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The evaluation of hemolysis index thresholds for significant hemolysis interference on routine biochemistry analytes

Rutin biyokimya testlerine hemolizin anlamlı interferansının tespiti için hemoliz indeksi eşik değerlerinin belirlenmesi

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Abstract: Objective: The hemolysis index (HI) is an objective, easy and inexpensive method for detection hemolysis. However, the clinical laboratories fully rely on the manufacturers of analytical systems for test-specific the HI thresholds at which the hemolysis significantly interferes with the analyte. In the present study, it was aimed to determine test-specific the HI thresholds for significant interference in hemolyzed specimens produced by shearing method that closely mimics actual hemolysis occurs during blood collection.

Methods: Whole anticoagulated bloods obtained from 34 healthy volunteers were repeatedly passed through a blood collection needle to produce hemolysis. 29 routine biochemistry analytes were assayed on the Roche Cobas 6000 c501 analyzer.

Results: The bias values determined for antistreptolysin-O, total bilirubin, chloride, C-reactive protein, gamma-glutamyltransferase, glucose, high-density lipoprotein, urea and uric acid did not achieve the allowable total error, even at the highest HI value (1550). In contrast to, HI thresholds for aspartate aminotransferase, direct bilirubin, lactate dehydrogenase, and potassium were observed as 50. Our data were generally in good agreement with the list of test-specific HI thresholds by the manufacturer. However, some assays including magnesium, total protein, rheumatoid factor, and sodium had lower the HI thresholds than those of recommended by the manufacturer.

Conclusion: We concluded that hemolysis is differently influence routine biochemistry tests and the HI can provide a data of which analyte is to be affected. The analyte-dependent rejection according to the HI thresholds may prevent prolongation of turnaround time for analyte unaffected by hemolysis. In addition, it was concluded that the HI thresholds might be different according to tolerable error limits selected to determine significant interference. Therefore, before the HI thresholds recommended by manufacturer is applied in laboratory, it should be noted the total allowable error limits used by manufacturer during determination of HI thresholds.

Keywords: hemolysis, hemolysis index, interference, pre-analytical variability

Özet: Amaç: Hemoliz indeksi, hemolizin tespiti için objektif, kolay ve nispeten düşük maliyetli bir yöntemdir. Bununla birlikte, klinik laboratuvarlar anlamlı hemoliz interferansının gözlendiği hemoliz indeksi eşik değerleri için neredeyse tüümüyle üretici firma verilerine güvenmektedir. Bu çalışmada, kan alma sırasında oluşan hemolizi en iyi şekilde taklit eden “shearing” yöntemi kullanarak oluşturulan hemolizli numunelerde 29 biyokimya testine özgü hemoliz indeksi eşik değerlerinin belirlenmesi amaçlanıdı.

Metod: 34 sağlıklı bireyden antikoagulandi (lityum heparin) tam kan numuneleri elde edildi. Hemoliz, ince işleçli (22 gauge) enjektörler aracılığıyla numunelerin tekrarlayan aspirasyonu ile oluşturuldu. 29 rutin biyokimya testinin analizi Roche Cobas c501 analizöründe gerçekleştirilmedi.
Bulgular: Antistreptolizin-O, total bilirubin, klor, C-reaktif protein, gama-glutamiltransferaz, glukoz, yüksek dansiteli lipoprotein, üre ve ürik asit testlerinin bias değerleri, en yüksek hemoliz indeks değerinde (1550) dahi izin verilebilebilecek toplam hatayı aşmamış olarak bulundu. Bunun aksine, aspartat aminotransferaz, direkt bilirubin, laktat dehidrogenaz ve potasyum testlerinin hemoliz indeksi eşik değeri oldukça düşük belirlendi (50). Bu çalışmada belirlenen hemoliz indeksi eşik değerleri, büyük çoğunlukla üretici firma tarafından önerilen değer ile uyumlu idi. Ancak; magnezyum, total protein, romatoid faktör ve sodyum için üretici firma tarafından önerilen değerlerden daha düşük hemoliz indeksi eşik değerlerinde anlamlı hemoliz interferansı görüldü.

