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RESEARCH ARTICLE

BLOOD COLLECTION TUBES: AN ASPECT OF PREANALYTICAL VARIATIONS OF COPPER AND ZINC ANALYSES

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ABSTRACT

Blood collection devices are essential for specimen quality. The aim of the present study is to examine the applicability of newly produced Barricor LH tubes for copper (Cu) and zinc (Zn) in view of influence due to tube additives and components. Comparison to already existing tubes for trace elements is made.

Blood is drawn from 32 volunteers into 3 types BD tubes: Barricor LH (REF 365032), K_2EDTA (REF 368381) and Trace element (TE) serum (REF 368380). Cu and Zn concentrations are measured by flame atomic absorption using AAnalyst 400, Perkin Elmer.

Statistically significant differences between Cu results exist depending on tube type ($P<0.001$). Median (IQR) perceived effort levels for the K_2EDTA, Barricor LH and TE tubes running trial are 19.06 (14.76 to 31.59), 19.43 (4.76 to 31.96) and 19.34 (15.01 to 35.23), respectively. Analysis points no statistically significant differences between mean Zn levels for three types of tubes ($P=0.753$). Median (IQR) perceived effort levels for the K_2EDTA, Barricor LH and TE tubes running trial are 10.74 (3.06 to 15.45), 10.25 (2.91 to 14.38) and 10.71 (2.91 to 16.14), respectively. Very strong correlation between the results in three types of tubes for both elements is established: Cu ($R>0.991$) and Zn ($R>0.851$).

Cu and Zn results for Barricor LH plasma tubes are comparable to those, designed for trace analysis: K_2EDTA plasma and TE serum. It could be recommended for each laboratory to test the preferred blood collected tubes for determination of Cu and Zn especially when low concentrations have been suspected.

INTRODUCTION

Laboratory diagnostics is a critical part of clinical decision making. The whole testing process comprises various phases that span from test ordering (pre-preanalytical phase), collection of diagnostic specimens (preanalytical phase), laboratory analysis (analytical phase), reporting of the results (postanalytical phase) and interpretation (post-postanalytical phase) [1]. Errors could arise in all these consecutive steps and they could generate adverse consequences for both results quality and patient safety. International standard for medical laboratories accreditation ISO 15189/2012 posits the necessity of establishment of quality indicators for performance monitoring and evaluation in all critical aspects of pre-examination, examination and post-examination processes [2]. It has been strongly evidenced that the vast majority of all laboratory errors arise in preanalytical phase because of manually intensive activities and lack of standardization there and they comprise up to 75% of total error volume [2]. Proper handling with blood collection devices in prenalaytical phase is essential for specimen quality. It requires an understanding of the complex interactions between the blood collection tubes (BCTs) and blood specimens as potential source of preanalytical variables and errors [3, 4]. Standardization of this initial phase inflicts evaluation of each kind of tubes in the labs, even for particular LOT, thus the preanalytical variation, due to biological material, can be controlled [5]. Working group of International Federation of Clinical Chemistry and Laboratory Medicine (IFCC WG "Laboratory Errors and Patient safety") has developed a proposal for the use of quality indicators for continuous monitoring of the results quality as part of the error management and control the risk for patient safety [2]. Several quality indicators are related to the control of the sample contamination and stability: type of sample, type of containers (for collection, preparation and storage tubes), sample volume, duration and temperature of transport and storage [2].

In determination of trace elements in blood, many possible preanalytical errors exist [6]. Cardinal importance for the results accuracy is the issue of the sample contamination which can arise at any time of the collection, transport and storage, as well as the process of testing itself [7]. All tube components could leach the element into a specimen and/or adsorbs the element from a specimen [3, 6]. Non proper blood collection and handling may affect specimen stability or lead to hemolysis thus resulting in false concentrations. In general, special BCTs are recommended for measurement of trace elements to avoid contamination. The Working Group for the Preanalytical Phase (WG-PRE) develops guidance on validation of BCTs to promote the importance of quality in the preanalytical phase [8]. In 2016 Becton Dickinson launched Barricor™ Lithium Heparin Plasma (Barricor LH) tubes with mechanical separator. Up to then, especially for trace analyses, the following BD tubes were in use: K_2 EDTA Trace Element (K_2EDTA) and Serum Trace Element (TE serum).

