 8.06    | 12.4    | 1.79       |    |
| AFP     | µg/L | Se    | 10.6    | 35.5    | 3.65       |    |
| ALB-pl  | g/L  |       | 5.07    | 10.2    | 30.8       |    |
| ALB-se  | g/L  |       | 6.16    | 10.8    | 31.6       |    |
| ALP     | U/L  |       | 8.91    | 20.0    | 96.3       |    |
| ALT     | U/L  |       | 9.60    | 54.6    | 35.5       |    |
| AMY     | U/L  |       | 4.79    | 24.6    | 79.5       |    |
| APOA1   | g/L  |       | 9.39    | 20.3    | 1.23       |    |
| AST     | U/L  |       | 6.60    | 34.7    | 33.3       |    |
| B12     | pmol/L |       | 15.3    | 44.3    | 457       |    |
| Ca      | mmol/L |       | 5.12    | 7.75    | 1.97       |    |
| CHOL    | mmol/L |       | 4.61    | 17.1    | 3.82       |    |
| CK      | U/L  |       | 4.18    | 63.3    | 116       |    |
| Cl      | mmol/L |       | 4.17    | 5.33    | 106       |    |
| CO2     | mmol/L |       | 12.0    | 16.3    | 18.1       |    |
| CORT    | µmol/L |       | 18.3    | 45.9    | 169       |    |
| CREA    | µmol/L |       | 8.11    | 18.4    | 96.8       |    |
| CRP     | mg/L  |       | 8.20    | 1.71    | 52.0       |    |
| E2      | ng/L  | Se    | 22.7    | 66.4    | 30.1       |    |
| FOL     | mmol/L |       | 20.2    | 69.5    | 457       |    |
| FSH     | U/L  |       | 12.5    | 32.9    | 30.7       |    |
| GGT     | U/L  |       | 6.30    | 37.7    | 104       |    |
| GLU     | mmol/L |       | 3.93    | 13.1    | 4.66       |    |
| HPT     | g/L  |       | 5.21    | 55.7    | 2.06       |    |
| hSfTnT  | ng/L  |       | 7.08    | 84.8    | 115       |    |
| IGA     | g/L  |       | 5.91    | 16.1    | 1.92       |    |
| IGG     | g/L  |       | 4.41    | 13.2    | 7.61       |    |
| IGM     | g/L  |       | 7.64    | 18.1    | 0.89       |    |
| IRON    | µmol/L |       | 10.2    | 74.2    | 14.0       |    |
| K       | mmol/L |       | 4.17    | 13.4    | 3.78       |    |
| LDH     | U/L  |       | 5.22    | 89.6    | 502       |    |
| LIP     | U/L  |       | 7.44    | 24.4    | 63.8       |    |
| LH      | U/L  |       | 12.9    | 65.0    | 16.3       |    |
| Mg      | mmol/L |       | 5.99    | 11.6    | 0.71       |    |
| Na      | mmol/L |       | 3.07    | 3.49    | 141       |    |
| NTPBNP  | ng/L  |       | 8.66    | 29.0    | 1788       |    |
| PALB    | g/L  |       | 11.9    | 32.5    | 0.18       |    |
| PHOS    | mmol/L |       | 4.78    | 23.1    | 1.09       |    |
| PROG    | µg/L  |       | 19.8    |        |           |    |
| TG      | mmol/L |       | 6.79    | 55.6    | 1.33       |    |
| TP-pl   | g/L  |       | 3.43    | 8.36    | 56.5       |    |
| TRF     | g/L  |       | 7.60    | 11.3    | 2.22       |    |
| UA      | µmol/L |       | 4.37    | 24.2    | 240       |    |
| UR      | mmol/L |       | 7.08    | 34.6    | 6.80       |    |

