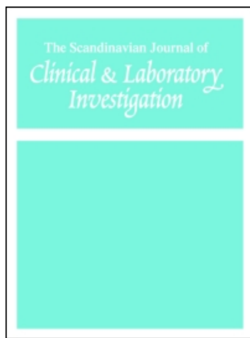

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ORIGINAL ARTICLE

Influence of hemolysis on clinical chemistry parameters determined with Beckman Coulter tests – detection of clinically significant interference

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ABSTRACT

The aim of this study was to examine the influence of hemolysis on 25 clinical chemistry parameters and to compare the resulting bias with clinically significant differences and the manufacturer's specifications. Using freeze-thawing of the treated blood aliquot of each subject ($N = 17$), four hemolysis levels were prepared with hemolysis index (HI) and hemoglobin concentration as follows: (+)=0.5–0.99 g/L, (2+)=1–1.99 g/L, (3+)=2–2.99 g/L and (4+)=3–4.99 g/L. All analytes were tested on the Beckman Coulter AU480 analyzer using proprietary reagents. It was considered that the interference was detected if the 95% confidence interval for mean differences (%) between hemolyzed and non-hemolyzed samples did not include zero. Clinically significant interference was judged against reference change value (RCV). Hemolysis interference was detected for: alpha-amylase, alkaline phosphatase (ALP), aspartate aminotransferase (AST), total and conjugated bilirubin, creatine kinase (CK), CK-MB, ɤ-glutamyltransferase (GGT), iron, lactate dehydrogenase (LD), magnesium, potassium, total protein and uric acid at HI=(1+); alanine aminotransferase (ALT) and phosphate at HI=(2+); urea at HI=(3+); albumin and cholinesterase at HI=(4+). Even at the greatest hemolysis degree, HI=(4+), no interference was detected for calcium, chloride, creatinine, C-reactive protein (CRP), glucose and sodium. Clinically significant difference was exceeded for LD at HI=(1+); CK-MB at HI=(2+); AST and potassium at HI=(3+); total bilirubin at HI=(4+). The presented results did not support the manufacturer's claim for CK and GGT. Establishing HI thresholds for reporting or suppressing test results is the responsibility of each laboratory, taking into account the manufacturer's data, but also its own investigations.

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Pre-analytical phase; hemolysis; hemoglobin; clinical chemistry testing; bias

Introduction

Hemolysis is the most frequent interference in the clinical laboratory [1,2] and one of the biggest challenges to laboratory specialists. Protocols for management of hemolyzed samples should include a systematic process for the detection and reliable quantification of hemolysis in each sample received, as well as an objective definition of the maximum allowable bias for hemolysis interference based on the clinical application of the tests [3–5]. In the present era, automated assessment of hemolysis via hemolysis index (HI) has become available on a large number of laboratory analyzers. Measurement of HI provides several advantages over the visual assessment of the degree of hemolysis, since it is objective, more reproducible and reduces the possibility of errors in the pre-analytical phase of laboratory testing [6,7]. Although there are some potential issues, such as heterogeneity between manufacturers in sensitivity and methods of detection, and the false positive results, HI measurement is currently the optimal way of assessing hemolysis [5,8]. Despite this additional tool, it is not always easy to define HI thresholds for reporting or suppressing test results since manufacturers' declarations for hemolysis interferences often contain limited information. In addition, the results of individual studies published on this topic cannot be easily generalized and transferred to another laboratory because the hemolysis interference depends on the method and instrument used [9].

Studies show that the hemolysis prevalence is 3.3% of all laboratory samples, accounting for the majority (40–70%) of all unsuitable specimens [10]. Blood samples from emergency departments have a particularly high incidence of hemolysis with a prevalence ranging between 3 and 12.4% [9,11]. In emergency departments, re-collecting blood samples (the safest way to remove hemolysis interference) significantly prolongs the therapeutic turnaround time, leads to additional costs and sometimes litigation with clinicians [9,12]. In addition, if results of laboratory tests in the hemolyzed sample lay within a critical range or if *in vivo* hemolysis is suspected, this will open a dilemma between the reliability of laboratory results and the necessary clinical decision making that may affect the clinical outcome.

Therefore, the aim of this study was to investigate the influence of four hemolysis degrees on clinical chemistry tests that emergency departments usually request and to compare the obtained data with clinically significant differences and the manufacturer's specifications for hemolysis interference.

