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Handling of hemolyzed serum samples in clinical chemistry laboratories: the Nordic hemolysis project

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Abstract

Background: Some clinical chemistry measurement methods are vulnerable to interference if hemolyzed serum samples are used. The aims of this study were: (1) to obtain updated information about how hemolysis affects clinical chemistry test results on different instrument platforms used in Nordic laboratories, and (2) to obtain data on how test results from hemolyzed samples are reported in Nordic laboratories.

Methods: Four identical samples containing different degrees of hemolysis were prepared and distributed to 145 laboratories in the Nordic countries. The laboratories were asked to measure the concentration of cell-free hemoglobin (Hb), together with 15 clinical chemistry analytes. In addition, the laboratories completed a questionnaire about how hemolyzed samples are handled and reported.

Results: Automated detection of hemolysis in all routine patient samples was used by 63% of laboratories, and 88% had written procedures on how to handle hemolyzed samples. The different instrument platforms measured comparable mean Hb concentrations in the four samples. For most analytes, hemolysis caused a homogenous degree of interference regardless of the instrument platform used, except for alkaline phosphatase (ALP), bilirubin (total) and creatine kinase (CK). The recommended cut-off points for rejection of a result varied substantially between the manufacturers. The laboratories differed in how they reported test results, even when they used the same type of instrument.

Conclusions: Most of the analytes were homogeneously affected by hemolysis, regardless of the instrument used. There is large variation, however, between the laboratories on how they report test results from hemolyzed samples, even when they use the same type of instrument.

Keywords: analytical interference; hemoglobin; hemolysis; laboratory errors; preanalytical phase.

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Introduction

Hemolysis of blood samples is a pre-analytical challenge that often leads to sample rejection in medical laboratories [1]. Hemolysis occurs when blood cells break down and the intracellular contents leak into the surrounding fluid [2]. When hemolyzed blood samples are analyzed in medical laboratories, the content released from the blood cells can interfere with the measurement procedure, leading to erroneous results that may not reflect the patient's clinical condition. How, and to what extent, hemolysis may affect test results depends on the analyte and the measurement method used [1].

Interference studies are performed to establish how much the various analytes, when measured by different methods, will be affected by hemolysis [3]. Based on the

results, instrument-specific cut-off points are determined to prevent hemolysis from significantly affecting the clinical interpretation of laboratory results. Most instruments used in medical laboratories today can measure cell-free hemoglobin (Hb) in individual blood samples and transfer the result to the laboratory information system (LIS). These Hb results may be combined with predefined Hb cut-off points, enabling the laboratories to automatically reject or comment upon test results significantly affected by hemolysis [4].

Cut-off points for rejection of samples are commonly recommended by the manufacturers of *in vitro* diagnostic (IVD) analytical systems. The Clinical and Laboratory Standards Institute (CLSI) recommends that the laboratories verify the intended usefulness, strengths and limitations of manufacturer-derived cut-off points before they are implemented [5]. This is time and resource consuming for the laboratory, and may be difficult as manufacturers' package inserts often lack information about experiment design and how the cut-off points were defined [6–8]. Consequently, many laboratories use the manufacturers' cut-off points for hemolysis, without further verification studies [8].

The Nordic cooperation of External Quality Assurance (EQA) organizers, EQAnord, performed a large interference study in 2002 to obtain data on the effect of hemolysis on analytical performance on different clinical chemistry instruments [9]. The aims of the current study were (1) to obtain updated information about how hemolysis affects clinical chemistry test results on different instrument platforms, and (2) to obtain data on how test results from hemolyzed samples are reported in medical biochemistry hospital laboratories in the Nordic countries.

Materials and methods

Preparation of samples

The blood was collected from eight healthy donors with identical ABO and RhD group at the Haukeland University Hospital blood bank, Bergen, Norway. The donors had provided informed written consent to the donation of blood. Ethical approval was not required for this quality assurance survey [10]. One Fenwal blood bag containing no anticoagulant (Fenwal Laboratories, Deerfield, IL, USA) and one lithium-heparin blood tube (BD Vacutainer Lithium-Heparin [17 IU/mL], BD, Plymouth, UK) were drawn from each blood donor. The blood bags were centrifuged after 3 h clotting time at room temperature. After centrifugation, serum from all donors was mixed and divided into four pools.

The hemolysate added was prepared with lithium-heparin blood from the blood donors, stored for 2 h at −80 °C. The tubes were thawed for 1 h at 21 °C and centrifuged for 10 min at 1300 *g* at 21 °C. The top layers from the eight tubes were mixed and used as hemolysate [11, 12]. The Hb concentration in the hemolysate and the four identical samples distributed to the participants was measured using a point-of-care instrument HemoCue Hb 201+ (HemoCue, Ängelholm, Sweden). The instrument has an analytical coefficient of variation (CV) of 0.5% and is monitored twice a year by external quality controls. The Hb concentration in the hemolysate was 140.5 g/L (n = 2). Sample 1 had no hemolysate added (baseline sample, mean Hb: 0.3 g/L, n = 3). Sample 2 had 2.8 mL hemolysate added to 394 mL serum corresponding to a calculated Hb concentration of approximately 1 g/L (mean Hb: 1.3 g/L, n = 3), Sample 3 had 5.7 mL hemolysate added to 391 mL serum corresponding to a calculated Hb concentration of approximately 2 g/L (mean Hb: 2.3 g/L, n = 3) and Sample 4 had 11.3 mL hemolysate added to 386 mL serum corresponding to a calculated Hb concentration of approximately 4 g/L (mean Hb: 5.0 g/L, n = 3).

The four samples containing different degrees of hemolysis were distributed with cooling elements to the various EQA organizations with express shipment. Further shipment to the various laboratories was performed at ambient temperature.

