Kocaeli Med J 2019; 8; 2:46-53

Orta ve uzun süreli saklanan kan örneklerinde etanol düzeyleri nasıl etkileniyor?

What happens to ethanol levels during medium and long-term storage of blood specimens?

Nahide Ekici Günay
Sağlık Bilimleri Üniversitesi, Kayseri Şehir Eğitim ve Araştırma Hastanesi, Klinik Biyokimya Departmanı, Kayseri, Türkiye

ÖZ

GİRİŞ VE AMAÇ: *Etanol analizinde işleme yöntemleri ve depolama sürelerinin standardizasyonu adli ve toksikolojik disiplin için önemlidir. Bu geriye dönük çalışma, farklı depolama süreleri için +40C'de saklandıktan sonra serum örneklerinde etanol davranışını araştırmak için yürütülmüştür.*

GEREÇ VE YÖNTEMLER: *Travma ve adli nedenlerle etanolün ölçülmesi gereken 183 olgudaki kan örnekleri kullanıldı. Yedi çalışma grubu oluşturuldu (1 ile 36 ay arasında değişen). Aynı tüpler yeniden analiz edildi ve sonuçlar ilk ölçümlerle karşılaştırıldı.*

BULGULAR: *Koruyucu kullanılmayan kan etanol numuneleri +40C 'de saklandığında 3 yıl civarında stabil olarak kalabildiği kaydedilmiştir. İlk ayda % 4.1 oranında düşüş olmuştur. Ardından, etanol konsantrasyonları için 5 aylık dönemden itibaren 26 aya yükseliş eğilimi başlamış ve daha sonra durağan sürece girilmiştir (26 ve 36 ay, %7.4 ve % 7.4). Negatif numuneler için orta ve uzun vadeli süreli gruplarda etanol üretimi gözlenmemiştir. Düşük konsantrasyonlu etanol numuneleri nispeten daha fazla azalma göstermiştir. Kan etanol konsantrasyonları yaralanma ciddiyet puanlaması (YCP) ve Glasgow Koma Ölçeği Puanları (GKÖP) ile anlamlı bir şekilde ilişkili değildi (P <0.862, P <0.675).*

TARTIŞMA ve SONUÇLAR: *Çalışmanın yapıldığı laboratuvarda, katkısız numuneler için saklama süresinin 3 yıla kadar olabileceği önerildi. Ek olarak, şahit örnek olarak ideal bir örnek türü olmasa da, zorunlu durumlarda orta ve uzun süreli depolama için serum örnekleri kullanılabilir.*

Anahtar Kelimeler: *etanol, preanalitik, depolama, adli*

ABSTRACT

BACKGROUND: *The standardization of processing methods and storage duration for ethanol analysis is important for the forensic sciences and toxicology. This retrospective study was conducted to investigate ethanol stability in serum specimens stored at 4°C for distinct periods.*

MATERIAL AND METHODS: *The study was carried out by using blood specimens from 183 patients in whom ethanol measurement was ordered due to trauma and forensic causes. Seven study groups were created (ranging from 1 to 36 months). The same tubes were re-analyzed for blood ethanol concentration and the results were compared to the first measurements.*

RESULTS: *It was recorded that the stability was 3 years around for non-preservative blood ethanol specimens at +4oC. There was a 4.1% decrease in the first month. Then, an elevation trend for ethanol concentration was observed from month 5 to 26 and ethanol concentration was stabilized thereafter for month 26 and 36: 7.4% and 7.4% respectively. No ethanol increase was observed for negative specimens in medium- and long-term storage groups. Ethanol specimens with lower concentration exhibited relatively lower reduction. No significant association was shown between blood ethanol concentration and Glasgow Coma Scale Scores (GCSS) and/or Injury Severity Score (ISS) (P<0.862 and P<0.675).*

