UC 577,1; 61 ISSN 0354-3447

Jugoslov Med Biohem 24: 45–49, 2005 *Originalni naučni rad*
Original paper

REFERENCE INTERVAL FOR CALCULATED TOTAL IRON-BINDING CAPACITY USING OLYMPUS AU2700 ANALYZER

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Summary: Total iron-binding capacity (TIBC) values are determined on Olympus AU2700 automated chemistry analyzer as the sum of serum iron and unsaturated iron-binding capacity (UIBC) – (calculated TIBC, $TIBC_{cal}$). Considering that Olympus AU2700 automated analyzer was recently brought in function and TIBC values calculated from serum iron and UIBC values were significantly lower than those obtained by a direct and fully automated TIBC assay, it was necessary to determine the reference interval for TIBC, according to the recommendation that every laboratory should have its own reference ranges. The »calculation method« of TIBC determination showed satisfactory accuracy ($p > 0.001$) and precision, with CV values ranging from 0.91% to 1.63% within-run and from 2.30% to 2.80% day-to-day. The correlation between the TIBC values obtained with the »calculation method« using Olympus AU2700 analyzer (y) and those obtained with the direct method (x) was: $y = 0.919x + 2.319$ μmol/L ($r = 0.980$; $S_{y,x} = 1.814$; $p < 0.001$; $N = 85$). The reference interval for TIBC was determined using sera collected from 125 healthy individuals of both sexes, 15 to 80 years old. Since the values did not depend on sex, the reference interval calculated for the whole studied population ranged between 42.0 μmol/L and 64.3 μmol/L.

Key words: reference interval, total iron-binding capacity, Olympus AU2700, unsaturated iron-binding capacity, calculation method

Introduction

Transferrin is the primary serum iron-transport protein, because over 95% of serum non–heme iron is bound by transferrin. Normally, only about one third of the iron-binding sites of transferrin are occupied by Fe^{3+} ions. That is why serum transferrin has considerable reserve iron-binding capacity. This is called serum *unsaturated iron binding capacity* (UIBC). *Total iron binding capacity* (TIBC) is a measurement of the maximum concentration of iron that serum proteins, principally transferrin, can bind. Measurements of serum iron, TIBC and the percentage of iron saturation of transferrin are useful for the clinical diagnosis of iron-deficiency anemia and chronic inflammatory disorders or malignancies, and as screening tests in suspected cases of hereditary hemochromatosis (1).

TIBC is routinely determined by direct methods (2–5). This includes saturation of transferrin with an excess predetermined amount of iron, removal of the unbound iron and measurement of the iron that is dissociated from transferrin. The difference between various direct methods is in the way of removing the excess iron after the transferrin saturation. For removal of the unbound iron, »light« magnesium carbonate (2), ion-exchange resin (3), alumina columns (4) or magnetic particles (5) are used.

Most direct TIBC measurements require manual procedures that involve centrifugation or pretreatment of serum samples. As an alternative to direct measurements methods, TIBC values are also calculated from sum of iron and UIBC, which is determined automatically by colorimetric methods (»cal-

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culation method«). Fully automated direct TIBC measurement methods are also commercially available (6–8).

Yamanishi et al. (7, 9) reported recently that TIBC values calculated from serum iron and UIBC values were significantly lower than those obtained by a direct and fully automated TIBC assay. Considering that on Olympus AU2700 chemistry analyzer (Olympus Diagnostica GmbH, Hamburg, Germany) TIBC values are obtained by the »calculation method«, and according to the recommendation that every laboratory should have its own reference ranges, the reference interval for TIBC is established in this study. Also, the comparison was made between the results of determination of TIBC by Olympus »calculation method« and by the direct method, together with the evaluation of imprecision and inaccuracy of the two methods.

Materials and Methods

Iron and UIBC values used for TIBC calculation were determined with Olympus colorimetric tests. Iron was measured using Iron-Olympus System Reagent (Olympus Diagnostica GmbH, Hamburg, Germany; Cat. No OSR6286) based on the TPTZ method. Fe^{3+} is reduced to Fe^{2+}, which forms a blue complex with 2,4,6-Tri(2-pyridyl)-5-triazine (TPTZ). This complex shows a maximum absorption at 590 nm. We used for UIBC determination, UIBC-Olympus System Reagent (Olympus Diagnostica GmbH, Hamburg, Germany; Cat. No OSR6124), based on the Nitroso-PSAP method. After the specific binding of Fe^{3+}, added to serum at alkaline pH, at unsaturated transferrin iron-binding sites, remaining unbound Fe^{3+} reacts with the Nitroso-PSAP to form an intense green complex with maximum absorption at 800 nm. The difference between the resulting change in the measured absorbance and the absorbance from the total amount added to serum is equivalent to the quantity bound to transferrin, which represents UIBC.