Recruitment of participants and sample analysis

Invitation to participate in the study was sent to laboratories participating in clinical chemistry EQA-schemes in the Nordic countries by the local EQA organizations; the Danish Institute for External Quality Assurance for Laboratories in Health Care (DEKS), the External Quality Assurance in Laboratory Medicine in Sweden (Equalis), Icelandic Society for Clinical Biochemistry and Laboratory Medicine (ISLM), Labquality and the Norwegian Organization for Quality Improvement of Laboratory Examinations (Noklus). A total of 294 Nordic medical biochemistry laboratories were invited. Of these, 145 agreed to participate and were sent four samples containing different degrees of hemolysis in November 2014. The laboratories were asked to measure and report the concentration of Hb and the following 15 clinical chemistry analytes: alkaline phosphatase (ALP), bilirubin (total), calcium, chloride, cobalamin, creatine kinase (CK), folate, free thyroxine (FT4), γ-glutamyltransferase (GGT), glucose, lactate dehydrogenase (LDH), potassium, sodium, thyroid-stimulating hormone (TSH) and uric acid.

All the laboratories were asked to analyze the samples in duplicate, on the fourth day after preparation of the samples, and report the mean value of each analyte. In addition, the laboratories completed a questionnaire about their procedures for handling and reporting hemolyzed samples, including two case studies (Figure 1).

Data analysis

Analyte concentration in Sample 1 (i.e. without hemolysis) was used as the baseline concentration. For each analyte, percent change (bias%) was calculated according to the formula: bias% = 100 * (measured concentration − baseline concentration)/baseline concentration, and a 95% confidence interval (CI) for the bias% was calculated. If the 95% CIs of bias% of two instrument groups did not overlap, the difference was considered statistically significant.

Question 1A: If Sample 2 was analyzed in your laboratory, which action would you take for the following analytes.

Question 1B: Please answer this question if you chose to reject some of the results in question 1A: Imagine that Sample 2 was from a 2-year-old child admitted to the Oncologic Department in your hospital. Thirty minutes after rejecting some of the results do you receive a call from the requesting physician who asks for the non-reported results, arguing that the sampling was extremely difficult and claims that it is not possible to get a new sample. Please specify below which ADDITIONAL results (if any) you would report.

Question 2A: If Sample 3 was analyzed in your laboratory, which action would you take for the following analytes.

Question 2B: Please answer this question if you chose to reject some of the results in question 2A: Imagine that Sample 3 was from a 60-year-old man who was admitted to the emergency department with acute chest pain. Ten minutes after rejecting some of the results do you receive a call from the requesting physician who asks for the non-reported results, arguing that rapid results are very important for further treatment of the patient. Please specify below which ADDITIONAL results (if any) you would report.

Figure 1: Case studies.

The laboratories were asked to report the concentration of Hb in either a quantitative (g/L) or a semi-quantitative value (e.g. +/++/+++, etc.), as applicable. All semi-quantitative Hb results were converted into quantitative values for further calculations. For example, if a laboratory with Beckman Coulter AU reported ++, which equals 1.00–2.00 g/L [13], the Hb result was converted into the mean value of the corresponding interval; i.e. 1.50 g/L. It was defined as "handled in accordance with manufacturer's cut-off" if the laboratory reported or reported with comment when the measured Hb value was below the manufacturer's cut-off point or if the laboratory rejected or rejected with comment when the measured Hb value was above the manufacturer's cut-off point. Manufacturer's cut-off points were stated by the manufacturers in personal communications to the authors, however, the bias corresponding to the cut-offs and consequently regarded as acceptable by the manufacturers, were not communicated. To check for consistency regarding the magnitude of bias regarded acceptable by manufacturers, we calculated the Hb concentrations giving a 10% bias for three analytes; CK, LDH and potassium, and investigated whether the manufacturers' recommended cut-off points for rejection of a test result corresponded to the 10% bias. A 10% bias was chosen as example as this value is commonly used as a cut-off point by the manufacturers of IVD analytical systems [14]. The concentration of Hb giving a bias of 10% was calculated using simple linear regression. Linearity was verified with plots and R^2.

Data analyses were performed using Excel 2010.

Results

Procedures for detecting and handling hemolysis

Of the 294 laboratories invited to take part, 143 (49%) responded to the survey. The response rate in Denmark was 67% (32/48), Finland 22% (25/112), Iceland 100% (4/4), Norway 95% (53/56) and Sweden 39% (29/74) (see Supplementary Table 1 for distribution of instrument platforms). Of these, 122 (85%) performed automated measurement of the Hb concentration in the four blood samples received and reported a quantitative or semi-quantitative Hb concentration. The laboratories were asked in the questionnaire how they investigate and detect interference by hemolysis in routine samples. Ninety laboratories (63%) stated that they measure Hb routinely in all patient samples; 88% (28/32) in Denmark, 69% (20/29) in Sweden, 57% (30/53) in Norway, 44% (11/25) in Finland and 25% (1/4) in Iceland. Manual inspection to detect hemolysis was performed by 22 laboratories (15%), whereas 9% used manual inspection, followed by automated detection if hemolysis was suspected (Figure 2). Eighty-eight percent of the laboratories had written procedures for how to handle hemolyzed samples; 100% (32/32) in Denmark, 96% (28/29) in Sweden, 88% (22/25) in Finland, 81% (43/53) in Norway and 25% (1/4) in Iceland.

Influence of hemolysis on analyte concentration

Clinical chemistry measurements in the four identical samples were carried out on 11 different instruments, produced by seven manufacturers; Abbott Architect (n = 23)

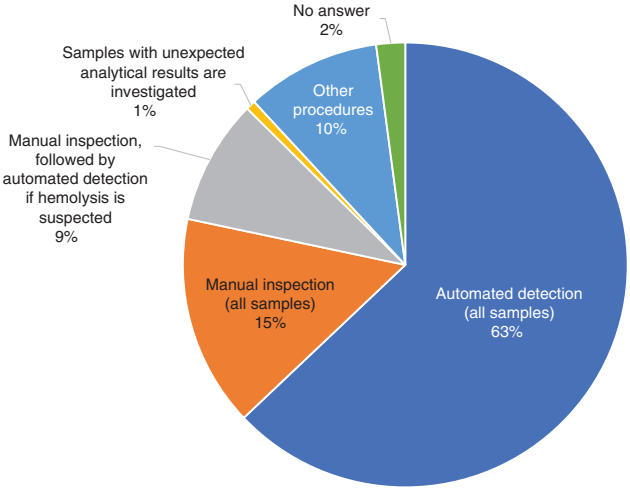

Figure 2: How laboratories investigate and detect hemolysis interference in patient samples received for analysis.