DISCUSSION AND CONCLUSIONS: *In the laboratory where the study was carried out, it was proposed that the storage period could be up to 3 years for samples without additives. Additionally, although it is not an ideal sample type as a split sample, serum samples are usable for medium and long-term storage in obligatory situations.*

Keywords: *ethanol; pre-analytical; storage; judicial.*

İletişim / Correspondence:

Dr. Nahide Ekici Günay
Sağlık Bilimleri Üniversitesi, Eğitim Ve Araştırma Hastanesi, Klinik Biyokimya Departmanı, Kayseri, Türkiye
E-mail: edihan30@yahoo.com
Başvuru Tarihi: 24.04.2018
Kabul Tarihi: 09.05.2019

INTRODUCTION

In many hospitals, blood ethyl alcohol measurements are routinely performed in medical laboratories serviced to emergency department and may be required both in criminal cases and after medical treatment1,2.

Prolonged forensic procedures and/or some laboratory manuals necessitate that specimens must be stored for a long period of time under appropriate conditions, which can lead to various difficulties such as equipment, location, environmental conditions and technical requirements during the storage of "split specimens"3.

If ordered by the judicial authorities, request for analysis and re-evaluation of blood specimen may be made, which is a commonly encountered situation4.

The judging and insurance processes related to a serious accident may depend almost exclusively on the level of ethanol determined by analysis. Obtained test results can sometimes vary depending on the preanalytical storage duration of the blood specimens, which may make it difficult for specialists to interpret the results of these reanalyzed ethanol concentrations5. The ethanol concentration may vary depending on elapsed time after the incident and the request for analysis by the court. In this process, assessment of the stability of the specimens during storage is considerable.

It has been reported that changes of ethanol levels occur in blood specimens that have been stored until analysis. This change is related to the length of the waiting period, whether or not a preservative is used in the specimen tube, the type of preservative and the storage temperature6, 7.