Sonuç: Sonuç olarak hemoliz rutin biyokimya testlerini farklı şekilde etkilemektedir. Hemolizin hangi analitleri etkilediği hemoliz indeksi eşik değerleri kullanılarak belirlenebilmiştir. Hemolizli numunelerin hemoliz indeksi eşik değerlerine göre redükte edilmesi düşünülmektedir. Ayrıca bu çalışmada, hemoliz indeksi eşik değerlerinin, anlamlı interferans tespiti için seçilen toplam izin verilebilir hata sınırlarına göre değişiklik gösterebilir. Bu nedenle her bir laboratuvar, üretici firma tarafından önerilen hemoliz indeksi eşik değerlerini belirlemek için üretici firma tarafından verilen toplam izin verilebilir hata sınırlarına dikkat etmelidir.

Anahtar Kelimeler: hemoliz, hemoliz indeksi, interferans, preanalitik değişkenlik

Materials and Methods

Sample collection and experimental design

The venous blood was collected into two 4.0-mL vacuum tube containing dry lithium heparin (Vacuette; Greiner Bio-One GmbH, Kremsmünster, Austria) from 34 healthy volunteers, who given explicit informed consent for the study. The blood samples were immediately divided in six aliquots of 1.25 mL each to obtain non-hemolyzed and hemolyzed samples. The first aliquot did not undergo further manipulation, while the remaining aliquots were passed through a blood collection needle to lyse the cells by mechanical trauma. To produce the hemolyzed samples ranging from slight to high, five samples were aspirated 1, 2, 3, 5, and 7 times respectively through a small-gauge needle (22G, 0.70×38 mm).

All aliquots were centrifuged at 1000 g for 10 minutes. Plasma samples were separated and the HI was measured. Samples were divided groups according to HI values. Then, routine biochemistry analytes were analyzed in all samples included the study.
Analytical methods

The HI was automatically estimated by bichromatic wavelength paired measurement at 570 and 600 nm on the Roche Cobas c501 analyzer (Roche Diagnostic GmbH, Mannheim, Germany). This system is also able to make correction for absorption due to lipemia [9].

29 routine biochemistry analyte were analyzed on the Cobas 6000 c501 analyzer (Roche Diagnostic GmbH, Mannheim, Germany) by methods indicated in Table 1.

The determination of hemolysis index thresholds

To determine test-specific HI thresholds for significant interference in hemolyzed specimens, the relative percent change was calculated as: Relative Percent Bias% = 100× (test result in hemolyzed sample − test result in non-hemolyzed sample) ÷ test result in non-hemolyzed sample. Relative percentage bias was compared with the desirable specification for allowable total error based on the biological variation of the analyte [10]. Significant interference was defined when relative percent bias was found exceeded

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**Table 1:** Analytical methods for biochemistry test parameters measured in the present study.

| Test parameters       | Analytical methods                                                                 |
|-----------------------|------------------------------------------------------------------------------------|
| Albumin               | Bromocresol green colorimetric method                                               |
| Alkaline phosphatase (ALP) | International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method with aminomethyl-propanol buffer |
| Alanine aminotransferase (ALT) | IFCC method without pyridoxal phosphate activation                               |
| Amylase               | IFCC method, enzymatic colorimetric                                               |
| Aspartate aminotransferase (AST) | IFCC method without pyridoxal phosphate activation                           |
| Antistreptolysin O (ASO)     | Immunoturbidimetric method                                                        |
| Direct bilirubin       | Diazo method                                                                       |
| Total bilirubin        | Diazo method                                                                       |
| Calcium               | Colorimetric method, o-cresolphthalein complex                                     |
| Total cholesterol      | Enzymatic colorimetric method                                                     |
| Chloride              | Indirect method using ion-selective electrodes                                     |
| Creatine kinase (CK)  | N-acetylcysteine-activated IFCC method                                              |
| Creatinine            | Jaffe kinetic method                                                               |
| C-reactive protein (CRP) | Immunoturbidimetric method with expanded particle surface                      |
| Gamma-glutamyltransferase (GGT) | Szasz-Persijn method                   |
| Glucose               | Enzymatic, hexokinase method                                                      |
| High-density lipoprotein (HDL) | Homogeneous enzymatic colorimetric method                                       |
| Iron                  | Ferrozine method                                                                   |
| Lactate dehydrogenase (LDH) | IFCC method                               |
| Magnesium             | Chlorophosphonazo method                                                           |
| Inorganic phosphorus   | Ammonium molybdate method                                                          |
| Potassium             | Indirect method using ion-selective electrodes                                     |
| Rheumatoid factor (RF) | Immunoturbidimetric method                                                        |
| Sodium                | Indirect method using ion-selective electrodes                                     |
| Total protein         | Colorimetric                                                                       |
| Triglyceride          | Enzymatic colorimetric                                                             |
| Unsaturated iron-binding capacity (UIBC) | Ferrozine method                   |
| Uric acid             | Uricase/peroxidase enzymatic method                                                |
| Urea nitrogen         | Urease/glutamate dehydrogenase kinetic method                                       |