(Abbott Laboratories, Abbott Park, IL, USA), Beckman Coulter AU (n = 5) (Beckman Coulter, Brea, CA, USA), Beckman Coulter UniCel (n = 4) (Beckman Coulter, Brea, CA, USA), Ortho Vitros (n = 15) (Ortho Clinical Diagnostics, Raritan, NJ, USA), Perkin Elmer AutoDelfia (n = 1) (Perkin Elmer, Turku, Finland), Roche Cobas (n = 52) (Roche Diagnostics, Mannheim, Germany), Roche Integra (n = 3) (Roche Diagnostics, Mannheim, Germany), Roche Modular (n = 8) (Roche Diagnostics, Mannheim, Germany), Siemens Advia (n = 17) (Siemens Healthcare Diagnostics, Deerfield, IL, USA), Siemens Dimension (n = 10) (Siemens Healthcare Diagnostics, Deerfield, IL, USA) and Thermo Scientific Konelab (n = 5) (Thermo Scientific, Vantaa, Finland).

Hb was measured on the same clinical chemistry instruments as the other 15 analytes and the mean Hb concentrations were in accordance with the theoretical Hb concentrations calculated from the amount of Hb added to the sample material (see Methods section); Sample 1 (n = 122): 0.02 g/L (95% CI −0.03, 0.07), Sample 2: 1.19 g/L (95% CI 0.64, 1.74), Sample 3: 2.17 g/L (95% CI 1.08, 3.26) and Sample 4: 4.18 g/L (95% CI 3.36, 5.00).

Table 1 shows the mean Hb concentrations and the average effect of hemolysis on test results (bias%) for Sample 2 and 3 for each of the 15 analytes for instrument groups with more than five participants. The interference caused by hemolysis on the mean test results for the different instruments was statistically significantly different for ALP, bilirubin (total) and CK in samples with Hb 1 g/L and 2 g/L (Table 1, Figure 3A, Supplementary Figure 1A and B). As an example, Sample 3 had a hemolysis of 2 g/L, which would cause the CK result to increase between 1.5% (Abbott Architect) and 25.6% (Siemens Dimension). For LDH, Ortho Vitros showed significantly lower concentrations compared to the other instrument groups (Figure 3B). The results for all instruments and all samples are shown in Supplementary Table 2. Interferographs for all analytes are shown in Figure 3 and Supplementary Figure 1.

Cut-off points recommended by the manufacturers

Even though hemolysis affected most analyte results to the same degree throughout different instrument platforms, the recommended cut-off points for rejection of a result varied substantially between the manufacturers (Supplementary Table 3). Using potassium as example, the bias from baseline in Sample 2 (i.e. 1 g/L) varied between 6.7% (Roche Modular) to 7.3% (Ortho Vitros) (Figure 3C). Simultaneously did the manufacturers' recommended cut-off points for rejection of a test result vary from 0 (avoid all hemolyzed samples) (Siemens Advia/Dimension) to 1.25 (Abbott Architect).

Hb concentrations giving a 10% bias were calculated across instrument platforms for CK, LDH and potassium. The manufacturers' recommended cut-offs did commonly not coincide with the Hb concentration causing 10% bias as calculated by linear regression (Tables 2–4). Using LDH as example, Hb concentrations between 0.12 and 0.18 g/L correspond to a 10% bias on the different instrument platforms (Table 3), while the manufacturers' recommended cut-off points varied from 0 (i.e. avoid all hemolyzed samples; Abbott Architect, Siemens Advia and Dimension) to 1.00 g/L (Beckman Coulter). Linearity for CK, LDH and potassium was verified with plots and R^2 and found good ($R^2 > 0.95$).

How do laboratories handle results affected by hemolysis?

The laboratories report in the questionnaire that they would take some kind of action due to hemolysis in 1%–2% of routine samples. The proportion of samples handled in accordance with the manufacturers' cut-off points (see Table 5) varied between 65% (folate) and 100% (FT4, GGT and TSH) in Sample 2 (Hb concentration 1 g/L). For Sample 3 (Hb concentration 2 g/L), the proportion varied between 59% (CK) and 98% (TSH). Tables 2–4 show that even laboratories using the same instrument platform would take very different actions, for CK, LDH and potassium, upon receiving identically hemolyzed samples. For example, all but two Roche Cobas instruments (48 out of 50) measured a Hb concentration above 2 g/L in Sample 3, which exceeds the manufacturer's recommended rejection limit when measuring CK. Table 2 shows, however, that the laboratories could take any of the following actions; report (n = 7), report with comment (n = 12), reject (n = 5) and reject with comment (n = 24). Sample 3 would cause the LDH result to increase between 114.8% and 152.3%, depending on the instrument used (Figure 3B). Twelve out of 122 laboratories (10%) would report this LDH concentration (Table 3), and comment on the result. The degree of hemolysis in Sample 2 (Hb concentration 1 g/L) would increase the potassium result by a maximum of 7.3% (Figure 3C), and 88 laboratories (72%) would reject the test result or reject the test result with comment (Table 4).

Laboratories that initially rejected the result due to hemolysis in the sample, would only to some extent release the result after a call from the requesting physician (Table 6). One-hundred of the 143 responding

Table 1: Measured Hb (with 95% CI) and the effect of Hb (bias%) with 95% CI on test results for 15 analytes in Sample 2 with Hb 1 g/L and Sample 3 with Hb 2 g/L.