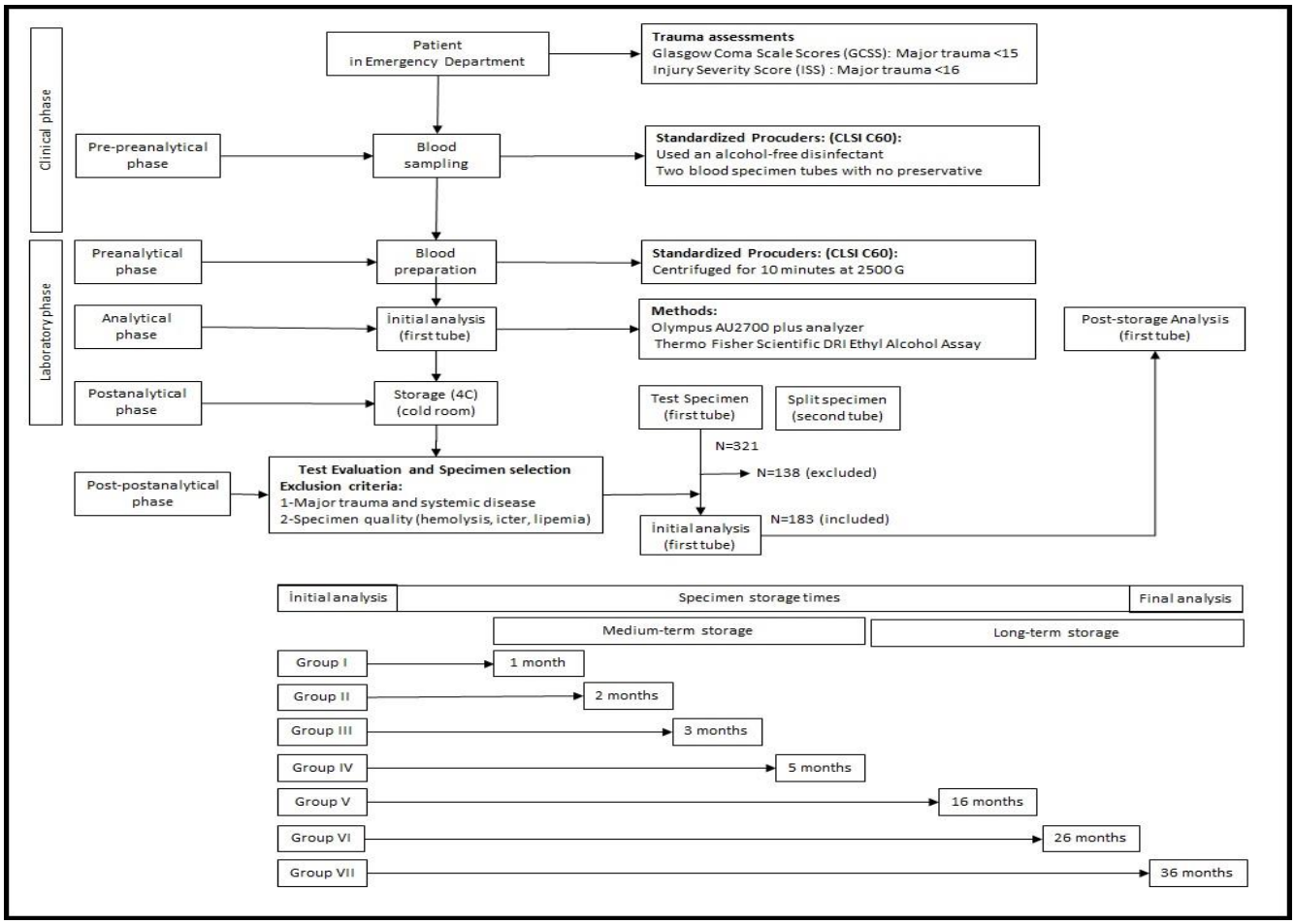
The change is mostly reported in the examples held that the level of ethanol has a tendency to decrease, which was shown to be due to variety of reasons such as evaporation, chemical oxidation and microbial alcohol metabolism8. In this current study, we aimed to determine changes in ethanol concentrations in blood serum specimens after medium- and long-term storage durations. Based on these results, we attempted to establish a local protocol for storage durations and preservation of split specimens for ethanol measurements.

MATERIALS AND METHODS

Study Design

This retrospective study was conducted on 183 blood ethanol specimens obtained from cases, in which ethanol analysis was ordered, at the XXX Training and Research Hospital with collaboration of Biochemistry and Emergency Medicine Clinics collaboration. Prior to the study, institutional scientific research ethics committee approval was obtained to review the clinical records of cases. Personal identifiers were entirely removed and the records were analyzed anonymously. For inclusion to the study, a lower cut-off concentration was set at 0.5 promille (50 mg/dL), which is permitted legal blood ethanol limit for private car driving in Turkey. The upper limit was set at 4.5 promile (450 mg/dL) due to presence of a few cases where ethanol levels exceed this limit.

The study design is shown in Flowchart 1. Once the selection of the specimens was completed, seven groups were identified. The groups were formed based on the time elapsed between the first and second analysis. The storage times ranged from one (1 month–Group I) to 36 month (Group VII) after first measurement. The group names were given based on the elapsed time between the first and second analyses. Medium-term storage groups were formed as 1, 2, 3, 5 months while long-term storage groups as 16, 26 and 36 months. Medium-term storage duration of blood ethanol groups were determined as Group I (1 month), Group II (2 months), Group III (3 months) and Group IV (5 months) while long-term duration were determined as Group V (16 month), Group VI (26 month) and Group VII (36 month).