**Table 2:** The selection of groups according to hemolysis index values.

| Groups   | The range of HI values |
|----------|------------------------|
| Group 1, n=34* | 0–50                   |
| Group 2, n=18  | 50–200                  |
| Group 3, n=27  | 200–400                 |
| Group 4, n=23  | 400–600                 |
| Group 5, n=15  | 600–800                 |
| Group 6, n=17  | 800–1000                |
| Group 7, n=17  | 1000–1250               |
| Group 8, n=12  | >1250                   |

*: While comparing group 1 with other groups, the test results of some participants in group 1 were not used during the calculation of the mean values of test parameters. Group 1 was separately constituted for each group (from 2 to 8) according to the number of participant.
The levels of analytes are given as mean±SD. To determine clinically meaningful variation, relative percentage bias and allowable total error based on biological variation (11) are also located. HI: Hemolysis index; Err: These values of analytes were excluded due to the analyzer gave warning messages related with analytical method in all aliquots.

Table 3: The effects of hemolysis on routine biochemistry analytes.

| Analytes                        | Group 1  | Group 2  | Group 3  | Group 4  | Group 5  | Group 6  | Group 7  | Group 8  | Group 9  | Group 10 | Group 11 | Group 12 | The allowable total error |
|---------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------------------------|
|                                 | n=18     | n=27     | n=23     | n=15     | n=17     | n=17     | n=12     |          |          |          |          |          | ±4.07%                                     |
| Albumin, g/L                    | 46.4±2.8 | 45.8±2.8 | 46.7±2.5 | 45.9±2.6 | 46.4±2.5 | 45.6±2.8 | 47.1±1.6 | 45.5±1.8 | 46.9±1.8 | 45.2±2.4 | 46.8±2.7 | 44.3±2.6 | 47.7±1.7 | 43.9±2.2 |
|                                 | -1.29%   | -1.71%   | -1.72%   | -3.4%    | -3.54%   | -5.34%   | -8.1%    |          |          |          |          |          | ±12.04%                                     |
| Alkaline phosphatase, U/L       | 76±26    | 73±26    | 86±29    | 78±28    | 80±27    | 67±27    | 77±28    | 57±28    | 84±25    | 62±25    | 87±32    | 59±30    | 79±29 | 46±25 | ±14.6% |
|                                 | -0.04%   | -9.4%    | -16.52%  | -24.78%  | -26.42%  | -32.23%  | -41.65%  |          |          |          |          |          | ±27.48%                                     |
| Alanine aminotransferase, U/L   | 18±9     | 19±8     | 17±6     | 18±6     | 17±8     | 18±7     | 17±7     | 18±6     | Err      | Err      | Err      | Err      | ±16.69%                                     |
|                                 |          |          |          |          |          |          |          |          |          |          |          |          | ±14%                                        |
| Amylase, U/L                    | 64.6±19.2| 62.3±19.6| 65.2±19.1| 61.8±18.7| 63.26±19.55| 58.39±18.68| 58.6±15.88| 52.2±14.97| 63.20±3.9 | 55.31±19.8| 57±16.55| 47.5±15.04|                      |
|                                 |          |          |          |          |          |          |          |          |          |          |          |          | ±10%                                           |
| Aspartate aminotransferase, U/L | 19±5     | 28±5     | 19±3     | 37±8     | 19±3     | 48±7     | 18±4     | 50±7     | Err      | Err      | Err      | Err      | ±26%                                        |
|                                 |          |          |          |          |          |          |          |          |          |          |          |          | ±2.55%                                      |
| Bilirubin, direct, µmol/L       | 3.42±2.74| 9.58±2.22| Err      | Err      | Err      | Err      | Err      | Err      | ±44.5%   |          |          |          | 180%                                           |
| Total bilirubin, µmol/L         | 9.2±1.37 | 8.55±0.86| 9.23±4.28| 9.06±4.28| 8.72±4.1 | 8.72±3.76| 9.69±5.01| 9.04±2.82| 9.15±4.48| 9.41±4.17| 10.73±4.63| 10.71±4.46| 12.48±6.31| 11.32±4.31| ±26.94% |
| Calcium, mmol/L                 | 2.42±0.07| 2.41±0.09| 2.41±0.08| 2.39±0.09| 2.39±0.08 | 2.38±0.10| 2.43±0.06| 2.41±0.08| 2.36±0.10 | 2.41±0.06 | 2.34±0.07 | 2.41±0.08 | 2.31±0.08| 2.55%                                         |
|                                 | -0.52%   | -0.83%   | -0.52%   | -1.96%   | -1.79%   | -2.92%   | -3.97%   |          |          |          |          |          |          | ±9.01%                                       |
| Cholesterol, mmol/L             | 4.59±0.83| 4.62±0.86| 4.31±0.74| 4.38±0.73| 4.33±0.81 | 4.51±0.78| 4.35±0.80| 4.52±0.75| 4.33±0.81| 4.63±0.82| 4.28±0.74| 4.62±0.74| 4.01±0.65| 4.56±0.60| ±9.01% |
Table 3: The effects of hemolysis on routine biochemistry analytes.