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Materials and methods

Subjects and samples

The study was conducted in the Department of Laboratory Diagnostics, Dubrovnik General Hospital, Dubrovnik, Croatia and approved by the Institutional Ethical Committee. Blood samples were collected from 17 female subjects from laboratory personnel, median age (range) 34 (23–49) years. The participants were informed of the study purpose and they all signed informed consent. In order to obtain a greater range of initial results, the study also included the use of stored serum pools with high or low levels of analytes.

Blood collection and hemolysate preparation

The venous blood samples (~7 mL) were drawn from the antecubital vein of subjects, using 20 G straight needle (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria), directly in the plastic vacuum tube (8 mL) with a clot activator and a gel-separator (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria). Immediately after blood collection, ~1 mL of the serum pool was added to each blood collection tube, and then the tubes were mixed according to the manufacturer's recommendation. From each prepared blood sample, 1 mL of blood was taken for hemolysate preparation by rapid freeze-thaw method. Blood collection tubes were left in an upright position at room temperature for about 30 min and then centrifuged at 1500 *g* for 10 min. The obtained non-hemolyzed serum from each tube was divided into five test tubes. Aliquots of whole blood treated by freeze-thawing were centrifuged under the same conditions and hemolyzed serum samples were obtained from each blood sample. Four hemolysis levels were prepared by adding hemolyzed serum to its non-hemolyzed serum aliquots. Before performing the analysis of the investigated analytes, the HI value was determined in all prepared degrees of hemolysis on the AU480 analyzer (Beckman Coulter, Brea, CA) according to the manufacturer's specifications and using proprietary reagent (LIH reagent with sodium chloride). The relationship between HI value and concentration of free hemoglobin on the Beckman Coulter analyzers was classified as follows:

$$\mathrm{HI}\,(+) = 0.5\text{–}0.99 g/\mathrm{L},\ \ \mathrm{HI}\,(2+) = 1\text{–}1.99\,\mathrm{g/L},\ \ \mathrm{HI}\,(3+) = 2\text{–}2.99\,\mathrm{g/L},\ \text{and}\ \ \mathrm{HI}\,(4+) = 3\text{–}4.99\,\mathrm{g/L}.$$

Sample analysis

During the study, internal quality control was carried out using the same lot of manufacturer's controls. The samples were processed in the same way as all routine samples. The following analytes were measured on the AU480 analyzer (Beckman Coulter, Brea, CA) according to the manufacturer's specifications and using proprietary reagents:

- albumin – bromocresol green colorimetric method
- alpha-amylase – IFCC method employing 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α-D-maltoheptaoside (EPS-G7-PNP)
- alkaline phosphatase (ALP) – IFCC method with p-nitro-phenylphosphate (pNPP)
- alanine aminotransferase (ALT) – IFCC UV method without pyridoxal phosphate (PP)
- aspartate aminotransferase (AST) – IFCC UV method without pyridoxal phosphate (PP)
- bilirubin, total – colorimetric diazo method employing 3,5-dichlorophenyl diazonium tetrafluoroborate (DPD) with caffeine and surfactant
- bilirubin, conjugated – colorimetric diazo method employing 3,5-dichlorophenyl diazonium tetrafluoroborate (DPD)
- calcium, total – colorimetric method with arsenazo III
- chloride – indirect method using ion-selective electrodes
- cholinesterase – colorimetric method with butyrylthiocholine and ferricyanide (III)
- creatine kinase (CK) – N-acetylcysteine-activated IFCC UV method
- creatine kinase MB isoenzyme (CK-MB) – N-acetylcysteine-activated IFCC UV method after inhibition of M subunit activity
- creatinine – enzymatic method
- C-reactive protein - latex immunoturbidimetry
- ɣ-glutamyltransferase (GGT) – IFCC method using L-γ-Glutamyl-3-carboxy-4-nitroanilide
- glucose – hexokinase UV method
- inorganic phosphorus – ammonium molybdate method
- iron – colorimetric method with 2,4,6-Tri-(2-pyridyl)-5-triazine (TPTZ)
- lactate dehydrogenase (LD) – IFCC UV method (lactate-pyruvate)
- magnesium, total – colorimetric method with xylidyl blue
- potassium – indirect method using ion-selective electrodes
- protein, total – method with biuret reagents
- sodium – indirect method using ion-selective electrodes
- urea – UV method with urease and glutamate dehydrogenase
- uric acid – uricase/peroxidase colorimetric method

Statistical analysis

The percentage difference between the results in the hemolyzed samples and non-hemolyzed samples was determined according to the equation:

$$\text{difference}\quad (\%) = [(X_{\mathrm{H}} - X_{\mathrm{nH}})/X_{\mathrm{nH}}] \times 100$$

where X_{H} is the value in hemolyzed samples and X_{nH} is the value in non-hemolyzed samples. The mean difference with the 95% confidence interval was calculated between samples for each analyte. It was considered that the hemolysis interference was detected if the confidence interval for mean

Table 1. Influence of hemolysis on 25 clinical chemistry parameters determined by the Beckman Coulter tests.