*ACL, Analytical change limit = 1.96*√2*(CVa; RCV, Reference change value = 1.96*√2*(CVa² + CVw²); Baseline pool concentration (non-hemolyzed); Roche hemolysis cut-offs (package inserts). % Δ, percentage of variation; CVa, between-run imprecision; CVw, average within-subject variation; (D), Decreased; HI, hemolysis index; (I), Increased; Min, minimum; Max, maximum; nd, not determined; ns, not significant; Pl, plasma; Se, serum.
of interference. Smoothing spline model was used for mean regression curves, using SigmaPlot® software (Systat Software Inc., USA), which allows to locate precisely the x- (HI, as Hb concentrations, in mg/dL) and y- (± change %) coordinates on the curves. The HI corresponding to the 10% changes (± 10%Δ) were considered as significant [14]. In order to define hemolysis allowable limits based on analytical variation, we determined HI corresponding to the analytical change limit (ACL) equal to 1.96*√2*CVa, wherein 1.96*√2 corresponds to the standard deviation for the bidirectional probability of change fixed at 95%, and CVa to the inter-assay imprecision, as previously described [15]. For each parameter, CVa was calculated from Roche or Biorad Quality Control values, with the closest concentrations to those of the baseline pool, and collected over a 6-month period. Moreover, we determined the allowable HI which takes into account both analytical plus within-individual biological variations, namely the reference change value (RCV) [16], and equal to 1.96*√2/(CVa2 + CVw2), wherein CVw is the average within-subject variation listed by Ricós et al. [17].

**Determination of the relative frequency distribution of tests per degree of hemolysis**

(a) For each parameter, plasma or serum HI of samples from hospitalized patients were extracted using the MPL®evo middleware (Roche Diagnostics), in order to calculate the relative frequency distribution (% RFD) of hemolyzed tests performed over a 25-day period, i.e. exceeding the three cut-offs: > 10%Δ, > ACL and > RCV.

(b) For each parameter, we tested the hypothesis that ACL- or RCV-related % RFD were equal to the 10%Δ-based % RFD, using the one proportion Z-test (MedCalc® Software, Belgium) in which ACL- or RCV-related % RFD were the observed proportions, and considering that this hypothesis is rejected if the p-value is less than 0.05.

**Results**

The Hb baseline concentrations of the overloaded plasma and serum specimens range were 6.04 and 8.94 mg/dL, respectively. The baseline pools were considered as free of interferences, regarding their total bilirubin (TBIL) and triglyceride (TG) concentrations: TBIL = 4.50 and 6.50 μmol/L, TG = 1.33 and 0.98 mmol/L for plasma and serum, respectively. The Hb concentrations and corresponding visual aspects are given in Supplementary Figure A (available online at http://informahealthcare.com/doi/abs/10.3109/00365513.2014.993691).

**Determination of hemolysis indexes giving significant change of concentration**

Table I lists all the analytes, their baseline pool concentration (BPC), their minimum and maximum % changes over all the range, and the HI recommended by Roche Diagnostics, as well as those determined for the 10%Δ, ACL and RCV of concentration. Considering the ± 10%Δ as maximum allowable bias, the analytes whose concentration was highly enhanced by hemolysis were the following (Figure 1A), in decreasing order of intensity: LDH, AST, FOL, K, and CK (+ 10%Δ HI = 32, 42, 56, 121 and 135 mg/dL, respectively). Those slightly increased by hemolysis were: PHOS, IRON, ALT, LIP, MCA and TG (+ 10%Δ HI = 251, 258, 264, 422, 425 and 432 mg/dL, respectively), as shown in Figure 1B. Conversely, the analytes whose concentration decreased with hemolysis were (Figure 1C): HPT, hsTnT, and ALP (− 10%Δ HI = 169, 282 and 416 mg/dL, respectively).

Considering the ACL percentage as maximum allowable bias, some additional analytes varied significantly for very high level of hemolysis (Table I): CHOL, Cl, Na and TP-pl, whereas only four analytes remained sensitive to hemolysis when considering RCV: AST, K, LDH and Na.

**Determination of the relative frequency distribution of tests per degree of hemolysis.**

As shown in Table II, over a 25-day period, the % RFD of tests for which a change in concentration due to hemolysis is greater than 10% were: 6.79% for LDH, 4.30% for FOL, 4.03% for AST, 1.16% for IRON, and <1% for all other analytes.