| Analytes                  | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | The allowable total error |
|---------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------------------------|
|                           | HI:9±7  | HI:14±9 | HI:10±6 | HI:28±6 | HI:50±8 | HI:71±5 | HI:90±3 | HI:11±8 | HI:114±87 |
| Chloride, mmol/L          | 102.4±2 | 103.5±2 | 101.3±2 | 102.2±2 | 101.6±2 | 102.4±2 | 101.6±2 | 101.4±2 | 101.2±2 | ±1.5% |
|                           | 102.4±2 | 103.5±2 | 101.3±2 | 102.2±2 | 101.6±2 | 102.4±2 | 101.6±2 | 101.4±2 | 101.2±2 | ±1.5% |
| Creatinine, µmol/L        | 70.7±10 | 72.5±10 | 74.3±11 | 76.9±12 | 74.3±11 | 77.8±12 | 72.5±11 | 74.8±12 | 81±15 | ±8.87% |
| C-reactive protein, mg/L  | 3.1±6.5 | 3.2±6.4 | 5.8±9.8 | 5.7±9.6 | 4±6.5   | 3.9±6.3 | 4.2±10.5 | 4.1±10.3 | 6.9±11 | ±56.6% |
| Gamma glutamyltransferase, U/L | 14.5±4.4 | 13.9±4.6 | 15.1±6.4 | 15.7±5  | 14.2±5.0 | 15.4±7.9 | 14.5±7.3 | 17.9±7.3 | 15.9±6.2 | ±22.11% |
| Glucose, mmol/L           | 4.9±0.58 | 4.8±0.58 | 5.2±0.96 | 5.2±0.96 | 5.0±0.96 | 5.0±0.96 | 5.1±0.96 | 5.1±0.96 | 5.1±0.96 | ±6.96% |
| HDL-cholesterol, mmol/L   | 1.3±0.3  | 1.3±0.3  | 1.2±0.3  | 1.2±0.3  | 1.2±0.3  | 1.2±0.3  | 1.2±0.3  | 1.2±0.3  | 1.2±0.3  | ±11.63% |
| Iron, µmol/L              | 18.3±11.2 | 20.4±10.6 | 19.9±9.7 | 22.0±9.2 | 21.6±9.3 | 24.9±9.4 | 20.4±11.5 | 27.5±9.6 | 20±9.8  | ±30.7% |
| Lactate dehydrogenase, U/L| 165±36  | 347±82  | 168±32  | 519±105 | 173±27  | 778±104 | 151±34  | 917±67  | 178±26  | ±11.4% |

The levels of analytes are given as mean±SD. To determine clinically meaningful variation, relative percentage bias and allowable total error based on biological variation (11) are also located.