				Mean difference with 95% confidence intervals (%)[c]				
Analyte (unit)	Beckman Coulter data	RCV (%)	Initial results at HI (N) (range)	HI (+) 0.5 to 0.99 g/L Hb	HI (2+) 1 to 1.99 g/L Hb	HI (3+) 2 to 2.99 g/L Hb	HI (4+) 3 to 4.99 g/L Hb	HI cut-off for CSD[e]
Albumin[a]	<10% up to	9.2	34. to 49.5	−0.9	−0.2	1.0	**3.0**	–
(g/L)	4.5 g/L Hb	–	–	(−1.7 to 0.0)	(−0.9 to 0.4)	(−0.1 to 2.0)	(2.2 to 3.9)	–
Alpha-amylase[b]	<10% up to	20.5	35 to 139	**−2.3**	**−3.6**	**−5.6**	**−8.5**	–
(U/L)	5 g/L Hb	–	–	(−3.0 to −1.6)	(−4.4 to −2.8)	(−7.0 to −4.2)	(−10.6 to −6.4)	–
ALP[b]	<10% up to	15.4	46 to 531	**−2.1**	**−3.8**	**−6.4**	**−10.5**	–
(U/L)	4.5 g/L Hb	–	–	(−3.0 to −1.2)	(−5.5 to −2.2)	(−8.5 to −4.2)	(−13.8 to −7.2)	–
ALT[a]	<10% up to	53.9	7 to 179	0.9	**2.4**	**5.6**	**8.5**	–
(U/L)	5 g/L Hb	–	–	(−0.5 to 2.4)	(0.4 to 4.4)	(1.8 to 9.5)	(4.4 to 12.6)	–
AST[b]	/	28.9	14 to 169	**13.8**	**25.2**	**44.6**	**80.2**	3+
(U/L)	–	–	–	(7.2 to 20.5)	(16.6 to 33.7)	(30.2 to 59.0)	(53.5 to 106.9)	–
BIL total[b]	<10% up to	50.9	7.8 to 213.2	**−10.4**	**−24.8**	**−41.5**	**−63.7**	4+
(µmol/L)	0.5 g/L Hb	–	–	(−13.9 to −7.0)	(−31.9 to −17.6)	(−52.6 to −30.4)	(−79.9 to −47.6)	–
BIL conjugated[b]	/	85.9	0.9 to 109.1	**−23.1**	**−35.9**	**−42.0**	**−48.6**	–
(µmol/L)	–	–	–	(−32.3 to −13.9)	(−45.3 to −26.6)	(−52.4 to −31.6)	(−58.7 to −38.5)	–
Calcium[a]	<3% up to	6.1	2.18 to 2.54	−0.8	−0.8	−0.8	−1.0	–
(mmol/L)	5 g/L Hb	–	–	(−1.8 to 0.2)	(−1.7 to 0.2)	(−1.8 to 0.2)	(−2.3 to 0.2)	–
Chloride[a]	/	3.7	98 to 105	−0.3	−0.2	0.1	0.1	–
(mmol/L)	–	–	–	(−0.9 to 0.2)	(−0.6 to 0.2)	(−0.6 to 0.9)	(−0.8 to 1.0)	–
Cholinesterase[a]	<3% up to	17.4	4248 to 10039	−0.2	−0.3	−0.3	**−0.8**	–
(U/L)	5 g/L Hb	–	–	(−0.5 to 0.2)	(−0.7 to 0.1)	(−1.0 to 0.4)	(−1.5 to −0.1)	–
CK[a]	<10% up to	63.3	48 to 430	**1.7**	**3.9**	**7.2**	**12.6[d]**	–
(U/L)	5 g/L Hb	–	–	(0.4 to 3.1)	(1.9 to 6.0)	(4.1 to 10.3)	(7.3 to 18.0)	–
CK-MB[a]	/	54.8	8 to 33	**31.5**	**60.2**	**116.1**	**222.5**	2+
(U/L)	–	–	–	(15.3 to 47.6)	(35.6 to 85.9)	(72.8 to 159.5)	(148.1 to 297.0)	–
Creatinine[a]	<5% up to	16.7	54 to 284	0.2	−0.2	0.5	1.5	–
(µmol/L)	5 g/L Hb	–	–	(−0.5 to 1.0)	(−0.9 to 0.5)	(−0.4 to 1.5)	(0.0 to 2.9)	–
CRP[a]	<5% up to	117.1	0.2 to 71.9	1.3	0.6	2.1	3.9	–
(mg/L)	5 g/L Hb	–	–	(−6.2 to 8.8)	(−4.8 to 6.0)	(−6.0 to 10.1)	(−6.6 to 14.5)	–
GGT[a]	<10% up to	37.3	13 to 495	**−7.4**	**−17.2[d]**	**−20.4[d]**	**−21.9**	–
(U/L)	3.5 g/L Hb	–	–	(−13.2 to −1.5)	(−24.9 to −9.4)	(−28.9 to −11.8)	(−31.1 to −12.7)	–
Glucose[a]	<3% up to	15.6	4.1 to 14.4	0.3	−0.2	0.0	0.3	–
(mmol/L)	5 g/L Hb	–	–	(−0.4 to 1.0)	(−0.8 to 0.4)	(−0.5 to 0.5)	(−0.3 to 1.0)	–
Iron[a]	<10% up to	73.5	7.8 to 24.4	**0.7**	**1.5**	**2.8**	**5.5**	–
(µmol/L)	1 g/L Hb	–	–	(0.2 to 1.3)	(0.8 to 2.2)	(1.9 to 3.8)	(4.0 to 6.9)	–
LD[b]	/	20.2	118 to 416	**40.6**	**70.0**	**124.4**	**217.0**	(1+)
(U/L)	–	–	–	(19.2 to 62.0)	(55.9 to 84.1)	(97.7 to 151.1)	(166.2 to 267.8)	–
Magnesium[a]	<10% up to	10.4	0.70 to 0.98	**1.2**	**3.2**	**5.4**	**9.3**	–
(mmol/L)	1.5 g/L Hb	–	–	(0.3 to 2.2)	(1.8 to 4.5)	(4.3 to 6.6)	(7.9 to 10.8)	–
Phosphate[a]	<10% up to	22.8	0.83 to 1.48	1.0	**3.6**	**5.8**	**9.0**	–
(mmol/L)	3.5 g/L Hb	–	–	(−0.1 to 2.1)	(2.6 to 4.5)	(4.6 to 7.0)	(7.2 to 10.7)	–
Potassium[b]	/	10.8	3.9 to 5.1	**4.6**	**9.5**	**16.7**	**30.3**	(3+)
(mmol/L)	–	–	–	(3.2 to 6.0)	(7.4 to 11.7)	(13.3 to 20.0)	(23.1 to 37.6)	–
Protein, total[a]	<10% up to	8.2	59 to 79	**0.8**	**1.8**	**2.6**	**4.5**	–
(g/L)	3 g/L Hb	–	–	(0.3 to 1.4)	(1.2 to 2.4)	(1.9 to 3.4)	(3.4 to 5.6)	–
Sodium[a]	/	2.2	133 to 142	−0.2	−0.3	−0.3	−0.4	–
(mmol/L)	–	–	–	(−0.7 to 0.3)	(−0.7 to 0.0)	(−0.9 to 0.4)	(−1.1 to 0.3)	–
Urea[a]	<10% up to	33.6	4.4 to 20.5	0.3	0.8	**1.3**	**2.4**	–
(mmol/L)	2.5 g/L Hb	–	–	(−0.6 to 1.2)	(0.0 to 1.6)	(0.3 to 2.3)	(1.3 to 3.4)	–
Uric acid[a]	<5% up to	23.9	197 to 459	**−0.7**	**−1.1**	**−1.7**	**−2.9**	–
(µmol/L)	5 g/L Hb	–	–	(1.1 to −0.2)	(−1.5 to −0.8)	(−2.5 to −0.9)	(−4.0 to −1.7)	–