Analyte	Sample 2: Hb 1 g/L						Sample 3: Hb 2 g/L					
	Abbott Architect	Ortho Vitros	Roche Cobas	Roche Modular	Siemens Advia	Siemens Dimension	Abbott Architect	Ortho Vitros	Roche Cobas	Roche Modular	Siemens Advia	Siemens Dimension
	n=23	n=15	n=52	n=8	n=17	n=10	n=23	n=15	n=52	n=8	n=17	n=10
Hb, g/L	**M=1.10**	**M=1.09**	**M=1.09**	**M=1.00**	M=++	M=++++	**M=2.10**	**M=2.03**	**M=2.10**	**M=1.96**	M=+++	M=++++
	(0.83, 1.38)	(0.75, 1.43)	(1.00, 1.17)	(0.90, 1.11)	1.57	1.25	(1.66, 2.53)	(1.33, 2.74)	(1.93, 2.28)	(1.73, 2.18)	2.93	1.45
					(0.48, 2.66)	(1.21, 1.28)					(0.81, 5.05)	(0.50, 2.39)
ALP	**−1.6%[a]**	**−15.1%[a]**	**−3.0%[a]**	**−2.5%[a]**	**−5.7%**	**−1.8%[a]**	**−5.0%[a]**	**−17.8%[a]**	**−11.7%**	**−9.2%**	**−18.1%[a]**	**−3.6%[a]**
Mb=58 U/L	(−5.4, 2.2%)	(−21.6, −8.6%)	(−7.0, 1.0%)	(−4.2, −0.8%)	(−10.2, −1.2%)	(−6.1, 2.5%)	(−9.4, −0.6%)	(−27.3, −8.3%)	(−19.3, −4.1%)	(−11.8, −6.6%)	(−25.6, −10.6%)	(−8.1, 0.9%)
Bilirubin (total)	**−1.0%[a]**	**45.9%[a]**	**1.5%**	**5.2%**	**−6.4%[a]**	**12.8%**	**−3.8%[a]**	**112.3%[a]**	**1.2%[a]**	**−1.4%[a]**	**−3.9%[a]**	**5.0%[a]**
Mb=8 µmol/L	(−5.8, 3.8%)	(9.7, 82.1%)	(−10.4, 13.4%)	(−12.4, 22.8%)	(−17.3, 4.5%)	(−2.6, 28.2%)	(−9.6, 2.0%)	(46.5, 178.1%)	(−13.1, 15.5%)	(−4.8, 2.0%)	(−13.1, 5.3%)	(−4.7, 14.7%)
Calcium	**−0.2%**	**0.9%**	**−0.3%**	**0.4%**	**0.0%**	**0.3%**	**−0.8%**	**0.0%**	**−0.6%**	**1.2%**	**−0.3%**	**−0.2%**
Mb=2.30 mmol/L	(−1.0, 0.6%)	(−0.2, 2.0%)	(−1.9, 1.3%)	(−2.2, 3.0%)	(−3.6, 3.6%)	(−3.9, 4.5%)	(−2.5, 0.9%)	(−1.5, 1.5%)	(−2.6, 1.4%)	(−2.1, 4.5%)	(−3.52, 2.9%)	(−4.1, 3.7%)
Chloride	**0.1%**	**0.7%**	**−0.5%**	**−1.0%**	**−0.8%**	**−0.3%**	**0.0%**	**0.5%**	**−0.6%**	**−0.7%**	**−0.9%**	**−0.1%**
Mb=102.5 mmol/L	(−1.0, 1.2%)	(−0.4, 1.8%)	(−1.7, 0.7%)	(−1.3, −0.7%)	(−1.9, 0.3%)	(−2.9, 2.3%)	(−1.1, 1.1%)	(−0.6, 1.6%)	(−1.8, 0.6%)	(−2.3, 0.9%)	(−2.0, 0.2%)	(−4.0, 3.8%)
Cobalamin	**−1.0%**		**0.2%**	**1.1%**		**3.3%**	**−2.8%**		**−0.6%**	**1.9%**		**5.4%**
Mb=359 pmol/L	(−9.7, 7.7%)		(−6.5, 6.9%)	(−1.4, 3.6%)		(−0.2, 6.8%)	(−11.3, 5.7%)		(−9.7, 8.5%)	(−1.7, 5.5%)		(1.1, 9.7%)
CK	**0.6%[a]**	**0.6%**	**3.7%**	**5.4%**	**6.9%**	**13.0%[a]**	**1.5%[a]**	**5.4%[a]**	**9.0%[a]**	**13.1%[a]**	**16.3%[a]**	**25.6%[a]**
Mb=128 U/L	(−1.3, 2.5%)	(−4.7, 5.9%)	(0.7, 6.7%)	(0.4, 10.4%)	(3.5, 10.3%)	(4.2, 21.8%)	(−1.2, 4.2%)	(0.6, 10.2%)	(4.4, 13.6%)	(4.6, 21.6%)	(11.4, 21.2%)	(11.1, 40.1%)
Folate	**15.0%**		**14.7%**	**14.2%**		**13.9%**	**30.5%**		**26.6%**	**28.3%**		**25.6%**