For pretreatment of samples for TIBC determination by the direct method IL Test™ TIBC Sample Pretreatment Kit (Instrumentation Laboratory SpA, Milano, Italy; Cat. No 181730–00) was used. Specimens were treated with a Fe (III) solution to saturate the unbound iron-binding sites of transferrin and the excess iron was removed by precipitation with magnesium carbonate hydroxide. The total iron present in the supernatant represents the TIBC. Concentration of iron was measured on the ILab™ 1800 Clinical Chemistry System (Instrumentation Laboratory SpA, Milano, Italy) using IL Test™ Iron assay (Instrumentation Laboratory SpA, Milano, Italy; Cat. No 182509–10) which is based on the formation of a blue colored complex between iron and Ferene® in an acidic solution. Absorbance measurements were taken at a reading wavelength of 600 nm with a blanking wavelength of 700 nm.

The reference interval for calculated TIBC values was determined using sera collected from 125 healthy individuals of both sexes, 15 to 80 years old.

For imprecision studies three serum pools were used with low, normal and high TIBC values. Inaccuracy was studied by analyzing two control sera: normal (Randox Multisera – bovine NORMAL; Cat. No AN1026) and elevated (Randox Multisera – bovine ELEVATED; Cat. No AE1032).

Statistical analysis included the calculation of means ($\bar{x}$), standard deviations (SD) and coefficients of variation (CV), as well as Student's t-test, Chi-square test and the least square regression analysis for correlation assessment. Reference interval for TIBC was calculated by the nonparametric method (10, 11). Statistical package SPSS for Windows 11.5 (Chicago, IL, USA) was used for data processing.

Results

Imprecision studies

Before determination of the reference interval for TIBC determined using Olympus AU2700 analyzer, imprecision of the two TIBC determination methods, »calculation« and the direct method, was studied in the series and on day-to-day basis. TIBC values were determined in each serum pool with 10 repeated determinations in each sample. The same serum pools, stored at –20 °C, were used for deter-

Table I Within-run and between-day imprecision studies for TIBC determination by »calculation method« using Olympus AU2700 analyzer and by the direct method

pool	Within-run						Between-day					
	»Calculation method«			Direct method			»Calculation method«			Direct method		
	$\bar{x}$ (μmol/L)	SD (μmol/L)	CV (%)	$\bar{x}$ (μmol/L)	SD (μmol/L)	CV (%)	$\bar{x}$ (μmol/L)	SD (μmol/L)	CV (%)	$\bar{x}$ (μmol/L)	SD (μmol/L)	CV (%)
low	31.06	0.498	1.60	32.49	0.483	1.49	30.60	0.858	2.80	32.33	0.551	1.71
normal	50.09	0.457	0.91	53.40	0.440	0.82	49.69	1.306	2.63	52.67	0.665	1.26
high	73.96	1.208	1.63	80.57	0.776	0.96	71.97	1.687	2.34	78.87	1.146	1.45

Table II Inaccuracy studies for TIBC determination by »calculation method« using Olympus AU2700 analyzer and by the direct method

Control serum	»Calculation method«					Direct method				
	$\bar{x}$ (μmol/L)	SD (μmol/L)	CV (%)	t	$t_{0.001}$	$\bar{x}$ (μmol/L)	SD (μmol/L)	CV (%)	t	$t_{0.05}$
Normal	38.84	0.733	1.91	2.675	4.781	39.41	0.601	1.52	1.631	2.262
Elevated	52.03	0.918	1.76	1.619	4.781	52.80	0.669	1.27	1.418	2.262