RCV: reference change value; HI: hemolysis index; Hb: hemoglobin; CSD: clinically significant difference
[a]Analytes where the direction of change was a two-way.
[b]Analytes where the direction of change was always one-way.
[c]Bold values for mean difference represent detected interference (95% confidence interval did not contain zero).
[d]Mean difference (%) is greater than manufacturer's data for hemolysis interference.
[e]HI threshold where the mean difference (%) is greater than RCV.

difference did not include the value of zero. Clinically significant interference was assessed by comparing the mean difference between hemolyzed and non-hemolyzed samples with calculated reference change value (RCV). RCV was calculated according to the equation:

$$\mathrm{RCV} = 2^{1/2} \times Z \times (\mathrm{CV}_{\mathrm{A}}^{2} + \mathrm{CV}_{\mathrm{I}}^{2})^{1/2}$$

where Z is 1.96 for the analytes with bidirectional change and 1.65 for the analytes with unidirectional change for the desired probability of $p<.05$. CV_{A} is the analytical coefficient of variation calculated from our laboratory's daily internal quality control results and CV_{I} is the within-subject biological variation derived from Westgard database [13]. Statistical analyses were performed using the MedCalc statistical software version 16.4.3 (MedCalc, Ostend, Belgium).

Results

Detection of hemolysis interference

The results of the study are presented in Tables 1 and 2. By using stored serum pools with high or low levels of

Table 2. Influence of hemolysis on 15 clinical chemistry tests according to the levels of the analytes.