Flowchart. The study design

Subjects

Study exclusion criteria of the specimens were made in two stages. In the first stage, the absence of systemic disease and no known clinically relevant observations was confirmed on the cases review once the presence of major trauma was established. Major trauma assessment was performed with Glasgow Coma Scale Scores (GCSS) and/or Injury Severity Score (ISS). A GCSS below 15 points, and/or an ISS of 16 points were classified as major trauma9. Each coma scores was recorded based on the Trauma Registry System at Level I trauma center of our hospital. An exclusion criterion in the second stage is that if there is hemolysis of the blood specimens received, or the specimen has been identified as having a lipemia/icteric appearance10. After the initial analysis made at the time of arrival at the hospital, blood specimens were stored in appropriate conditions11. In the study, background check of the case data showed that those conditions acting on the metabolism of alcohol or the presence of any systemic disease had been excluded.

Blood sampling

The blood collection and handling process was standardized as recommended by the Clinical and Laboratory Standards Institute (CLSI)12. Peripheral venous blood specimens were drawn from the antecubital fossa of the forearm (each with two tubes of blood) and were collected in 10 ml red-top vacutainer tubes with no additive (Becton, Dickinson and Company Franklin Lakes, NJ, USA). During all analyzes, an alcohol-free disinfectant was used in all equipment surface cleaners and personal hygiene. The venipuncture zone was primarily cleaned with baticcon and then dried with sterile dry gauze13. The vacutainer tube immediately closed and all blood tubes were filled to the indicated line. Tubes were centrifuged for 10 minutes at 2500 G and analyzed within an hour. For the second analysis, to make sure that the working specimens were not degraded, blood specimens at the end of the first study were closed tightly. One tube from each case was analyzed and all blood specimens were then stored at +4oC in a cold room until the second analytical measurement.

It has been noted that the tube levels are maximally and uniformly on the same line14. The cold room temperature, moisture and lighting conditions were followed in accordance with established standards of quality control. The relative humidity of the cold room was monitored as by ensuring that 45±5% with a digital humidity meter connected to a power source. The blood specimens were placed in boxes designed to keep them in the dark during storage periods for order to protect the specimens from light. The tubes opened more than one, hemolyzed, lipemic, icteric, and specimens of those with systemic disease that could affect ethanol metabolism were excluded from the study.

METHODS

The ethanol concentrations were measured on an Olympus AU2700 plus analyzer (Beckman Coulter, Tokyo, Japan) using Thermo Fisher Scientific DRI Ethyl Alcohol Assay (Microgenics, USA) by commercial bioenzymatic reagents according to the established alcohol dehydrogenase method. The enzymatic method used in the assays is based on the principle of acetaldehyde fermentation using coenzyme nicotinamide adenine dinucleotide (NAD) reduced to form NADH simultaneously with alcohol dehydrogenase catalysis. The enzymatic reaction was monitored spectrophotometrically at 340 nm. Thermo Fisher Microgenics TRI brand controls (0 mg/dL and 300 mg/dL) and calibrators (0 mg/dL and 100 mg/dL) were used. The measuring range of the method is 10 mg/dL to 600 mg/dL, and shows a linear regression with a correlation coefficient of 0.982. According to the analysis, severe hemolysis (800 mg/dL), icterus (30/mg/dL) or lipemia (1000 mg/dL) interferences are not shown. The intra-assay coefficients of variation (CVs) of the ethanol measurement method were found to be 1.1 for 100 mg/dL and 0.5 for 300 mg/dL, respectively. The tests were worked in duplicate manner and internal quality control specimens were made to run to for each analysis. The opened tubes were reanalyzed for ethanol measurement by the same laboratory that performed the initial testing using the same method and same instrumentation.