Objectives

The main objectives of this research are to study the applicability of Barricor LH tubes in Cu and Zn analyses and to compare the results for both elements obtained by three types BCTs: Barricor LH, K_2EDTA and TE serum vacutainers in aspect of the influence of tube additives and components on the results.

MATERIALS AND METHODS

Study design

This study is conducted in Clinical laboratory Department in St. Ivan Rilski University Hospital, Medical University – Sofia in 2016. Blood is drawn simultaneously from 32 volunteers (males: females = 21:11; age 57±13 yr.) into 3 types BD tubes: Barricor LH plasma (REF 365032), K_2EDTA plasma 10.8 mg (REF 368381) and TE serum (REF 368380). Signed informed consent from all participants has been obtained. The order of blood drawn is based on Clinical and Laboratory Standards Institute (CLSI) guidelines to minimize cross-contamination from tube additives[9]: first - serum, second - LH plasma membrane separator and third - EDTA plasma. All tubes are centrifuged at room temperature and ≤1300 x g; Barricor LH - for 3 min, K_2EDTA and TE serum – for 10 min. No specimens with hemolysis, lipemia or hyperbilirubinemia are observed. All samples (serum/plasma) are treated by similar manner and immediately tested after separation in one and the same analytical run. Cu and Zn concentrations are measured by flame atomic absorption be dropped the acronim (FAAS) using AAnalyst 400, Perkin Elmer, USA.

Statistical analysis

Descriptive statistics is used with calculated of the mean, standard deviation (SD), standard error of the mean (SEM), median, minimum and maximum calculated for all variables. Normality of distribution for investigated parameters is tested by D'Agostino - Pearson test. Difference significance is tested by Friedman test and to examine where the difference actually occurs Wilcoxon signed - rank test is applied. P <0.05 is considered statistically significant. To investigate the correlation between the variables, Pearson correlation coefficient is employed. The data are analyzed by SPSS software, version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

All data, present in Table 1, do not point normal distribution (P<0.05) for both tested analytes, Cu and Zn.

Copper results

Statistical significant difference in Cu concentrations between different types of BCTs is established (P<0.001). Post hoc analysis with Wilcoxon signed - rank tests with a Bonferroni correction applied points a significance level set at P < 0.05. Median (IQR) perceived effort levels for K_2EDTA, Barricor LH and TE tubes running trial are 19.06 (14.76 to 31.59), 19.43 (4.76 to 31.96) and 19.37 (15.01 to 35.23), respectively. Differences in Cu concentrations between all types tested tubes are obviously statistically significant (Tabl. 2).

Very strong correlation between the results in three tube types for both elements is established: for Cu (R>0.991) and Zn (R>0.851).

Table 1 Descriptive analysis of Cu and Zn data in the examination of BCTs

Analyte	*Cu μmol/L*			*Zn μmol/L*		
BCTs	**K_2EDTA**	**Barricor LH**	**TE serum**	**K_2EDTA**	**Barricor LH**	**TE serum**
n	32	32	32	32	32	32
Mean	19.78	20.10	20.48	10.49	10.40	10.64
Median	19.06	19.43	19.37	10.74	10.25	10.71
SD	3.97	4.13	4.50	2.17	2.29	2.39
SEM	0.70	0.73	0.79	0.38	0.40	0.42
Minimum	14.76	14.76	15.01	3.06	2.91	2.91
Maximum	31.59	31.96	35.23	15.45	14.38	16.14

Table 2 Test statistic Wilcoxon signed – rank test in Cu and Zn analysis

		Blood collection tube		
		Barricor LH to K_2EDTA	***K_2EDTA to TE tubes***	***Barricor LH to TE tubes***
Cu	Z	-3.431	-4.752	-2.974
	P-value	0.001	0.003	0.000
Zn	Z	-.679	-.412	-.977
	P value	0.497	0.681	0.328

Zinc results

Friedman test points no statistically significant differences between Zn levels for three types of tubes (P=0.753). Wilcoxon signed - rank test shows similar results with all P-values being >0.05 (Tabl. 2). Median (IQR) perceived effort levels for the K_2EDTA, Barricor LH and TE tubes running trial are 10.74 (3.06 to 15.45), 10.25 (2.91 to 14.38) and 10.71 (2.91 to 16.14), respectively.