Analyte (unit)	Beckman Coulter data	RCV (%)	Initial results (range) at HI (N)[a] Number of results	Mean difference with 95% confidence intervals (%)[b]				HI cut-off for CSD[d]
				HI (+) 0.5 to 0.99 g/L Hb	HI (2+) 1 to 1.99 g/L Hb	HI (3+) 2 to 2.99 g/L Hb	HI (4+) 3 to 4.99 g/L Hb	
Albumin	<10% up to	9.2	41.7 to 49.5	−0.8	−0.1	0.8	**2.8**	–
(g/L)	4.5 g/L Hb		N = 12	(−2.1 to 0.4)	(−0.9 to 0.6)	(−0.5 to 2.1)	(1.9 to 3.8)	–
–	–	–	34.1 to 40.6	−1.0	−0.5	1.4	**3.5**	–
–	–	–	N = 5	(−2.1 to 0.0)	(−2.3 to 1.4)	(−1.1 to 3.8)	(0.6 to 6.5)	–
ALP	<10% up to	15.4	56 to 82	**−2.6**	**−4.8**	**−8.3**	**−13.1**	–
(U/L)	4.5 g/L Hb	–	N = 8	(−4.2 to −1.1)	(−7.5 to −2.1)	(−10.8 to −5.9)	(−15.5 to −10.7)	–
–	–	–	145 to 531	−0.9	**−1.8**	**−3.0**	**−4.7**	–
–	–	–	N = 7	(−1.8 to 0.0)	(−3.4 to −0.2)	(−6.3 to −0.2)	(−8.5 to −0.9)	–
ALT	<10% up to	53.9	10 to 22	2.3	4.8	**10.9**[c]	**15.2**[c]	–
(U/L)	5 g/L Hb	–	N = 7	(−1.4 to 6.0)	(−0.1 to 9.7)	(2.1 to 19.7)	(8.4 to 21.9)	–
–	–	–	38 to 179	0.0	0.8	**2.1**	**2.7**	–
–	–	–	N = 9	(−0.9 to 1.0)	(0.0 to 1.5)	(0.8 to 3.5)	(1.1 to 4.3)	–
AST	/	28.9	14 to 26	**23.2**	**37.1**	**71.2**	**130.0**	(2+)
(U/L)	–	–	N = 7	(8.4 to 38.1)	(19.0 to 55.2)	(48.9 to 93.6)	(89.1 to 170.8)	–
–	–	–	33 to 169	**7.2**	**16.8**	**26.0**	**45.4**	(4+)
–	–	–	N = 10	(5.3 to 9.2)	(12.0 to 21.6)	(20.1 to 31.9)	(34.8 to 56.0)	–
BIL total	<10% up to	50.9	7.8 to 19.9	**−13.6**	**−31.6**	**−53.4**	**−82.8**	(3+)
(μmol/L)	0.5 g/L Hb	–	N = 12	(−16.9 to −10.2)	(−38.1 to −25.1)	(−61.0 to −45.8)	(−87.5 to −78.2)	–
–	–	–	65.2 to 213.2	**−2.9**	**−8.2**	**−13.0**	**−17.9**	–
–	–	–	N = 5	(−5.8 to −0.1)	(−10.8 to −5.7)	(−21.4 to −4.6)	(−29.1 to −6.7)	–
BIL conjugated	/	85.9	0.9 to 3.6	**−26.2**	**−38.8**	**−43.2**	**−46.4**	–
(μmol/L)	–	–	N = 12	(−39.0 to −13.4)	(−51.7 to −25.8)	(−57.3 to −29.1)	(−59.9 to −32.8)	–
–	–	–	26.9 to 109.1	**−15.6**	**−29.2**	**−39.1**	**−53.9**	–
–	–	–	N = 5	(−26.4 to −4.8)	(−41.2 to −17.2)	(−59.1 to −19.1)	(−72.8 to −35.0)	–
CK	<10% up to	63.3	48 to 138	**1.9**	**4.8**	**8.5**	**15.5**[c]	–
(U/L)	5 g/L Hb	–	N = 13	(0.2 to 3.6)	(2.7 to 7.0)	(5.0 to 11.9)	(9.7 to 21.4)	–
–	–	–	162 to 430	1.1	0.9	3.0	3.2	–