Mb=20.8 nmol/L	(8.6, 21.4%)		(5.8, 23.6%)	(7.1, 21.3%)		(9.7, 18.1%)	(25.1, 35.9%)		(17.7, 35.5%)	(8.7, 47.9%)		(16.8, 34.4%)
FT4	**−1.0%**		**0.3%**	**−0.8%**		**1.2%**	**−1.6%**		**−0.3%**	**−0.7%**		**1.2%**
Mb=15.2 pmol/L	(−5.6, 3.6%)		(−2.9, 3.5%)	(−3.4, 1.8%)		(−1.6, 4.0%)	(−6.2, 3.0%)		(−3.3, 2.7%)	(−2.1, 0.7%)		(−2.8, 5.2%)
GGT	**−1.5%**	**−1.0%**	**−8.3%**	**−9.5%**	**−8.7%**	**−9.2%**	**−4.6%**	**6.2%**	**−6.9%**	**−7.3%**	**−8.2%**	**−10.0%**
Mb=22 U/L	(−5.7, 2.7%)	(−16.9, 14.9%)	(−14.7, −1.9%)	(−15.2, −3.8%)	(−30.7, 13.3%)	(−16.7, −1.7%)	(−8.8, −0.4%)	(−25.4, 37.8%)	(−13.3, −0.5%)	(−36.1, 21.5%)	(−40.2, 23.8%)	(−17.5, −2.5%)
Glucose	**−0.2%**	**−1.0%**	**0.2%**	**0.2%**	**0.5%**	**−1.4%**	**−0.5%**	**−0.9%**	**−0.1%**	**−0.2%**	**1.0%**	**−2.7%**
Mb=5.8 mmol/L	(−1.7, 1.3%)	(−2.7, 0.7%)	(−2.0, 2.4%)	(−2.7, 3.1%)	(−1.8, 2.8%)	(−5.3, 2.5%)	(−2.4, 1.4%)	(−2.4, 0.6%)	(−2.7, 2.5%)	(−4.6, 4.2%)	(−1.8, 3.8%)	(−7.3, 1.9%)
LDH	**73.7%**	**58.6%[a]**	**74.9% [a]**	**74.5%**	**75.5%[a]**	**76.1%[a]**	**146.8%[a]**	**114.8%[a]**	**149.2%[a]**	**148.9%[a]**	**147.9%[a]**	**152.3%[a]**
Mb=156 U/L	(65.8, 81.6%)	(49.4, 67.8%)	(71.5, 78.3%)	(66.4, 82.6%)	(72.7, 78.3%)	(71.1, 81.1%)	(129.9, 163.7%)	(105.4, 124.2%)	(143.4, 155.0%)	(135.7, 162.1%)	(142.6, 153.2%)	(146.0, 158.6%)
Potassium	**7.0%**	**7.3%**	**6.9%**	**6.7%**	**6.8%**	**6.9%**	**13.8%**	**14.5%**	**13.8%**	**13.3%**	**13.3%**	**13.6%**
Mb=4.1 mmol/L	(5.5, 8.5%)	(4.9, 9.7%)	(4.7, 9.1%)	(4.6, 8.8%)	(4.5, 9.1%)	(5.5, 8.3%)	(11.9, 15.7%)	(12.3, 16.7%)	(11.0, 16.6%)	(10.7, 15.9%)	(10.8, 15.8%)	(11.6, 15.6%)
Sodium	**−0.4%**	**−0.6%**	**−0.2%**	**−0.5%**	**−0.4%**	**−0.3%**	**−0.6%**	**−0.9%**	**−0.4%**	**−0.8%**	**−0.7%**	**−0.4%**
Mb=142.1 mol/L	(−1.4, 0.6%)	(−1.7, 0.5%)	(−1.2, 0.8%)	(−1.7, 0.7%)	(−1.7, 0.9%)	(−1.2, 0.6%)	(−1.6, 0.4%)	(−1.8, 0.0%)	(−1.6, 0.8%)	(−2.5, 0.9%)	(−2.0, 0.6%)	(−1.5, 0.7%)
TSH	**−0.5%**		**−0.8%**	**−0.6%**		**−0.7%**	**−1.3%**		**−1.1%**	**−1.6%**		**−2.1%**
Mb=1.57 mIU/L	(−4.9, 3.9%)		(−4.6, 3.0%)	(−2.3, 1.1%)		(−4.4, 3.0%)	(−5.9, 3.3%)		(−3.7, 1.5%)	(−5.4, 2.2%)		(−5.6, 1.4%)
Uric acid	**−0.3%**	**−0.2%**	**−1.1%**	**−1.4%**	**−1.3%**	**1.0%**	**−0.6%**	**−1.4%**	**−1.4%**	**−1.9%**	**−1.6%**	**0.7%**
Mb=314 µmol/L	(−1.1, 0.5%)	(−1.1, 0.7%)	(−2.9, 0.7%)	(−4.3, 1.5%)	(−4.3, 1.7%)	(−1.5, 3.5%)	(−2.7, 1.5%)	(−4.0, 1.2%)	(−3.6, 0.8%)	(−4.3, 0.5%)	(−7.6, 4.4%)	(−1.1, 2.5%)