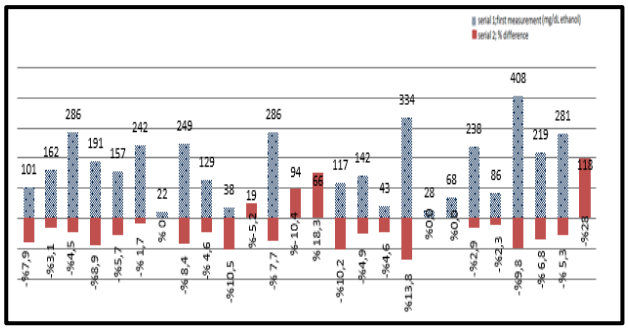
Statistical analysis

The normality of distribution of all variables was tested using the Shapiro-Wilk test. Data were described by the mean±standard deviation (SD) for data with normal distribution. All analyses were performed by using SPSS software (version 21.0 for Windows; SPSS, Inc., Chicago, IL, USA). The intergroup differences were assessed by ANOVA test. A paired student t-test was applied to all groups, aiming for a 95% confidence level. The relationships between the mean concentrations and deviations from the initial concentrations (%) were analyzed by Spearman's rank correlation coefficient. Associations between first ethanol concentrations and coma scores (ISS and GCSS) were also assessed by using same correlation coefficient. In each group, the percentage difference between first and second ethanol readings was calculated with excel program.

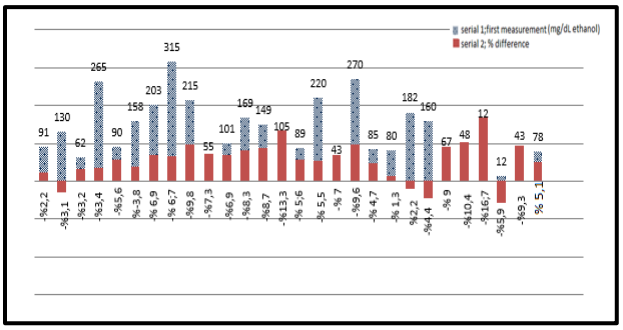
RESULTS

During study period, blood ethanol concentrations from 321 cases were screened and 183 specimens were included in the study according to the exclusion criteria. The remaining 138 judicial specimens were, analyzed and stored as required, but not included in the study. The study groups consisted of 16 trauma patients and 167 non-traumatic judicial cases. For these patients (2 women, 181 men) the age range was between 18 and 45 years. All specimens were analyzed for their ethanol concentrations at admission to emergency room. The lowest ethanol reading was 12 mg/dL, while the highest level was measured as 483 mg/dL. The distribution of results for each specimen within the group, percentage changes (Graph I-VII), and the first-final mean ethanol measurements for each group are shown in Table 1 and Graph VIII.

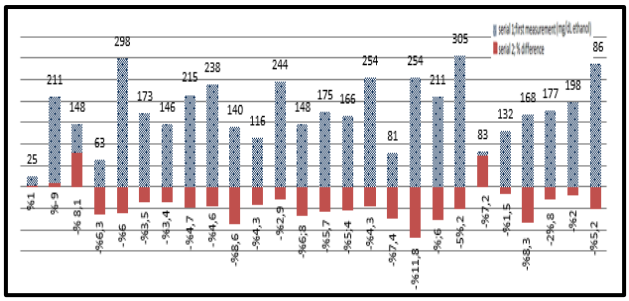
There was no significant correlation between blood ethanol concentration and GCCS (13(4-15)) $P<0.675$) or ISS (8.14(0-75), $P<0.862$). In each group, two ethanol negative specimens were also tested and no ethanol increase was observed in medium- and long-term duration groups.



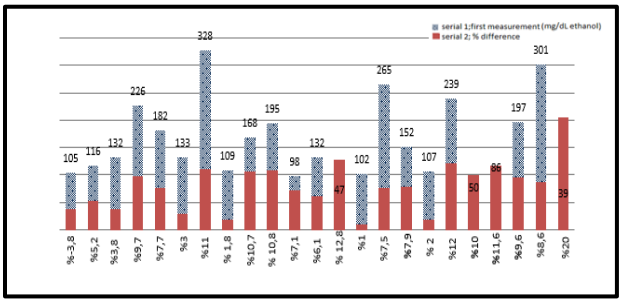
Percent Differences Against Ethanol Concentrations Graph – Group I (n:26)