–	–	–	N = 4	(−1.6 to 3.7)	(−5.5 to 7.4)	(−6.3 to 12.3)	(−7.6 to 14.0)	–
CK-MB	/	54.8	8 to 25	**34.5**	**61.9**	**125.4**	**241.6**	(2+)
(U/L)	–	–	N = 14	(14.9 to 54.1)	(30.1 to 93.6)	(73.3 to 177.5)	(153.1 to 330.1)	–
–	–	–	27 to 33	17.4	**52.5**	**72.8**	**133.6**	(3+)
–	–	–	N = 3	(−1.6 to 36.4)	(18.5 to 86.5)	(11.6 to 134.1)	(73.3 to 193.8)	–
Creatinine	<5% up to	16.7	54 to 87	0.7	0.3	**1.3**	**2.8**	–
(μmol/L)	5 g/L Hb	–	N = 11	(−0.3 to 1.8)	(−0.5 to 1.1)	(0.2 to 2.4)	(1.1 to 4.5)	–
–	–	–	96 to 284	−0.6	−1.1	**−0.9**	**−1.0**	–
–	–	–	N = 6	(−1.2 to 0.0)	(−2.3 to 0.0)	(−1.7 to −0.1)	(−1.6 to −0.4)	–
GGT	<10% up to	37.3	13 to 28	**−11.6**[c]	**−28.2**[c]	**−33.7**[c]	**−36.2**	–
(U/L)	3.5 g/L Hb	–	N = 9	(−22.7 to −0.5)	(−37.8 to −18.7)	(−41.2 to −26.2)	(−44.4 to −28.0)	–
–	–	–	43 to 495	**−2.6**	**−4.7**	**−5.4**	**−5.8**	–
–	–	–	N = 8	(−4.5 to −0.6)	(−7.3 to −2.1)	(−10.5 to −0.3)	(−11.3 to −0.4)	–
Glucose	<3% up to	15.6	5.1 to 6.2	0.4	0.1	0.4	0.4	–
(mmol/L)	5 g/L Hb	–	N = 10	(−0.7 to 1.4)	(−0.9 to 1.0)	(−0.3 to 1.2)	(−0.6 to 1.3)	–
–	–	–	6.5 to 14.4	0.1	−0.8	−0.4	0.5	–
–	–	–	N = 5	(−1.8 to 1.9)	(−1.9 to 0.4)	(−1.2 to 0.3)	(−0.9 to 1.8)	–
LD	/	20.2	118 to 230	**50.3**	**81.7**	**143.8**	**255.3**	(1+)
(U/L)	–	–	N = 11	(17.1 to 83.5)	(63.5 to 99.8)	(107.5 to 180.2)	(186.0 to 324.5)	–
–	–	–	254 to 416	**22.9**	**48.6**	**88.8**	**146.8**	(1+)
–	–	–	N = 6	(13.5 to 32.2)	(37.5 to 59.6)	(67.2 to 110.4)	(116.0 to 177.5)	–
Sodium	/	2.2	137 to 142	−0.3	−0.4	−0.2	−0.2	–
(mmol/L)	–	–	N = 12	(−0.9 to 0.3)	(−0.8 to 0.1)	(−1.1 to 0.7)	(−1.2 to 0.8)	–
–	–	–	133 to 136	0.0	−0.3	−0.4	**−0.9**	–
–	–	–	N = 5	(−1.3 to 1.3)	(−1.3 to 0.8)	(−1.5 to 0.6)	(−1.6 to −0.1)	–
Urea	<10% up to	33.6	4.4 to 7.5	0.5	0.9	**1.8**	**3.0**	–
(mmol/L)	2.5 g/L Hb	–	N = 12	(−0.7 to 1.7)	(−0.3 to 2.0)	(0.6 to 3.0)	(1.6 to 4.3)	–
–	–	–	8.5 to 20.5	0.0	0.6	−0.2	**1.0**	–
–	–	–	N = 5	(−1.6 to 1.6)	(−0.9 to 2.1)	(−1.9 to 1.5)	(0.1 to 1.8)	–
Uric acid	<5% up to	23.9	197 to 318	**−0.9**	**−1.3**	**−1.7**	**−2.8**	–
(μmol/L)	5 g/L Hb	–	N = 9	(−1.8 to −0.1)	(−1.8 to −0.7)	(−3.2 to −0.2)	(−5.1 to −0.6)	–
–	–	–	354 to 459	−0.4	**1.0**	**−1.7**	**−2.9**	–
–	–	–	N = 8	(−0.7 to 0.0)	(−1.6 to −0.4)	(−2.7 to −0.8)	(−4.0 to −1.8)	–