The effect of Hb is given as mean percent change (bias% [in bold], 95% CI) from the baseline sample (Sample 1, no hemolysis). Significant differences (non-overlapping 95% CI) between two or more instrument groups are marked with [a]. M, mean; Mb, mean concentration in baseline sample; n, number of respondents. Results are not shown if the number of test results are less than three. Results for all instruments and all samples are shown in Supplementary Table 2.

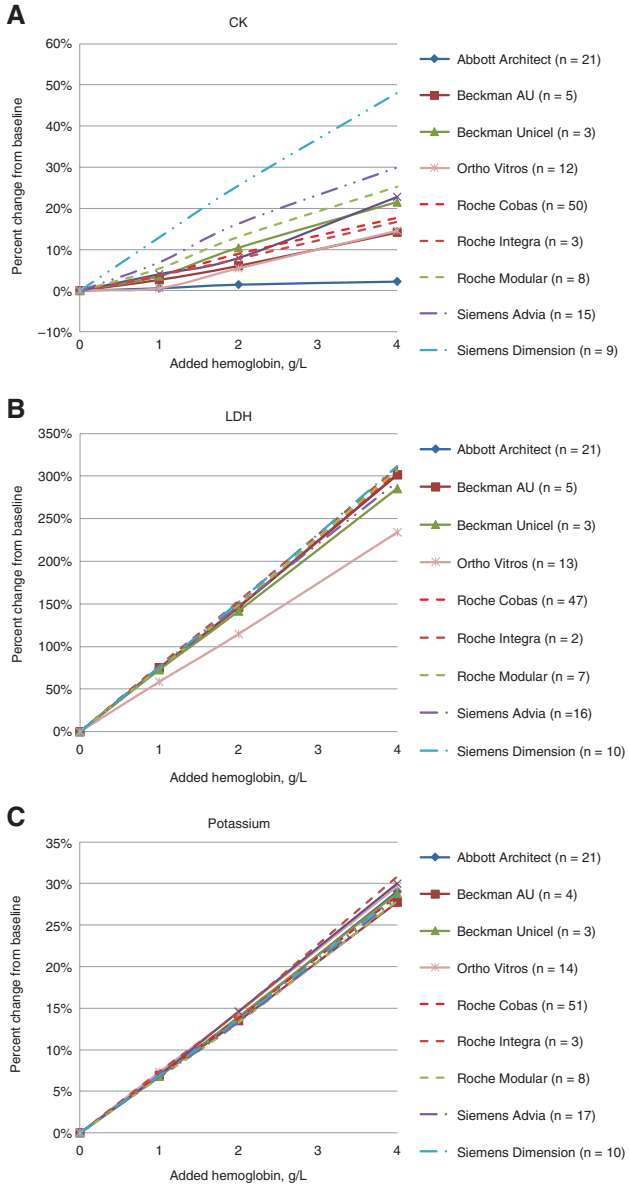

Figure 3: Percent change from baseline, i.e. hemolysis interference, presented as interferographs; A: CK, B: LDH, C: potassium.

laboratories (70%) would refuse to report a potassium result of 4.1 mmol/L in a hemolyzed sample causing the potassium result to increase by a maximum of 7.3% (Figure 3C). Even after learning from the requesting clinician that the sample was from a 2-year-old oncologic patient with a very difficult phlebotomy case (Figure 1), 59 (41%) would still refuse to report the result. A total of 117 (82%) laboratories would refuse to report a potassium result if it increased 14% due to hemolysis (Figure 3C). After learning the sample was from a 60-year-old man admitted to the emergency department with acute chest pain (Figure 1), 91 (64%) would still refuse to report the result.

Discussion

This study provides updated information on the effect of hemolysis on clinical chemistry test results. Most of the 15 analytes were similarly affected, regardless of the instrument used. However, the laboratories differed widely on how they handled and reported the results.

The laboratories in our study reported that they would take some form of action due to hemolysis in 1%–2% of the samples received. This is in line with previous studies, ranging from 0.05% to 3.3% [7]. Eighty-eight percent of the laboratories had written procedures for how to handle hemolyzed samples. This is higher than what was found by Lippi et al. in a national Italian study, where 67 of 107 laboratories (63%) followed a standardized procedure for management of hemolyzed specimens [15]. In the Italian study, 69% used visual inspection to detect hemolysis, while in our study, automated procedures were most commonly used. Guidelines are issued by different laboratory organizations [16–18], however, there is no harmonized procedure for how unsuitable samples should be handled across different laboratories worldwide [17]. However, there is a general consensus that automated detection of hemolysis should be used as visual inspection is highly unreliable [16, 17, 19].

The agreement between different instruments on measured Hb concentrations corresponds well with previous findings [7, 20–22]. To further harmonize measurement of cell-free Hb concentrations across instruments, we advocate that laboratories should participate in EQA programs for serum indices.

We observed that even if different instrument platforms were equally affected by hemolysis, the manufacturers gave different recommendations regarding when a sample should be rejected (see Tables 3 and 4 and Supplementary Table 3). This means that following the manufacturers' advice will not lead to harmonized handling of hemolyzed samples. The proportion of samples handled in accordance with manufacturers' cut-off points varied, even for laboratories using the same assay. This indicates that Nordic laboratories have implemented other analyte-specific cut-off points for hemolysis for some analytes than those provided by the manufacturer. This was also found in a Dutch multicenter evaluation [23]. In both an Australian and an American study, however, laboratories more often used the manufacturers' cut-off points [24, 25]. These findings demonstrate the lack of consensus on which acceptance criteria to use when determining cut-off points for rejection of samples. Acceptance limits can be based on clinical outcome, biological variation (reference change value [RCV]), imprecision of the assay (analytical

Table 2: Number of test results reported, reported with comment, rejected and rejected with comment for CK in samples with Hb 1 g/L and Hb 2 g/L among laboratories that reported a semi-quantitative or quantitative value of measured Hb (n = 122).

Instrument	Cut-off (Hb), g/L		CK											
	Based on 10% bias	Manufacturer's	Sample 2 with Hb 1 g/L						Sample 3 with Hb 2 g/L					
			Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturers' cut-off	Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturers' cut-off
			n	n	n	n	n	n (%)	n	n	n	n	n	n (%)
Abbott Architect	>4.00	10.00	17	0	0	1	0	17 (94)	14	1	0	3	0	15 (83)
Beckman Coulter AU	2.94	5.00	2	1	0	2	0	3 (60)	0	3	0	2	0	3 (60)
Beckman Coulter Unicel	1.96	5.00	2	0	0	0	1	2 (100)	0	0	0	2	1	0 (0)
Ortho Vitros	3.01	1.51	9	0	0	0	2	9 (100)	3	0	4	2	2	7 (78)
Roche Cobas	2.27	2.00	35	4	1	8	2	39 (81)	7	12	5	24	2	28 (58)
Roche Integra	2.46	1.00	0	2	0	0	0	0 (0)	0	1	0	1	0	1 (50)
Roche Modular	1.61	1.00	2	3	0	2	0	3 (43)	0	3	1	3	0	4 (57)
Siemens Advia	1.31	1.88	8	2	0	5	1	9 (60)	4	3	0	8	1	8 (53)
Siemens Dimension	0.78	2.00	0	1	0	9	0	1 (10)	0	1	0	9	0	2 (20)
Total			75	13	1	27	6	83 (72)	28	24	10	54	6	68 (59)

The laboratories' measured Hb value was compared to manufacturers' cut-off points. It was defined as "handled in accordance with manufacturer's cut-off" if the laboratory reported or reported with comment when the measured Hb value was below the manufacturers' cut-off points or if the laboratory rejected or rejected with comment when the measured Hb value was above the manufacturers' cut-off points. Cut-offs based on 10% bias are calculated based on the results in the study. Manufacturers' cut-offs are the Hb cut-off points stated by the manufacturers (see Supplementary Table 3).