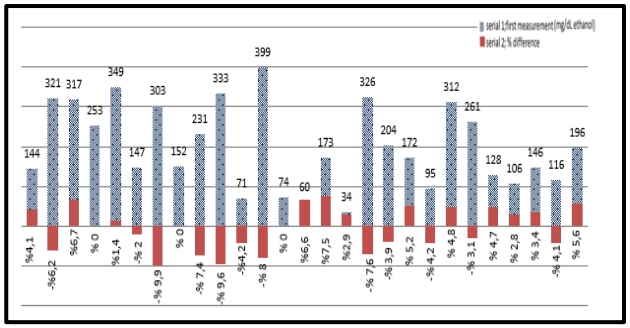
Percent Differences Against Ethanol Concentrations Graph – Group V (n:28)



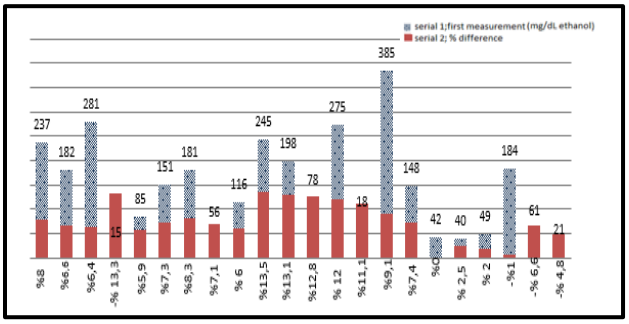
Percent Differences Against Ethanol Concentrations Graph – Group II (n:26)



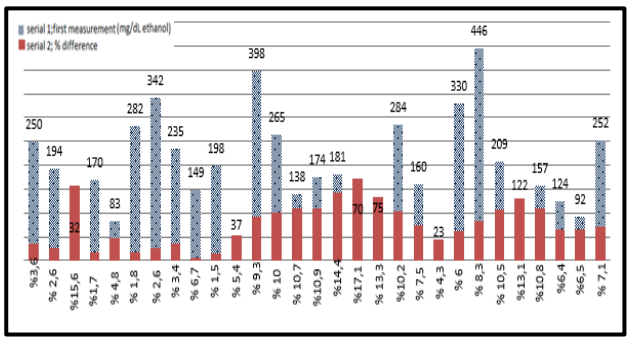
Percent Differences Against Ethanol Concentrations Graph – Group VI (n:23)



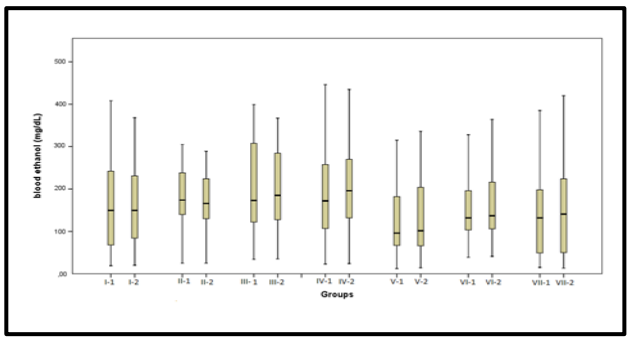
Percent Differences Against Ethanol Concentrations Graph – Group III (n:27)



Percent Differences Against Ethanol Concentrations Graph – Group VII (n:22)



Percent Differences Against Ethanol Concentrations Graph – Group IV (n:29)



Graph VIII. The Average Ethanol Concentrations of Groups at the First Measurement

