doi: 10.5505/tjb.2013.83702

Technical Report [Teknik Rapor]

Yayın tarihi 30 Eylül, 2013 © TurkJBiochem.com

[Published online 30 September, 2013]

An underestimated preanalytical error source: Centrifuge temperature

[Gözden kaçan bir preanalitik hata kaynağı: Santrifüj sıcaklığı]

Fatma Meriç Yılmaz[1,2],
Serkan Kıral[1],
Nihal Boğdaycıoğlu[1],
Sema Uysal[1]

[1]Ankara Numune Education and Research Hospital Clinical Biochemistry Laboratory
[2]Yıldırım Beyazıt University Medical Faculty, Clinical Biochemistry Department

Yazışma Adresi
[Correspondence Address]

Fatma Meriç Yılmaz, Ass. Prof.

Ankara Numune Education and Research Hospital
Clinical Biochemistry Laboratory
Altındağ, Ankara
Tel. 00 90 312 5084467
Fax. 00 90 312 3621364
E-mail. fatmamericyilmaz@hotmail.com

Registered: 3 December 2012; Accepted: 10 June 2013
[Kayıt Tarihi: 3 Aralık 2012; Kabul Tarihi: 10 Haziran 2013]

ABSTRACT

Objectives: The study demonstrates a gel problem sourced from the internal heat of centrifuges without temperature control. It was planned to investigate the internal temperatures of temperature controlled centrifuges and the centrifuges without temperature control and the effect of centrifuge temperature on enzymes and thyroid panel.

Design and Methods: Internal heat of the centrifuges were monitored in the most busy working hours. Blood samples were obtained from 42 patients to two separate biochemistry tubes. One sample of the same patient was centrifuged with a temperature-controlled centrifuge (Group 1) and the other was centrifuged with a centrifuge without temperature control (Group 2). AST, ALT, GGT, LDH, total protein, albumin, TSH, fT3 and fT4 levels were determined in the same run of the analyzer.

Results: ALT and TSH values in Group 2 were significantly lower, fT4 values were significantly higher than Group 1.

Conclusions: Laboratories should be aware of a possible internal heat production in centrifuges without temperature control and gel tubes should be centrifuged in temperature controlled centrifuges especially in centrifuge units with high working load.

Key Words: blood specimen collection, specimen handling, temperature

Conflict of Interest: The authors declare that there is no conflict of interest

ÖZET

Giriş ve amaç: Çalışma, ısı kontrollü olmayan santrifüjlerin iç sıcaklığından kaynaklanan bir jel problemine dikkat çekmekte ve ısı kontrollü olan ve olmayan santrifüjlerin iç sıcaklıkları ölçülerek santrifüj sıcaklığının enzim düzeyleri ve tiroid paneline olan etkisinin araştırılması amaçlanmaktadır.

Gereç ve Yöntem: Kan alımının ve santrifüjünün yoğun olduğu saatlerde ısı kontrollü olan ve olmayan santrifüjlerin iç sıcaklıkları ölçülerek kaydedildi. 42 hastadan eş zamanlı olarak düz biyokimya tüplerine alınan ikişer tüp kan, ısı kontrollü olan (Grup 1) ve olmayan (Grup 2) santrifüjlerde santrifüj edildi. Daha sonra eş zamanlı olarak AST, ALT, GGT, LDH, total protein, albumin, TSH, fT3 ve fT4 ölçümleri yapıldı.

Bulgular: Grup 2 deki ALT ve TSH değerleri Grup 1'den anlamlı derecede düşük, fT4 değerleri ise anlamlı şekilde yüksekti.

Sonuç: Isı kontrollü olmayan santrifüjlerin kullanımının gerek bu çalışmada değerlendirmeye alınmamış analitler üzerinde gerek jelli tüp kullanıldığında tüpün jel yapısı üzerine olumsuz etkileri olabileceği göz önünde bulundurulmalıdır.

Anahtar Kelimeler: kan numunesi toplanması, örnek işlenmesi, sıcaklık

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

There are three phases of laboratory testing known as preanalytical, analytical and postanalytical phases. Preanalytical phase include specimen collection, transport and processing and is the most vulnerable part of the total testing process. Centrifugation of the samples is a part of the preanalytical phase and can be performed with temperature–controlled centrifuges or classical multipurpose ones without temperature control.

Recently, we had a problem about electrolyte testing in our central biochemistry laboratory and it has been reported to be related with a gel problem from serum seperator tubes by the technical staff. After we began to investigate the source of the error, we have noticed that the centrifuges without temperature control, which we use in central specimen acceptance and processing unit, might generate internal heat which is not suitable for gel tubes. In order to clarrify the problem, we monitored internal heat of temperature controlled and classical centrifuges and we observed that the internal heat of classical centrifuges has rised to a maximum heat of 57 ^{0}C in the most busy hours. After we re-arranged the working plan in the processing unit to centrifuge the gel tubes in temperature-controlled centrifuges, the gel problem we had faced with in auto analyzers has solved. However an internal heat of 57 ^{0}C was much more higher than we expected and we thought that this high temperature could effect many analytes independent from the gel problem.