HI: Hemolysis index; Err: These values of analytes were excluded due to the analyzer gave warning messages related with analytical method in all aliquots.
### Table 3: The effects of hemolysis on routine biochemistry analytes.

| Analytes                        | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 | Group 11 | Group 12 | The allowable total error |
|---------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|----------|---------------------------|
|                                 | HI:9±7  | HI:149±28 | HI:10±6 | HI:286±49 | HI:505±58 | HI:711±47 | HI:901±58 | HI:11±8 | HI:1145±87 | HI:1550±215 | HI:15±7 | HI:15±7 | ±4.8%                      |
| Magnesium, mmol/L               | 0.86±0.06 | 0.87±0.07 | 0.85±0.07 | 0.87±0.07 | 0.84±0.05 | 0.90±0.12 | 0.85±0.08 | 0.90±0.07 | 0.85±0.06 | 0.90±0.07 | 0.85±0.06 | 0.97±0.22 | ±4.8%                      |
| Phosphorus, mmol/L              | 1.43%    | 1.93%    | 2.83%    | 7.35%    | 5.76%    | 5.35%    | 14.33%   |         |         |          |          |          | ±10.11%                   |
| Potassium, mmol/L               | 0.96±0.18 | 1.01±0.19 | 0.98±0.16 | 1.05±0.17 | 0.99±0.18 | 1.12±0.18 | 0.96±0.14 | 1.15±0.14 | 0.94±0.16 | 1.16±0.15 | 1.01±0.18 | 1.30±0.20 | ±10.11%                   |
| Sodium, mmol/L                  | 5.03%    | 7.59%    | 12.99%   | 19.5%    | 23.73%   | 28.13%   | 36.08%   |         |         |          |          |          | ±13.5%                    |
| Triglyceride, mmol/L            | 4.07±0.39 | 4.61±0.44 | 4.02±0.38 | 4.97±0.43 | 4.04±0.39 | 5.75±0.45 | 3.98±0.33 | 6.31±0.43 | 4.14±0.36 | 7.13±0.48 | 3.92±0.39 | 7.67±0.44 | ±5.61%                    |
| Rheumatoid factor, kU/L         | 13.27%   | 23.63%   | 42.33%   | 58.65%   | 72.2%    | 95.41%   | 121.01%  |         |         |          |          |          | ±3.63%                    |
| Total protein, g/L              | 77.6±4.9 | 78.7±5.2 | 77.7±4.1 | 79.1±4.2 | 77.2±3.4 | 80.6±3.6 | 77.1±2.95 | 82.0±5.19 | 77.7±5.90 | 84±1.15 | 78.5±1.28 | ±3.63%                    |
| Uric acid, µmol/L               | 304.5±73.2 | 302.8±72.6 | 303.9±66 | 302.2±64.8 | 306.3±70.2 | 304±67.8 | 302.8±58.3 | 305.7±60.7 | 304.2±52.8 | 307.5±50.4 | 297.8±67.5 | 301.1±61.9 | ±11.97%                   |
| Urea, mmol/L                    | 8.78±0.72 | 8.82±0.72 | 9.71±2.68 | 9.78±2.68 | 9.59±3 | 9.67±2.87 | 8.97±1.99 | 9.13±1.96 | 10.14±2.38 | 10.30±2.30 | 9.55±2.60 | 9.66±2.51 | ±15.55%                   |
| Unsatirated iron binding capacity, µmol/L | 22.07% | 46.25% | 83.88% | 125.5% | 151.88% | 192.34% | 290.6% |         |         |          |          |          | ±10%                      |

The levels of analytes are given as mean±SD. To determine clinically meaningful variation, relative percentage bias and allowable total error based on biological variation (11) are also located. HI: Hemolysis index; Err: These values of analytes were excluded due to the analyzer gave warning messages related with analytical method in all aliquots.
total allowable error limits. For the ASO and UIBC, evaluation was done according to ±10% since there are not the desirable specifications for allowable total error based on the biological variation. It was also investigated whether test-specific HI threshold values obtained from the present study were different from those of manufacturer or not.

**Results**

Of obtained 204 aliquots, 170 were subjected to multiple needle aspirations and the remained 34 samples were not performed further manipulation. According to the HI values, plasma samples were divided to from group 1 to 8. The range of HI values of each group was indicated in Table 2. The lower and upper HI values of groups were defined according to HI thresholds values of manufacturer [8].

Non-hemolyzed group was considered as Group 1. To determine accurately the relative percent bias, group 1 was constituted separately for each group. For example, during the selection of group 2, some participants were not included to group 2 due to no aliquot of these participants had 50–200 values of HI. Therefore, aliquots with non-hemolyzed of these participants were not included group1 during the comparison of group 2 with group 1.