By comparing ACL-based % RFD to 10%Δ-based % RFD (Table II), the proportions of significant hemolized samples were much higher for AST, CK, K, LDH, PHOS (p<0.0001), slightly higher for LIP and MCA (p<0.05), and significantly lower for FOL (p<0.0001).

Compared to the 10%Δ-based % RFD, the RCV-based % RFD were significantly decreased only for AST, K, LDH (p<0.001).

**Discussion**

For all the biochemical tests and immunoassays, the 10%Δ-based HI were close to those provided by the manufacturer, as well as to those corresponding to the ACL. Nevertheless, the ACL-based HI appear more restrictive, and differ substantially for some analytes. Using the combined analytical plus within-subject variations as allowable limits (RCV), most analytes known to be sensitive to hemolysis are no longer considered as such.
The three main mechanisms of interference from hemolysis are (i) Additive interference of released intracellular substances added to plasma or serum measurement (e.g. LDH, AST, K), (ii) spectral interference due to released hemoglobin (e.g. ALP, GGT), and (iii) chemical interference when intracellular substances interact with the measured analyte (e.g. CK) [18].

In Roche Diagnostics package inserts, the criteria of limit acceptance used to consider an absence of hemolysis interference is defined at ±10% from the baseline value free of interference, given as Hb concentration in mg/dL, i.e. the HI (Table I). Many of these Roche HI were based on the results from Glick et al., published in 1986 and assessed for serum on Hitachi® 705 and 737 chemistry analyzers (Boehringer, Mannheim Diagnostics, USA) [19]. Overall, our results showed similar hemolysis-linked variations of concentrations to those announced by Roche or those previously published [7,8], i.e. an increase for LDH, AST, FOL, K, CK, PHOS, IRON, ALT, LIP, Mg, TG, and a decrease for HPT, hsTnT and ALP, in descending order. However, our test showed no decrease due to hemolysis for GGT activity up to 451 mg/dL of hemoglobin, unlike Roche recommendation from package insert (200 mg/dL), but in accordance with the result from Ji and Meng (601 mg/dL) [7,8]. In the same way, our results show a slight but not significant decrease in CO2 concentration caused by hemolysis (−7.84%), in accordance with the Roche cut-off (799 mg/dL), but in contrast with that from Ji and Meng (130 mg/dL). Although an overall tendency towards agreement, our 10%Δ-based HI still differed substantially from those of Roche for ALT (264 vs. 60 mg/dL, respectively) and hsTnT (282 vs. 100 mg/dL, respectively). These two parameters are less sensitive to hemolysis in our experience than announced by Ji and Meng, who had found HI close to ours for ALT and TnT assayed on Roche Cobas 6000 system (235 and 290 mg/dL, respectively) [7]. Snyder et al. also indicate a negative interference of hemolysis on TnT (−10%), for a HI close to our result (250 vs. 282 mg/dL, respectively) [20], whereas Florkowski et al. found a stronger negative interference for hsTnT (−14 to −16% at 132 mg/dL Hb) [21]. Some other discrepancies were found between our 10%Δ-based HI results, Roche cut-offs and Ji and Meng values, in particular for CK (135, 100 and 400 mg/dL, respectively), IRON (258, 201 and 40 mg/dL, respectively), and K (121, 100 and 200 mg/dL, respectively), underlining the need to standardize protocols of hemolysis interference determination.

Using the ACL as significant limit, our HI results appeared lower than the 10%Δ-based ones, especially for CK (56 vs. 135 mg/dL, respectively), K (55 vs. 121 mg/dL, respectively), LDH (18 vs. 32 mg/dL, respectively) and PHOS (108 vs. 251 mg/dL, respectively), but contrary to FOL (155 vs. 56 mg/dL, respectively) because of its increased CVa (7.28%) giving a high ACL at 20.2. Moreover, by using ACL-based HI, our results showed that CHOL and TP-pl concentrations increase with hemolysis, and could be considered as more sensitive to this interference (280
Table II. Relative frequency distribution of hemolyzed samples for tests performed over a 25-day period, according to significant change limits.