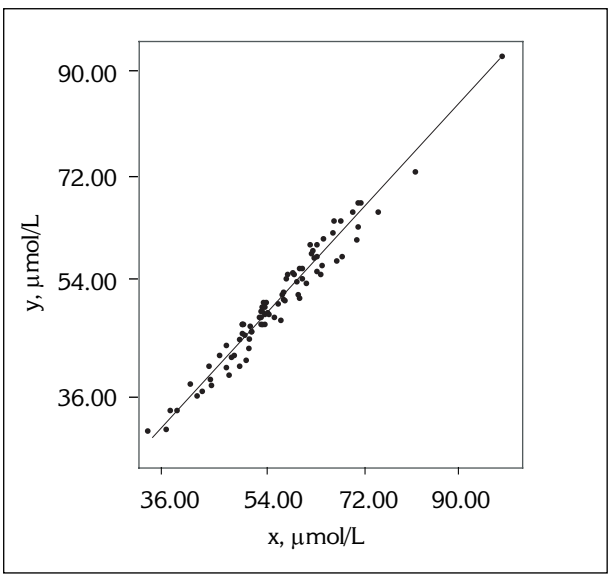

Figure 1. Correlation of the Olympus »calculation method« (y) with the direct method (x) for TIBC determination ($Y = 2.319 + 0.919\ X$; $S_{y,x} = 1.814$; $r = 0.980$; $t_{0.001}{}^{83} = 3.631$)

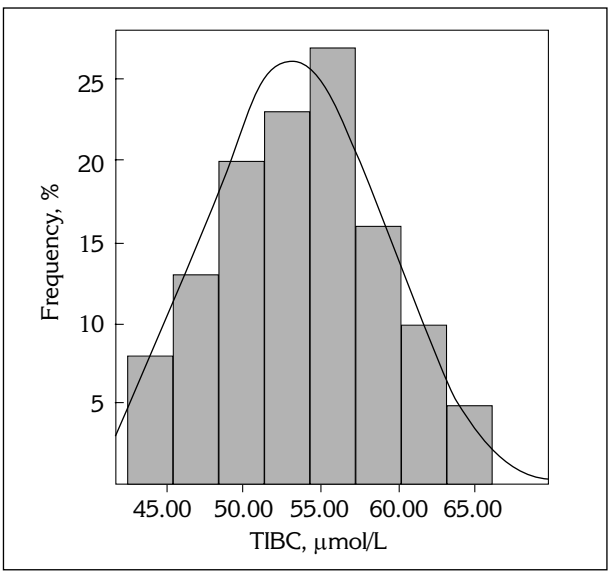

Figure 2. Frequency distribution of TIBC values in a selected population of 125 healthy individuals of both sexes determined by the »calculation method« using Olympus AU2700 automated analyzer

mination of between-day imprecision during the 10 day-interval. From the results presented in *Table I* it can be seen that »calculation method« yielded reproducible results, with CVs ranging from 0.91% to 2.80%, but the direct method achieved better precision, with CVs ranging between 0.82% and 1.71%.

Inaccuracy studies

Based on the data obtained analyzing the two control sera by each method, mean values, standard deviations and coefficients of variations were calculated. The obtained values were compared with declared values using Student's t-test. Accuracy of the »calculation« and the direct method were satisfactory, since statistically significant difference was not obtained for $p=0.001$ and $p=0.05$, respectively. The results are presented in *Table II*.

Correlation studies

For correlation studies 85 serum samples with TIBC values ranging from 30 μmol/L to 95 μmol/L were analyzed on the Olympus AU2700 by the »calculation method« and on the Ilab™ 1800 analyzer using direct method. The results are shown on *Figure 1*. The two methods were highly correlated ($r = 0.980$; $p<0.001$), but with a significant proportional bias (slope = 0.919, $SE_{slope} = 0.020$).

Reference interval

In order to determine reference interval for TIBC determination by »calculation method« using Olympus AU2700 analyzer, its values were determined in 125 serum samples obtained from healthy individuals of both sexes, 57 males and 68 females, with their age ranging between 15 and 80 years. Presentation of the obtained data in the form of histogram showed that the distribution of TIBC values for examined population was Gaussian (*Figure 2*), which is confirmed with Chi-square test. Since the application of Student's t-test evidenced no significant difference between values obtained for males and females ($p= 0.3289$), the unique reference interval for the completely studied population was introduced, using non-

parametric method (10, 11). The obtained reference interval ranged between 42.0 and 64.3 μmol/L. The confidence interval for the lower reference interval limit was 41.6 μmol/L – 44.0 μmol/L, and for the upper reference interval limit the confidence interval was 63.1 μmol/L – 69.7 μmol/L.

Discussion

The serum TIBC varies in disorders of iron metabolism. It is often increased in iron-deficiency anemia, second half of pregnancy, women taking oral contraceptives and viral hepatitis and decreased in chronic inflammatory disorders or malignancies, renal failures, thalasemias and also in hemochromatosis (1).