DISCUSSION

To avoid systemic contamination all the containers in determination of trace elements should be examined by application of specialized schemes for control, even in cases with changing the serial product number [11]. All components of blood collection tubes could be possible factors for interference on the final results: a wall of the tube, a rubber stopper, a lubricant, an anticoagulant, or an activator of clotting, gel separator and a surfactant [3]. Centrifugation is also of importance.

All studied tubes are made of polyethylene terephthalate, therefore, the wall material is not expected to affect, in comparison evaluation, the measured analyte concentrations substantially. In all tested samples in this work, no hemolysis has been observed. Membrane in Barricor LH tubes creates a stabile robust barrier between the plasma above and blood cells below. Mechanical separator and low zinc stopper improve separation time (to be just in 3 min), sample stability, enables reliable zinc testing and could eliminate possible interferences due to gel adsorption or to release gel particles or silicone oil into the sample. No observed hemolysis, even in single case, is in support of effective separation and keeping the integrity with no destroying of erythrocytes. This consideration is absolutely important for minimizing interferences, due to hemolysis, especially pronounced on Zn results because of strongly higher concentration of the element in these blood cells than in serum/plasma.

Plasma offers several advantages over serum as specimen in laboratory testing: less preanalytical delay, larger sample yield, minimizing of coagulation-induced influences, reduced in vitro hemolysis [11]. The type of the used anticoagulant is of a major importance. Most anticoagulants could express great risk for sample contamination because their high affinity to bind metal ions. Hence, they must with purity, acceptable enough for trace analysis. The most commonly used anticoagulants are ethylene-diamine-tetra-acetate (EDTA), heparin and citrate[3]. EDTA allows sample storage longer than 36 hours but it can bind certain metal ions. EDTA chelates copper ions (with higher affinity than for zinc) and draws water from cells to artificially dilute plasma due to osmotic redistribution, arising in addition of this anticoagulant. These both effects could explain lower Cu in EDTA plasma, observed by us, than in serum and LH plasma. Intriguingly, Cu values in plasma, obtained by Barricor LH tubes are higher than those in serum. It seems like membrane materials (elastomer or higher density base material or both) release certain amount, even small, of copper into plasma specimen. No significant differences in Zn results are established by us between three types of specimens although with tendency for higher Zn in EDTA plasma and serum (almost comparable) than in Barricor LH plasma. These observations might be attributed to negligible leaching of Zn by anticoagulant EDTA and releasing of the element from erythrocytes and thrombocytes during clot formation. Obviously, both effects are too small with no causing statistical differences between Zn results. Eventual impact of different clotting activators on the measurement of trace elements has not been reported in view point of contamination risk [3, 8], supported also by the present data. Employed lubricants and surfactants to facilitate specimen quality are no source of contamination, as seen also by our data.

There are no significant methodological problems or high risk of contamination for copper measurements in biological fluids.[9] Observed by us differences between Cu values for three types BCTs are significant, but as absolute concentration are less than 1 μmol / L (0.7 μmol/L) as being not essential for clinical interpretation in laboratory evaluation of either deficiency or overload. New membrane mechanical separation for heparin plasma is compatible with current preanalytical requirements in phlebotomy and clinical laboratory practice. Very low Zn content, confirmed by the present study, enables to achieve reliable results in Zn testing. Understanding the factors of biological and analytical variations in Cu and Zn analysis is fundamental for the interpretation of the results in certain clinical conditions. The medical importance of the trace elements for pathogenesis, diagnostics, predictive significance and the treatment extends over a wide range of socially important diseases such as diabetes mellitus, chronic hepatitis, neurodegenerative diseases, cardiovascular diseases, etc. Undoubtedly, standardizing Cu and Zn analyses, with special focus on preanalytical phase, is of a key importance for quality assurance in laboratory testing of both parameters. Such approach allows providing better health cares and improves patient safety.

CONCLUSION

The term “preanalytical phase” is firstly used in the 70ies of the twentieth century and continues to be a challenge in recent laboratory work [12]. Barricor LH plasma tubes give comparable results for Cu and Zn in consistency with declared by manufacturer specifications for trace analysis using K_2EDTA and TE serum tubes. The established differences in Cu concentrations are negligible small: less than 1 μmol /L. One limitation of the present research could be related to comparatively low number of the studied individuals. In any case, this study is an attempt for more detailed understanding of some prenalytical variables in Cu and Zn measurements. In addition, it could be recommended for each laboratory to test the preferred blood collected tubes for determination of both trace elements, especially when low concentrations have been suspected.

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