The effects of hemolysis interferences on routine biochemistry tests are presented in Table 3. The percent bias values for ASO, total bilirubin, chloride, CRP, GGT, glucose, HDL, urea and uric acid were within the allowable total error limits in all HI values, even at the highest HI value (1550). However; the relative percentage bias values for AST, LDH, direct bilirubin potassium and unsaturated iron binding capacity was beyond the allowable total error limits at hemolyzed samples with lowest HI value (50–200). For remained tests, the HI thresholds were observed at wide range (200, 400, 600, 800, 1000, and 1250). The HI threshold values determined in the present study as well as recommended by manufacturer are

| **Analytes**           | The HI values determined according to total allowable error based on biological variation | The HI values determined according to ±10 tolerable bias | The HI values recommended by manufacturer |
|------------------------|--------------------------------------------------------------------------------------------|--------------------------------------------------------|------------------------------------------|
| Albumin                | 901 | 1550 | 1000 |
| Alkaline phosphatase   | 286 | 286 | 200 |
| Alanine aminotransferase | 711 | 711 | 200 |
| Amylase                | 901 | 505 | 500 |
| Aspartate aminotransferase | 50 | 50 | 40 |
| Antistreptolysin O     | 1550 | 1550 | 1000 |
| Bilirubin, direct      | 50 | 50 | 25 |
| Total Bilirubin        | 1550 | 1550 | 800 |
| Calcium                | 901 | 1550 | 1000 |
| Total Cholesterol      | 1145 | 1145 | 700 |
| Chloride               | 1550 | 1550 | 1000 |
| Creatin kinase         | 711 | 286 | 200 |
| Creatinine             | 1145 | 1145 | 1000 |
| C-reactive protein     | 1550 | 1550 | 500 |
| Gamma glutamyltransferase | 1550 | 711 | 200 |
| Glucose                | 1550 | 1550 | 1000 |
| HDL-cholesterol        | 1550 | 1550 | 1200 |
| Iron                   | 505 | 50 | 200 |
| Lactate dehydrogenase  | 50 | 50 | 15 |
| Magnesium              | 505 | 1145 | 2000 |
| Phosphorus             | 286 | 286 | 300 |
| Potassium              | 50 | 50 | 100 |
| Sodium                 | 149 | 1550 | 1000 |
| Rheumatoid factor      | 149 | 50 | 300 |
| Total protein          | 286 | 1145 | 1000 |
| Triglyceride           | 1145 | 286 | 700 |
| Unsaturated iron binding capacity | 50 | 50 | 40 |
| Urea                   | 1550 | 1550 | 1000 |
| Uric acid              | 1550 | 1550 | 1000 |
shown in Table 4. For majority of evaluated tests, the HI threshold values obtained from the study were found to be compatible with the HI thresholds recommended by manufacturer [8]. However, the HI thresholds of magnesium, RF, sodium and total protein were found lower than those of the manufacturer.

Discussion

The hemolysis index is an objective, easy and inexpensive method for detection hemolysis. Although there are several obvious advantages in the using of the HI, the clinical laboratories fully relies on the manufacturers of analytical systems for test-specific the HI thresholds at which the hemolysis significantly interferes with the analyte. Manufacturers, unfortunately, usually give limited information about experimental design of hemolysis study, sample size and analyte concentrations [8].

In the present study, it was determined the HI thresholds at which relative percentage bias exceeded the allowable total error based on the biological variation. Thereafter, the test-specific HI threshold values obtained from the present study were compared with those of manufacturer. For most of the evaluated tests, it was determined that there was good agreement between the HI threshold values obtained from the study and those of recommended by manufacturer. However; for magnesium, RF, sodium and total protein, the HI thresholds determined in the study had lower than those of the manufacturer. A deviation within ±10% from nonhemolyzed sample for any analyte was accepted as tolerable bias by the manufacturer. This tolerable limit is higher than allowable limits based on biological variation for some tests including total protein, sodium and magnesium. On the other hand, for majority of evaluated tests, the allowable total errors based on biological variation are higher than ±10%. Therefore, HI thresholds were re-defined according to ±10% tolerable limit then compared those of the manufacturer (Table 4). The HI threshold values obtained from the present study were in good agreement with what gave the list of test-specific the HI thresholds by manufacturer, except iron, magnesium, RF and triglyceride. The difference of experimental design among two studies may explain this situation. In the present study, the shearing method by defined Dimeski [2] was chosen to produce hemolyzed samples because this method closely mimics the actual pathological processes of hemolysis [2,11]. In spite of shearing method used in the present study, the manufacturer evaluated hemolysis interference on biological analyte by added different concentrations of hemoglobin to non-hemolyzed samples. This method ignores the effects of additive, chemical, and dilutional hemolysis by derived from cell debris and intracellular compounds other than hemoglobin [2,11].