| Analyte | n\textsuperscript{a} | HI ≥ 10%Δ | % RFD | HI ≥ ACL | Z\textsuperscript{b} | p | HI ≥ RCV | Z\textsuperscript{b} | p |
|---------|----------------|-----------|------|---------|---------|----|---------|---------|----|
| ALP     | 6163            | ≥ 416     | 0.065 | ≥ 374   | 0.097   | 1.00 | 0.317   | ns      |     |
| ALT     | 8520            | ≥ 264     | 0.153 | ≥ 248   | 0.153   | 0.00 | 1.00    | ns      |     |
| AST     | 8508            | ≥ 42      | 0.403 | ≥ 31    | 7.28    | 15.2 | < 0.0001 | ≥ 130  | 0.540  | 16.4 | < 0.0001 |
| CHOL    | 1585            | ns        |      | ≥ 280   | 0.126   | ns   |         |         |     |
| CK      | 3145            | ≥ 135     | 0.668 | ≥ 56    | 3.05    | 16.4 | < 0.0001 | ns      |     |
| Cl      | 13962           | ns        |      | ≥ 351   | 0.079   | ns   |         |         |     |
| FOL     | 837             | ≥ 56      | 4.30  | ≥ 155   | 0.956   | 4.77 | < 0.0001 | ns      |     |
| HPT     | 1250            | ≥ 169     | 0.320 | ≥ 142   | 0.480   | 1.00 | 0.317   | ns      |     |
| hsTnT   | 1646            | ≥ 282     | 0.365 | ≥ 232   | 0.365   | 0.00 | 1.00    | ns      |     |
| IRON    | 1292            | ≥ 258     | 1.16  | ≥ 258   | 1.16    | ns   |         |         |     |
| K       | 14535           | ≥ 121     | 0.578 | ≥ 55    | 2.35    | 28.1 | < 0.0001 | ≥ 169  | 0.351  | 3.61 | 0.0003 |
| LDH     | 3095            | ≥ 32      | 6.79  | ≥ 18    | 21.8    | 33.2 | < 0.0001 | ≥ 242  | 0.194  | 14.6 | < 0.0001 |
| LIP     | 1929            | ≥ 422     | 0.052 | ≥ 290   | 0.156   | 2.00 | 0.045   | ns      |     |
| Mg      | 3968            | ≥ 425     | 0.025 | ≥ 346   | 0.076   | 2.00 | 0.046   | ns      |     |
| Na      | 14534           | ns        |      | ≥ 361   | 0.069   | ≥ 416 | 0.041  | na      | na     |
| PHOS    | 5860            | ≥ 251     | 0.154 | ≥ 108   | 0.734   | 11.3 | < 0.0001 | ns      |     |
| TG      | 1582            | ≥ 432     | 0.000 | ≥ 319   | 0.063   | na   | na      | na      |     |
| TP-pl   | 13927           | ns        |      | ≥ 238   | 0.151   | ns   |         |         |     |

\*Total number of tests performed over a 25-day period; \textsuperscript{b} one proportion Z-test testing the hypothesis that ACL- or RCV-based % RFD were equal to the 10%Δ-based % RFD (rejected hypothesis if \( p < 0.05 \)). % RFD, relative frequency distribution; ACL, analytical change limits; RCV, reference change value; HI, hemolysis-index (in mg/dL); na, not applicable; ns, not significant.

and 238 mg/dL, respectively) than Roche cut-offs (701 and 651 mg/dL, respectively) or Ji and Meng values (601 and 741 mg/dL, respectively). Since the ACL calculation depends from inter-assay imprecision (CVa), the lower the CVa, the more restrictive the ACL, as shown for Cl and Na, proved to decrease with severe hemolysis (351 and 361 mg/dL, respectively) whereas both are usually considered as not affected by this interference [8]. Recently, Dolci and Panteghini have suggested establishing the more appropriate allowable hemolysis-related bias based on analytical CV plus within-subject biological variation (i.e. RCV), rather than the common but arbitrary >10% cut-off [9]. In our study, by applying this RCV clinical approach, only four analytes were still considered as sensitive to hemolysis, with unexpected high values: AST (130 mg/dL), K (169 mg/dL), LDH (242 mg/dL) and Na (416 mg/dL). These unsuitable values were mainly due to the large average CVw provided by Ricos et al. considered for serial measurements and applicable at the individual scale. In laboratory practice, the ACL-based HI would be preferred as acceptance limit for hemolysis interference, for a given test, since it reflects the real field conditions, insofar as it is based on analytical variation including variations due to different calibrations, changes in reagent lots, maintenances of the analyzer, and conditions of temperature and humidity.