Table 1. Comparisons of initial and post-storage ethanol concentrations

	n	First analyse (M±SD)* (mg/dL)	Second analyse (M±SD)* (mg/dL)	T.dif	T. dif SD	T.dif %	r©	Sig.#	t	*P*
1 month (Group I)	26	160.9±78	154.4±88	- 6.6	17	-4.1	,999	,000	-1.9	0.063
2 month (Group II)	26	179±81	171±86	-8	8.2	-4.5	,992	,000	-4.8	<0.001
3 month (Group III)	27	200.8±107	195.6±114	-5.2	13.6	-2.6	,995	,000	-1.9	0.048
5 month (Group IV)	29	188.6±103	202±95	13.4	10.3	7.1	,994	,000	6.9	<0.001
16 month (Group V)	28	127.6±72	134.1±38	6.5	14.6	5.1	,980	,000	2.1	0.034
26 month (Group VI)	23	152.5±106	163.8±95	11.3	11	7.4	,999	,000	4.8	<0.001
36 month (Group VII)	22	138.5±81	148.8±82	10.3	12.7	7.4	,999	,000	3.7	<0.001

**: Mean and standard deviation of each group; T.dif SD: standard deviation of total difference; T. dif %: percentage of total difference; © 1st and 2nd measurement correlation coefficient; #:1st and 2nd measurement correlation; t: Paired sample t tests score*

DISCUSSION

In toxicological laboratory practice, the medically allowable limit for ethanol measurement is reported to be 9-15% according to guideline of the German medical association on quality assurance in medical laboratory examinations (Rili-BAEK)15. Similarly, allowable limit for ethanol was 15-25% according to The Clinical Laboratory Improvement Amendments of 1988 (CLIA), accredited by American College of Pathologists (CAP)16. In our study, the percentage of ethanol error in the follow-up for 3 years was maximum as 7.4% and it was below the allowable error limits. Although there was a statistical significant change from the 1st month onwards, the deviation in alcohol values in our study was considered to be within the acceptable range since it did not exceed these all limits.

Our study also had some main results obtained during the follow-up of ethanol treatment for 3 years. Firstly, when non-preservative blood ethanol specimens were stored at +4 oC, it was observed that the stability was around 3 years with a decreasing trend (4.1%) for one month. A fall of 4.1 percent in serum ethanol concentrations was interpreted as no statistically significant change after one month. The maximum ethanol loss in the retained tubes was observed on month 2 (4.5%). On month 3, reduction of 2.6% could be interpreted as a sign of degradation due to bacterial production-induced ethanol production, since the balance between ethanol loss and production balance began to change in-vitro. It was observed that ethanol concentrations were decreased up to 2 months and tended to increase thereafter when storage period was prolonged in non-preservative tubes used in this study.

Secondly, this study showed that the significant increase in ethanol concentration upon month 5 of storage. In our study, the month 5, with a 7.1% increase, was point in the beginning of in-vitro ethanol production. In other words, the month 5 was assessed as the time point at which the in-vitro increase of ethanol was started in untreated serum tube specimens. In addition, this increase was reflected in the same proportions as in 26 and 36 months (7.4%, 7.4% respectively).

Thirdly, if the second analysis is required, especially in judicial samples, measurements should not be made from the primary test tube due to the risk of contamination during airborne or autoanalyser pipetting on tubes with opened lid.

It was showed that ethanol concentrations with sodium fluoride tubes specimens decreased by 24% at room temperature when stored 10 years17. This change was reported as 1.8% decrease in 1 year when it was stored at +4°C in another study18. Dubowski et al. have suggested that whole blood specimens stored at +4 oC for 1 year are not statistically different in terms of ethanol concentrations19.

David et al. suggested that there are no significant changes in the specimens stored for 10 days20 regardless of collection tube and storage conditions while Winek et al. suggest this period as 14 days8. If blood specimens are to be kept for a month, this

study showed that using preservative/anticoagulant does not have a superior effect on ethanol results.

In our study, a statistically significant increase (5%) was observed in ethanol concentrations in serum specimens around the first year. The water contents of whole blood and serum specimens differ from each other21; thus, we can consider the different results obtained as an effect on bacterial contamination of water.