Table 3: Number of test results reported, reported with comment, rejected and rejected with comment for LDH in samples with Hb 1 g/L and Hb 2 g/L among laboratories that reported a semi-quantitative or quantitative value of measured Hb (n = 122).

Instrument	Cut-off (Hb), g/L		LDH											
	Based on 10% bias	Manufacturer's	Sample 2 with Hb 1 g/L						Sample 3 with Hb 2 g/L					
			Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturers' cut-off	Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturers' cut-off
			n	n	n	n	n	n (%)	n	n	n	n	n	n (%)
Abbott Architect	0.15	AHS	0	5	2	11	0	13 (72)	0	1	2	15	0	17 (94)
Beckman Coulter AU	0.15	1.00	0	1	0	4	0	3 (60)	0	0	0	5	0	5 (100)
Beckman Coulter Unicel	0.14	1.00	0	0	0	3	0	3 (100)	0	0	0	3	0	3 (100)
Ortho Vitros	0.18	0.51	0	2	4	3	2	7 (78)	0	1	4	4	2	8 (89)
Roche Cobas	0.14	0.15	0	7	3	36	4	39 (85)	0	4	5	37	4	42 (91)
Roche Integra	0.13	0.10	0	1	0	1	0	1 (50)	0	1	0	1	0	1 (50)
Roche Modular	0.15	0.10	0	3	0	4	0	4 (57)	0	3	0	4	0	4 (57)
Siemens Advia	0.12	AHS	0	2	0	14	0	14 (88)	0	2	0	14	0	14 (88)
Siemens Dimension	0.15	AHS	0	0	1	9	0	10 (100)	0	0	1	9	0	10 (100)
Total			0	21	10	85	6	94 (81)	0	12	12	92	6	104 (90)

The laboratories' measured Hb value was compared to manufacturers' cut-off points. It was defined as "handled in accordance with manufacturer's cut-off" if the laboratory reported or reported with comment when the measured Hb value was below the manufacturers' cut-off points or if the laboratory rejected or rejected with comment when the measured Hb value was above the manufacturers' cut-off points. Cut-offs based on 10% bias are calculated based on the results in the study. Manufacturers' cut-offs are the Hb cut-off points stated by the manufacturers (see Supplementary Table 3). AHS, avoid hemolyzed samples.

Table 4: Number of test results reported, reported with comment, rejected and rejected with comment for potassium in samples with Hb 1 g/L and Hb 2 g/L among laboratories that reported a semi-quantitative or quantitative value of measured Hb (n = 122).

Instrument	Cut-off (Hb), g/L		Potassium											
	Based on 10% bias	Manufacturer's	Sample 2 with Hb 1 g/L						Sample 3 with Hb 2 g/L					
			Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturers cut-off	Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturers cut-off
			n	n	n	n	n	n (%)	n	n	n	n	n	n (%)
Abbott Architect	1.41	1.25	4	3	2	9	0	9 (50)	1	5	2	10	0	12 (67)
Beckman Coulter AU	1.46	1.00	0	3	0	2	0	1 (20)	0	0	0	5	0	5 (100)
Beckman Coulter Unicel	1.42	1.00	0	0	0	3	0	3 (100)	0	0	0	3	0	3 (100)
Ortho Vitros	1.36	0.51	0	3	4	2	2	6 (67)	0	1	4	4	2	8 (89)
Roche Cobas	1.43	0.90	2	6	4	38	0	42 (84)	1	2	7	39	1	46 (94)
Roche Integra	1.33	1.00	0	2	0	0	0	0 (0)	0	0	0	2	0	2 (100)
Roche Modular	1.47	1.00	3	1	1	2	0	4 (57)	1	3	1	2	0	3 (43)
Siemens Advia	1.46	AHS	0	5	0	11	0	11 (69)	0	2	0	14	0	14 (88)
Siemens Dimension	1.43	AHS	0	0	1	9	0	10 (100)	0	0	1	9	0	10 (100)
Total			9	23	12	76	2	86 (72)	3	13	15	88	3	103 (87)

The laboratories' measured Hb value was compared to manufacturers' cut-off points. It was defined as "handled in accordance with manufacturer's cut-off" if the laboratory reported or reported with comment when the measured Hb value was below the manufacturers' cut-off points or if the laboratory rejected or rejected with comment when the measured Hb value was above the manufacturers' cut-off points. Cut-offs based on 10% bias are calculated based on the results in the study. Manufacturers' cut-offs are the Hb cut-off points stated by the manufacturers (see Supplementary Table 3). AHS, avoid hemolyzed samples.

Table 5: Number of test results reported, reported with comment, rejected and rejected with comment in samples with Hb 1 g/L and Hb 2 g/L among laboratories that reported a semi-quantitative or quantitative value of measured Hb (n = 122).