Regarding the RFD per day, on a total mean of 4254 daily measurements, all tests taken together, only 30 measurements (0.70%) would be considered as hemolyzed using the 10%Δ-based HI as significant cut-off, 75 measurements (1.77%) using the ACL-based HI, and 5 measurements using the RCV-based HI (0.12%). It seems that the use of the ACL-based cut-offs would be realistic, suitable in laboratory and safer for patients, whereas the RCV-based ones could be probably too permissive and potentially at risk for patients. Indeed, despite significantly higher percentages of hemolyzed samples for almost all parameters when using ACL-based HI compared to 10%Δ-based HI, the daily total percentage still remained acceptable compared to the 3.3% previously published [4]. Consequently, depending on the parameter, the ACL-based HI could be used as flag cut-off for comment to clinicians such as ‘Result increased or decreased by hemolysis interference’ along with the reported result, for example. Since less restrictive, the 10%Δ-based HI could be retained as decision cut-off for not reporting the hemolysis-impacted result, which could be replaced by a comment such as ‘Hemolyzed specimen’ as previously proposed [9,22]. Depending on the importance of the parameter in certain critical situations such as intensive care contexts, a %Δ-based HI giving a variation more than 10% could be still informative for diagnosis, and thus should be discussed with clinicians even if it should not be reported. For this purpose, we provide the detailed graphs of percentage changes of analyte concentrations according to the hemolysis interference degree (Supplementary Figure B, available online at http://informahealthcare.com/doi/abs/10.3109/00365513.2014.993691).

**Limits of the study**

In our study, the maximum HI was 451 mg/dL whereas the CLSI recommends testing up to 1000
mg/dL [23]. However, we preferred to focus our attention in the low hemolysis range in order to define the indexes with a high precision. Among all the measurements assayed over the 25-day period, all analytes sensitive to hemolysis taken together, less than 0.1% of tubes presented a HI > 451 mg/dL (i.e. 18/19832). Another limit of this study is that the hemolysis interference has been assessed only for one single level of concentrations, generally normal ones, whereas the CLSI recommends to test at least two medical decision concentrations [10]. Nevertheless, such recommendation requires selecting too many samples with appropriate levels of concentrations, i.e. below, within or above normal ranges depending on each analyte, which is very difficult to achieve for a large panel of analytes in routine laboratory. The analytes varying significantly with hemolysis in the present study could be reassessed according to such CLSI recommendation in a subsequent study, in particular for total and direct bilirubin (TBIL and DBIL). Indeed, since the baseline plasma pool required is free of icterus interference, i.e. with very low concentrations for these two parameters, a very slight increase or decrease of concentration inevitably results in a high coefficient of variation: from 4.5–6.0 μmol/L for mean plasma TBIL concentration (CV + 33.3%) and from 2.75–0.00 μmol/L for mean plasma DBIL concentration (CV – 100%) in the present study. Both these variations due to spectral interference of hemoglobin have been previously described on Roche Cobas 6000 analyzer, but without providing concentration levels, unfortunately [7]. Finally, one must remember that the mechanism of experimental hemolysis by osmotic shock and freezing is different from hemolysis causes in clinical practice [5,6], and that the protocol used in the present interference study did not allow distinguishing the effects of hemoglobin from those of released erythrocytic, leukocytic and trombocytic constituents.

Conclusion
The present study provides new hemolysis indexes reassessed in detail for a large panel of biochemical tests and immunoassays using Roche Diagnostics analyzers. In routine laboratory practice, the percentage of tests considered as hemolyzed varies significantly depending on the chosen allowable limits. This work aims to enrich current data by providing benchmarks for a future harmonization of interference hemolysis evaluations.

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Supplementary material available online

Supplementary Table A and Supplementary Figures A and B.