In this study our aim was to investigate whether the internal heat of centrifuges without temperature control effect specific analytes; namely AST, ALT, GGT, LDH, total protein, albumin, TSH, fT3 and fT4 levels which have protein structures and known to be effected by high temperatures.

Material and Methods

The study was performed in Ankara Numune Education and Research Hospital Central Biochemistry Laboratory. Internal heat of two centrifuges, one temperature-controlled (NF 1200R; Nüve, Turkey) and one centrifuge without temperature control (NF1200; Nüve, Turkey) were monitored in the most busy working hours using a calibrated digital thermometer (Thermomark; Germany). In order to investigate the temperature effect, blood samples were obtained from 42 patients to two separate biochemistry tubes (non-additive serum tubes without gel, Becton, Dickinson and Company, Franklin Lakes, NJ 07417 USA). Samples were obtained with 21 gauge needles immediately and consequtively after the tourniquet application in maximum 1 minute. One sample of the same patient was centrifuged in NF 1200R temperature-controlled centrifuge (Group 1) and the other was centrifuged in NF 1200 centrifuge without temperature control (Group 2) 10 min at 1300 *g* .

Routine chemistry and immunochemistry tests were studied on a Roche Modular PP (Roche Diagnostics Limited, England) and a Roche Cobas e-601 analyzer (Roche Diagnostics Limited, England) with original reagents, respectively. All of the tests were performed at the same day and paired tubes were studied duplicate in the same run of the analyzer.

This study was conducted at Ankara Numune Teaching and Research Hospital with approval of the local Ethics Committee and suitable with the Declaration of Helsinki.

Statistical Analysis

Values are reported as mean ± SD for quantitative variables. A *p* value less than 0.05 was considered statistically significant. Differences between the groups were compared using paired *t* test. All statistical calculations were made using SPSS[â] for Windows 13.0 (SPSS Inc. Headquarters, Chicago, lll., USA) software program. Percent differences for each analyte was calculated by the following formula: % difference= [(Concentration Group 2-Concentration Group 1)/ Concentration Group 1]*100. CLIA Acceptable Performance (AP) limits were used for the interpretation of the results [1].

Results

The internal heat values determined during the most busy working hours are given in Table 1. Centrifugation of the samples were performed in the 5th cycle, in which the temperature has been measured 19 ^{0}C for NF 1200R, 57 ^{0}C for NF 1200.

The results of the study are summarized in Table 2. According to the paired t test analysis ALT, fT4 and TSH values showed statistically meaningful difference between the groups. ALT and TSH values in Group 2 were significantly lower, fT4 values were significantly higher than Group 1 [Table 2]. According to the CLIA Acceptable Performance (AP) Criteria, 3 values for ALT, 5 values for AST, one value for albumin exceeded the AP limits.

Table 1. Minimum and maximum temperatures measured during centrifuge periods.

Hour	NF1200 (C^0)	NF1200R (C^0)
9.30 a.m.	26.4-40.7	15.8-21.7
10.30 a.m.	26.1-39.4	15.7-16.8
10.42 a.m.	35.2-42.4	16.6-22.6
10.52 a.m.	50.3-54.7	15.9-19.5

Table 2. Comparison of the determined analytes between the groups.
*Statistically significant ($p<0.05$)

Analytes	Group 1	Group 2	*p*	CVa	% Difference (%95 CI)	CLIA AP
ALT	26,9±18,3	26,1±18,1	0.04*	3.30	(-18.8/20.7)	±20%
AST	23,07±11,9	23,66±11,7	0.08	1.66	(-15.9/24.7)	±20%
GGT	25,6±22,3	25,5±22,40	0.79	2.73	(-15.1/8.27)	NA
LDH	403,1±183,4	402,8±187,9	0.96	3.18	(-10.5/12.7)	±20%
T-Protein	74,7±4,02	74,6±3,99	0.59	1.62	(-2.68/2.84)	±10%
Albumin	45,3±3,16	45,1±3,49	0.37	3.70	(-4.08/2.56)	±10%
fT3	3,25±0,39	3,23±0,39	0.31	2.65	(-3.85/4.16)	±3SD
fT4	1,18±0,18	1,19±0,18	0.002*	1.75	(-1.82/4.29)	±3SD
TSH	2,50±1,76	2,48±1,73	0.012*	1.00	(-4.08/2.56)	±3SD

Discussion

The aim in the laboratory testing is to obtain accurate and precise results in order to improve laboratory-related patient outcomes. In the recent years there have been improvements in laboratory performance, especially in analytical quality through proficiency testing. However, improvement of laboratory performance does not automatically indicate a reduction in the number of errors, both analytical and organizational [2]. In the studies performed on laboratory error rates, it has been shown that the largest percentage of laboratory errors occur in preanalytical phase [3-5].