Measurand	Sample 2 with Hb 1 g/L						Sample 3 with Hb 2 g/L					
	Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturer's cut-off	Report	Report with comment	Reject	Reject with comment	No answer	Handle in accordance with manufacturer's cut-off
	n	n	n	n	n	n (%)	n	n	n	n	n	n (%)
ALP	105	6	3	5	3	103 (87)	61	14	10	33	4	74 (63)
Bilirubin (total)	103	7	1	7	4	108 (92)	92	4	4	15	7	99 (86)
Calcium	116	0	0	1	5	116 (99)	109	2	2	1	8	111 (97)
Chloride	91	0	0	2	29	91 (98)	86	2	1	2	31	88 (97)
Cobalamin	84	2	1	12	23	81 (82)	75	2	2	17	26	71 (74)
CK	75	13	1	27	6	83 (72)	28	24	10	54	6	68 (59)
Folate	9	15	7	67	24	64 (65)	10	11	9	69	23	64 (65)
FT4	102	0	0	0	20	102 (100)	95	2	1	2	22	92 (92)
GGT	114	1	0	0	7	115 (100)	63	16	6	29	8	81 (71)
Glucose	118	0	0	1	3	118 (99)	104	0	2	8	8	106 (93)
LDH	0	21	10	85	6	94 (81)	0	12	12	92	6	104 (90)
Potassium	9	23	12	76	2	86 (72)	3	13	15	88	3	103 (87)
Sodium	114	2	0	3	3	116 (97)	105	4	1	5	7	109 (95)
TSH	108	0	0	0	14	108 (100)	102	1	1	1	17	103 (98)
Uric acid	114	2	0	2	4	107 (91)	95	2	4	14	7	100 (87)

The laboratories' measured Hb value was compared to the manufacturers' cut-offs. It was defined as "handled in accordance with manufacturer's cut-off" if the laboratory reported or reported with comment when the measured Hb value was below the manufacturer's cut-off or if the laboratory rejected or rejected with comment when the measured Hb value was above the manufacturer's cut-off.

Table 6: Number of test results reported, reported with comment, rejected and rejected with comment (n = 143) in samples with Hb concentration 1 g/L (1A) and Hb concentration 2 g/L (2A), as well as additional results reported after a call from the physician (1B and 2B).

Measurand	Sample 2 with Hb 1 g/L						Sample 3 with Hb 2 g/L					
	(1A) Initial action taken					(1B) Additional results reported (despite rejection initially)	(2A) Initial action taken					(2B) Additional results reported (despite rejection initially)
	Report	Report with comment	Reject	Reject with comment	No answer	n	Report	Report with comment	Reject	Reject with comment	No answer	n
	n	n	n	n	n		n	n	n	n	n	
ALP	120	8	4	6	5	5	70	17	11	39	6	12
Bilirubin (total)	115	11	2	7	8	2	101	7	5	19	11	4
Calcium	130	1	0	1	11	0	121	4	2	2	14	0
Chloride	100	0	0	2	41	0	94	2	1	3	43	0
Cobalamin	91	3	1	13	35	2	80	4	2	19	38	2
CK	86	15	2	29	11	8	35	27	11	59	11	16
Folate	11	17	8	71	36	20	10	12	11	75	35	9
FT4	115	1	0	0	27	n.a.	107	3	1	3	29	0
GGT	131	3	0	0	9	n.a.	75	19	6	33	10	9
Glucose	135	3	0	1	4	0	119	2	2	11	9	4
LDH	2	23	12	91	15	26	0	13	14	101	15	11
Potassium	13[a]	26	14	86	4	41	4[b]	17	16	101	5	26
Sodium	130	5	0	3	5	0	120	5	1	8	9	0
TSH	121	1	0	0	21	n.a.	114	2	1	2	24	0
Uric acid	130	5	0	2	6	1	109	5	4	16	9	3

The questions are presented in Figure 1. [a]Three of the laboratories would correct the result. [b]One of the laboratories would correct the result; n.a., not applicable, no results were rejected initially.

change limit [ACL] [3, 26]), as well as the arbitrary 10% cut-off point commonly used by manufacturers and laboratories [8, 27, 28].

Analytical test results from hemolyzed samples have also previously been reported heterogeneously. Lippi et al. [15] found that hemolyzed specimens were either reported with comment (54%), rejected and the requesting department contacted for a new sample (27%), rejected with comment (16%) or the result corrected, depending on the degree of hemolysis (3%). Laboratories in the UK and Ireland would reject 40% of results above the cut-off point automatically [29]. Nikolac et al. [30] showed that 136 out of 159 laboratories (86%) would reject a potassium result in a sample with hemolysis of 0.5 g/L, while in our study, 72% of laboratories would reject a potassium test result in a sample with hemolysis of 1 g/L. The different actions taken by laboratories may indicate that they might apply less stringent quality criteria for some analytes to prevent rejection of test results, and therefore to a larger extent rely on local performance studies or apply cut-off points based on clinical needs [23, 31–33].

In our study, more than 40% of laboratories still refused to report a potassium result that increased by 7%, even if they learned from the clinician that the result was important. It is questionable whether such a rigid practice is beneficial for the patient. Carraro et al. have argued that potassium results from hemolyzed samples should be reported with comment, due to the severity of a possible *in vivo* hemolysis [34]. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase states that it is acceptable to communicate hyper- or hypokalemia measured in mildly hemolyzed samples, when the bias is regarded as being not clinically relevant [17]. If a universal consensus for handling of hemolyzed samples should be agreed on, it is important that laboratories agree locally with clinicians on what interferences they can accept [35]. EQA organizers should offer schemes on interpretation and reporting of results from hemolyzed samples [36].

Limitations

We did not ask the laboratories or the manufacturers how they had defined their cut-off points. Another limitation was that only samples with analyte concentrations within normal intervals were used. However, spiking the samples to achieve abnormal concentrations could have added confounding interferences. Some of the 15 analytes (i.e. folate, LDH and potassium) are affected by hemolysis at lower thresholds than 1 g/L and including a sample with, for example, 0.5 g/L Hb, could have provided more accurate data for these analytes.

Conclusions

Regardless of the instrument used, most of the analytes were similarly affected by hemolysis. The cut-off points recommended by the manufacturers, however, varied significantly. The laboratories differed in how they reported test results, even when they used the same type of instrument. It appears to be difficult to harmonize actions on how analytical test results from hemolyzed samples should be handled, due to a lack of consensus on which acceptance criteria to use. If a universal consensus for handling of hemolyzed samples should be agreed on, it is important that laboratories agree locally with clinicians on what interferences they can accept.

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