RCV: reference change value; HI: hemolysis index Hb: hemoglobin; CSD: clinically significant difference

[a]Values in non-hemolyzed samples are classified according to reference intervals (RI) for women between 20 and 50 years (in case of less than two results outside RI, the results were not analyzed).

[b]Bold values for mean difference represent detected interference (95% confidence interval did not contain zero).

[c]Mean difference (%) is greater than manufacturer's data for hemolysis interference.

[d]HI threshold where the mean difference (%) is greater than RCV.

the analytes, the range of initial values in non-hemolyzed samples was obtained within and outside the reference intervals (RI) selected for women between 20 and 50 years for 21 of the 25 analytes (Table 1). Since the hemolysis interference was considered to be detected if the confidence interval for the mean difference between the samples did not include the value of zero, the results for 6 of the 21 analytes were not classified according to RI because they had less than two initial results outside RI. Therefore, Table 2 shows the hemolysis interference for only 15 analytes with initial values within and outside RI.

In all 17 tested samples, hemolysis interference was detected for: alpha-amylase, ALP, AST, total and conjugated bilirubin, CK, CK-MB, GGT, iron, LD, magnesium, potassium, total protein and uric acid at HI=(1+); ALT and phosphate at HI=(2+); urea at HI=(3+); albumin and cholinesterase at HI=(4+). Even at the greatest hemolysis degree, HI=(4+), no interference was detected for calcium, chloride, creatinine, CRP, glucose and sodium (Table 1). Classification of the results for 15 analytes, which was performed according to RI, showed that hemolysis interference was higher when the initial values were within or below RI than for values above RI (Table 2).

Thresholds of hemolysis index that produce clinically significant difference

Since interference can be considered clinically significant when it exceeds the total variation (analytical and intra-individual biological variation), the mean differences (%) were compared with the calculated RCV for each analyte. Taking into account all the results (Table 1), a clinically significant difference was exceeded for: LD at HI (1+); CK-MB at HI (2+); AST and potassium at HI (3+); total bilirubin at HI (4+). However, when the results of 15 analytes (including LD, CK-MB, AST and total bilirubin) were classified according to RI, the HI threshold for a clinically significant difference was not the same for CK-MB, AST and total bilirubin within and above RI (Table 2). Interestingly, mean differences (%) for total bilirubin above RI (range 65.2–213.2 µmol/L) did not exceed a clinically significant difference even at the greatest hemolysis degree, HI=(4+). On the other hand, the HI threshold for a clinically significant difference was the same for LD values within and above RI (Table 2).

A comparison of results with the manufacturer's data

The mean differences (%) between hemolyzed and non-hemolyzed samples in all 17 tested samples did not support data from Beckman Coulter Technical documents [14] for CK and GGT (Table 1). Classification of the results with respect to the level of the analyte showed that the results of this study are not in accordance with the manufacturer's claims for: ALT range 10–22 U/L, CK range 48–138 U/L and GGT range 13–28 U/L (Table 2).