Preanalytical phase is defined as the processes prior to the actual analysis of the sample and include steps needed to obtain the primary sample and to obtain the analytical specimen like plasma, serum, cells etc. Majority of the analysis in a clinical biochemistry laboratory requires serum or plasma as the analytical specimen and obtaining serum from a primary sample is an important step of preanalytical phase. Centrifuges are designed to seperate liquids of different densities by applying centrifugal force. Different types of rotors, swing-out and angle rotors, can be fitted to the centrifuges. Centrifuges can be designed as to be temperature-controlled or without temperature control. According to the NCCLS global consensus guideline, centrifuges can generate internal heat that may be inappropriate for analyte stability and laboratories should have temperature-controlled centrifuges for temperature-sensitive analytes [6].

Previously, effect of centrifuge temperature has been evaluated and it has been reported that routine coagulation testing has not been effected for centrifuge temperatures of +4 ^{0}C, +12 ^{0}C or +21 ^{0}C [7]. Centrifuge temperature has been reported to have a significant effect on flavanone distribution in the extracts [8].

Serum blood collection tubes can be either gel containing serum seperator tubes or non-gel serum tubes. The gel forms a physical barrier between serum or plasma and blood cells during centrifugation. The gel is said to be composed of inert components, which are part of a polyester-based proprietary formulation and gel containing tubes are recommended to be centrifuged 10 minutes at a centrifugal force of 1300-2000 g and a temperature of 20-25 C^0 by the manufacturer [9]. In a previous study, transport and temperature effects on measurement of serum and plasma potassium has been investigated and collection into gel-separation tubes has been recommended in order to ensure less variation due to temperature and time to centrifugation [10]. Biochemistry auto analyzers use the same sample prob for the analyses after a cleaning process following each pipetting. Decomposition of the gel barier due to high temperature exposure during centrifugation might occur and gel particles in the serum might effect the pippetting process. We have faced with the gel problem in electrolyte testing because sera is first pipetted in ISE unit of the analyser with electrolyte sample prob, than the sample is transferred to photometric unit. The technical report about the problem had mentioned about the gel contamination in the electrolyte sample prob and the laboratory has contacted with manufacturer of serum gel tubes (Becton, Dickinson and Company). Second technical report about the reason of gel decomposition pointed out the problem in our central laboratory specimen acceptance and processing unit. Our central laboratory is one of the biggest laboratories in Turkey and a number of 4000-5000 tubes are accepted and processed in a working day. There are four centrifuges

in the unit; two are temperature controlled, two are without temperature control. Our working plan had been arranged to use temperature controlled centrifuges for temperature sensitive analytes like ACTH, Vitamin D etc. However in the technical report the internal heat of four centrifuges were determined and has been reported that the centrifuges without temperature control have been used again and again without proper breaks because of the working load and they have generated internal heat which is not suitable for gel tubes. We re-arranged the working plan in processing unit to centrifuge the gel tubes in temperature controlled centrifuges; technical staff of the autoanalyzer cleaned the probes and after an observation period we decided that the problem has been solved and the source of the problem had been the internal heat of the centrifuges without temperature control.

Our hypothesis after this experience was analytes which have a protein structure could also be effected if the internal temperature rises so much, independent from the gel problem. The results of the study showed that although the magnitude does not seem to be clinically important according to the CLIA limits; ALT, fT4 and TSH levels has changed significantly with the use of centrifuges without temperature control.

In conclusion, laboratories should be aware of a possible internal heat production in centrifuges without temperature control and gel tubes should be centrifuged in temperature controlled centrifuges especially in centrifuge units with high working load.

Acknowledgements. *The authors thank* Çisem Tarhan Kıran for technical assistance.

Conflict of Interest: The authors declare that there is no conflict of interest.

References

[1] westgard.com/biodatabasel.htm Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation, **Updated for 2012. 10.11.2012, 00:25**

[2] Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in laboratory medicine. Clin Chem. 2002;48:691-8. [Pubmed: 11978595]

[3] Goldschmidt HMJ, Lent RW. Gross errors and work flow analysis in the clinical laboratory. Klin Biochem Metab. 1995;3:131–40.

[4] Stahl M, Lund ED, Brandslund I. Reasons for a laboratory's inability to report results for requested analytical tests. Clin Chem. 1998;44:2195–7. [Pubmed: 9761256]

[5] Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. Clin Chem 1997;43:1348–51. http://www.clinchem.org/content/43/8/1348.short

[6] Calam RR, Bessman JD, Ernst AJ, Smith S, Szamosi DI, Warunck DJ, Wiseman JD. Procedures for the handling and processing of blood specimens; Approved Guideline. NCCLS Document H18-A3. 2004; 24: 38.

[7] Lippi G, Salvagno GL, Montagnana M, Poli G, Guidi GC. Influence of centrifugetemperature on routine coagulation testing. Clin Chem. 2006;52:537-8

[8] Chebrolu KK, Jayaprakasha GK, Jifon J, Patil BS. Optimization of flavanones extraction by modulating differential solvent densities and centrifuge temperatures. Talanta. 2011 15;85:353-62.

[9] Becton, Dickinson and Company, Franklin Lakes, NJ 07417 USA. http://www.bd.com/

[10] Seamark D, Backhouse S, Barber P, Hichens J, Salzmann M, Powell R. Transport and temperature effects on measurement of serum and plasma potassium. J R Soc Med. 1999;92:339-41.