Most direct TIBC measurement methods require manual procedures that involve centrifugation or pretreatment of serum samples, which makes the automation of the procedure difficult. That is the reason why the »calculation method« is very often used on automated analyzers, even though it requires two separate analyses: serum iron and serum UIBC. That is the case with Olympus AU2700 analyzer. However, the results that we obtained (*Figure 1*) show that calculated TIBC values were significantly lower than those gained by the direct method were. The negative proportional bias was ~ 8%, meaning that results gained by the Olympus »calculation method« were 91.9% of the results obtained by the reference method. These findings are similar with those reported in other studies (9, 12–14).

The results of multiple regression analysis, done by Yamanishi et al. (9) suggest that the UIBC is independently associated with an increase in the difference between the TIBC values converted from transferrin concentrations and the calculated TIBC values. These results indicate that the difference in the direct and the calculated TIBC can be attributed to the low UIBC value determined by the colorimetric assay. The UIBC concentrations are determined as the difference between a known amount of iron added for saturation of transferrin and the amount of iron that was left after binding to unsaturated sites on transferrin in the colorimetric UIBC methods. However, the binding of iron to transferrin is not instantaneous and it takes many minutes to reach completion. As the number of unsaturated sites increases, more time is required for the reaction to come to completion. Therefore, the low UIBC result obtained by the automated UIBC method is attributable to insufficient time allowed for the saturation of transferrin (9, 14).

Also, Gambino et al. (13) reported that the difference between calculated TIBC values and those obtained directly is the consequence of one-point calibration, which is the case on the Olympus AU2700 analyzer. Their results suggest that a two-point calibration method with a protein-based calibrator should be used for colorimetric UIBC methods.

In conclusion, determination of TIBC values in healthy individuals of both sexes by »calculation method« using Olympus AU2700 analyzer enabled determination of the reference interval ranging from 42.0 μmol/L to 64.3 μmol/L (*Figure 2*). The Olympus »calculation method« showed analytical performance similar to that of the direct method and is a precise and accurate procedure (*Tables I* and *II*). The reference interval for TIBC obtained by the direct method in our laboratory is ranging between 44.8 μmol/L and 75.1 μmol/L. Our results confirmed that reference interval for calculated TIBC using Olympus AU2700 analyzer gives lower TIBC values than determined by direct method.

Acknowledgements: The present work was supported by the Ministry for Science, Technologies and Development of Serbia, on the basis of Contract N° 1260.

REFERENTNI INTERVAL UKUPNOG KAPACITETETA VEZIVANJA GVOŽĐA ODREĐIVANOG »METODOM IZRAČUNAVANJA« NA ANALIZATORU OLYMPUS AU2700

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Kratak sadržaj: Ukupni kapacitet vezivanja gvožđa (TIBC) se određuje na analizatoru Olympus AU2700 kao zbir serumskog gvožđa i slobodnog kapaciteta za vezivanje gvožđa (UIBC) – (izračunat TIBC, $TIBC_{cal}$). S obzirom da je analizator Olympus AU2700 nedavno uveden u rad i da rezultati istraživanja poslednjih godina pokazuju da se »metodom izračunavanja« dobijaju vrednosti TIBC-a koje su značajno niže od onih dobijenih direktnim i potpuno automatizovanim metodama, bilo je neophodno da se odredi referentni interval za TIBC, a

prema preporuci da svaka laboratorija treba da utvrdi svoje referentne vrednosti. »Metoda izračunavanja« TIBC-a je pokazala zadovoljavajuću tačnost ($p > 0,001$) i preciznost, sa koeficijentima varijacije od 0,91% do 1,63% pri određivanju u seriji i od 2,30% do 2,80% pri određivanju iz dana u dan. Korelacija između vrednosti TIBC-a dobijenih »metodom izračunavanja« na analizatoru Olympus AU2700 (y) i onih dobijenih direktnom metodom (x) predstavljena je sledećim parametrima: $y = 0,919x + 2,319$ μmol/L ($r = 0,980$; $S_{y,x} = 1,814$; $p < 0,001$); N = 85). Referentni interval je određivan u uzorcima seruma 125 zdravih osoba oba pola, starosti između 15 i 80 godina. Pošto je utvrđeno da vrednosti TIBC-a ne zavise od pola, određen je jedinstven referentni interval za celu ispitivanu populaciju i nalazi se u rasponu od 42,0 μmol/L do 64,3 μmol/L.

Ključne reči: referentni interval, ukupni kapacitet vezivanja gvožđa, Olympus AU2700, slobodan kapacitet vezivanja gvožđa, »metoda izračunavanja«

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Received: September 15, 2004

Accepted: November 15, 2004