Discussion

The main finding of our research was the detection of clinically significant interferences for the analytes most sensitive to hemolysis interference: LD, CK-MB, AST, potassium and total bilirubin. Also, the results of our study show that the HI threshold for clinically significant interferences is greater for pathological levels of AST, CK-MB and total bilirubin, which is particularly important for urgent samples with high or critical values and for pediatric samples with high bilirubin results. However, it should be noted that the presented results may only be useful to laboratories using Beckman Coulter assays/analyzers and the same methods. For example, a significant decrease of total bilirubin with hemolysis is in accordance with finding in previous study conducted with Beckman Coulter assays [15], while this effect was not found for the same method with the Roche assay/analyzer, even up to 20 g/L Hb [16]. Obviously, the pseudo-peroxidase activity of free hemoglobin in the Beckman Coulter assay inhibits the diazonium color formation, while this effect is not present in the Roche assay.

Chemical and spectrophotometric interferences of hemolysis have been recognized for a large number of analytes including bilirubin, AP, GGT (negative interference) and CK, creatinine and iron (positive interference) [10]. For LD, AST, potassium, ALT, magnesium, phosphate and urea, hemolysis interference is a consequence of the intracellular components release into serum/plasma, while the effect of dilution can affect albumin, glucose, sodium and chlorides [10,17]. Our study shows the mean differences with the 95% confidence intervals between 17 hemolyzed and non-hemolyzed samples for 25 urgent clinical chemistry testing, including normal and pathological levels for 15 of the 25 analytes tested. In order to avoid detection of interference due to the analytical imprecision of the method, it was considered that the interference was detected only if the confidence interval did not include the value of zero. For the range of initial values obtained from all 17 samples, according to the above criteria, no hemolysis interference was detected for calcium, chloride, creatinine, CRP, glucose and sodium up to 5 g/L Hb i.e. HI (4+). However, negative interference was observed for sodium below RI, at HI (4+).

As previously mentioned, manufacturers' declarations for hemolysis interferences usually contain limited information, and for some assays (such as AST, CK-MB, LD and potassium) the hemolysis interference data are not available. In fact, the criterion of significant interference is debatable and manufacturers should not define criteria for significant interference. For example, the criterion of significant interference for albumin and total bilirubin cannot be the same (10%) taking into account the biological variation. According to the Clinical and Laboratory Standards Institute (CLSI) C56-A document, manufacturers are obliged to declare the results of their interference studies by reporting the percentage of the bias observed, as well as the concentration of analyte and concentration of interfering product. It also recommends the interference testing to be done at two medical decision

levels of the analyte and to be performed up to 10 g/L of hemoglobin [18]. In addition to this, useful information would also be a protocol used to test hemolysis interference (e.g. paired-difference testing according to CLSI document EP7-A2 [19]).

It is important to note that the preparation of test solutions for hemolysis interference testing according to CLSI document EP7-A2, using the same prepared erythrocyte hemolysate in distilled water, cannot differentiate between interference effects of hemoglobin and other erythrocyte constituents, such as potassium, LD and AST. Therefore, in accordance with CLSI EP7-A2 recommendations, for analytes that are the major constituents of erythrocytes, the interferences should be tested by parallel analysis of naturally hemolyzed patient specimens [19]. For this reason, for each blood sample, we prepared the initial hemolysate from its own erythrocytes and serum, not using distilled water. Only assuming that the Beckman Coulter procedure for hemolysis interference testing follows the CLSI EP7-A2 protocol, the reason for the discrepancy between manufacturer's statement and our results for ALT, CK and GGT may be the use of different procedures for hemolysis interference testing. Also, the reason may lie in the levels of the analytes tested (normal or pathological).

Given that all samples were tested in the same way as all routine samples using Beckman Coulter LIH reagent, the limitation of this study is a semi-quantitative determination of hemoglobin concentration. Another limitation of this study is that the hemolysis interference testing has been performed only up to 5 g/L Hb, which is the threshold value in our laboratory for suppressing all tested results (determined according to manufacturer's declarations).

Conclusion

The manufacturers' data should contain more information on the hemolysis interference testing: procedure used, concentrations of both hemoglobin and tested analyte in various hemolysis degrees, and the observed bias. Determination of HI thresholds for reporting or suppressing test results is the responsibility of laboratory specialists taking into account the methods used and the manufacturers' data but also their own investigation and experiences for hemolysis interference. Since hemolysis is the most common interference in laboratory work, the guidelines for verifying the manufacturers' data would be very useful.

Disclosure statement

The authors declare that there are no conflicts of interest.

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