In a study performed by Ji et al. [12], they assessed the impacts of hemolysis on several laboratory tests in hemolyzed samples with different the HI values. Hemolysis had prepared by freezing-thawing of washing packed red cells in their study. Our findings were in accordance with those of their study for most of the evaluated tests. On other hand, unlike our findings, albumin, calcium, creatinine, magnesium and sodium were reported as unaffected by hemolysis at highest HI value by Ji et al. This may due to be difference of allowable error limits used to detect significant variation from non-hemolyzed sample. They defined significant hemolysis interference when the change of the analyte value exceeded ±10% of the baseline value. The allowable total error limits derived from biological variation for these analyte were lower than ±10%. Therefore, the significant variation from baseline value might be determined in hemolyzed samples with lower the HI values in our study. In addition to, Ji et al. [12] defined significant hemolysis interference at very low the HI value for total bilirubin in contrast to our findings. Manufacturers may sometimes produce different generations of a reagent that have different properties. There are two reagent of total bilirubin in different generation, produced by Roche Diagnostics. We used third generation of total bilirubin in the present study however any data was not obtained about which generation used in their study.

In conclusion, it was found that hemolysis is differently affect routine biochemistry tests and the HI can provide a data of which analyte is to be affected by hemolysis. The analyte-dependent rejection according to the HI thresholds may prevent the prolongation of turnaround time for analyte unaffected by hemolysis. In addition, it was concluded that the HI thresholds might be different according to tolerable error limits selected to determine significant interference. Therefore, before the test-specific HI thresholds recommended by manufacturer is applied in laboratory, it should be noted the total allowable error limits used by manufacturer during determination of HI thresholds.

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References

[1] Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, et al. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. Clin Chem Lab Med 2008; 46(6):764–72.
[2] Dimeski G. Interference testing. Clin Biochem Rev 2008; 29 Suppl 1:43–8.
[3] Lippi G, Plebani M. Continuous-flow automation and hemolysis index: a crucial combination. J Lab Autom 2013; 18(2):184–8.
[4] Simundic AM, Nikolac N, Ivankovic V, Ferenc-Ruzic D, Magdic B, Kvatarnik M, et al. Comparison of visual vs. automated detection of lipemic, icteric and hemolyzed specimens: can we rely on a human eye? Clin Chem Lab Med 2009; 47(11):1361–5.
[5] Clinical Laboratory Standards Institute. Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interferencen Clinical Laboratory Analysis; Approved Guideline. CLSI C56-A document. Clinical Laboratory Standards Institute, Wayne, Pennsylvania, USA, 2012.
[6] Söderberg J, Jonsson PA, Wallin O, Granqvist K, Hultdin J. Haemolysis index – an estimate of preanalytical quality in primary health care. Clin Chem Lab Med 2009; 47(8):940–4.
[7] Dolci A, Panteghini M. Harmonization of automated hemolysis index assessment and use: Is it possible? Clin Chim Acta 2014; 432:38–43.
[8] F. Hoffmann-La Roche Ltd. List of interferences based on serum indices for serum and plasma (not applicable for urine). www.rochediagnostics.ch/content/dam/corporate/rochedia_ch/documents/serumindices/Interferences_c311_c501_c502.pdf (Last accessed: October 2014).
[9] F.Hoffmann-La Roche Ltd. Serum Indices: Reduction of clinical errors in laboratory medicine. www.rochediagnostics.fr/Htdocs/media/pdf/actualites/2b_SI_Brochure_2007.pdf (Last accessed: September 2014).
[10] Westgard QC. Quality Requirements: Desirable biological variation database specifications. www.westgard.com/biodatabase1.htm (Last accessed: December 2014)
[11] Lippi G, Musa R, Avanzini P, Aloe R, Pipitone S, Sandei F. Influence of in vitro hemolysis on hematological testing on Advia 2120. Int J Lab Hematol 2012; 34(2):179–84.
[12] Ji JZ, Meng QH. Evaluation of the interference of hemoglobin, bilirubin, and lipids on Roche Cobas 6000 assays. Clin Chim Acta 2011; 412(17-18):1550–3.