In fact, bacterial contamination and ethanol production is expected in no-additive tubes 22. On the other hand, we suggest that serum specimens may be considered as an alternative to NaF tubes when stored for long periods of time in obligatory situations. As a matter of fact, the gray cap (NaF/EDTA) tube hasn't been available for a long time due to production and import supply restriction. Therefore, ethanol analyzes were performed on unpreserved tube specimens in our study.

In the long term storage results of the study, it was observed that the upward trend started at the end of the medium term durations (at the 5 month) and continued until month 26. We can define as the transition from fall to rise between 3 and 5 month, and it can be argued that the change of direction is possible by competitive in-vitro mechanisms acting on ethanol. The percentage of increase during months 26 and 36 didn't change. According to our findings, we can suggest that the ethanol concentration change is in the same range at two different measurements, such as such as month 36 and 5. From here, the change in serum ethanol specimens was interpreted to enter the stationary process after the second year in serum specimens.

The lower ethanol specimens showed a further decrease in mid-term storage, but the number of specimens with low ethanol concentration was insufficient to draw definitive conclusion (12% for 183 cases, 22 specimens).

All groups were initially analyzed on the Beckman Olympus 2700 analyzer using acceptable calibration data, with the same production lot number control, kit and calibrator (within same day for the second measurements), under supervision of same specialist. The %CV of ethanol measurement method was 1.1 in 100 mg/dL and 0.5 in 300 mg/dL, respectively. Therefore, measured increasing and decreasing values in ethanol concentrations was considered to have no effect on statistical significance.

Contradictory results are encountered when analysis is carried out by gas chromatography mass spectrometry (HS-GC/MS) method, so a validation test is recommended23. Despite HS-GC/MS remains to be gold standard for analyzing ethanol for judicial medicine purposes, ethanol measurement in routine clinical laboratories is usually used by enzymatic techniques24. Our laboratory also did not have ready access for verification by HS-GC/MS methods.

The limited number of specimens that could be taken into account for financial reasons was a limitation of our study. Secondly, we have linked the increase in ethanol concentrations to possible bacterial and fungal production in long term storage25; however, we had no facility of studying blood culture. Furthermore, due to retrospective nature of the study, data of serum glucose levels which the main substrate of fungal cells, could not be assessed because data could not be obtained from all cases.

There are no clear and precise standards guidelines for application as well as no literature data in our country for re-analysis process and storage conditions of split specimens. Therefore, for inquiries that can be executed on the grounds of judicial authorities, each laboratory should determine the maximum duration of specimen storage on their own. This period was determined by the laboratory experts considering the technical infrastructure.

In our country, based on general legal information on the principles and procedures of ethanol analysis in blood specimens; serum specimens can be stored at +4 °C for up to 14 days (Circular number:2017/12/95966346). However, in the case of mandatory measurement, we recommend this time as approximately three years for the serum specimens. In this study, we showed behavior of split specimens when stored over 3 years. Accordingly, serum ethanol concentrations decreased within first month; then increased from month 5 to two years, and remained stable during third year.

The author have no financial conflicts of interest.

REFERENCES

1. Dixon MA, Chartier KG. Alcohol Use Patterns Among Urban and Rural Residents: Demographic

and Social Influences. Alcohol Res. 2016; 38:69-77.

2. Kelly AT, Mozayani A E. An overview of alcohol testing and interpretation in the 21st century. J Pharm Pract 2012; 25:30-6.
3. Stark M. Clinical Forensic Medicine: A physician's guide. Forensic Analysis. Margaret M. Stark., 2nd Ed, London; Humana Press; 2005. p.111.
4. Beutler HO. Ethanol. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis. Vol VI, 3rd edition. New York Academic Press 1984, pp.598-606.
5. Higuchi A, Kurihara R, Yoshimoto T, Sano T, Katsumata K, Ishii A, Katsumata Y. Problems in blood alcohol testing of severely injured drivers brought to emergency departments in Japan. Leg Med (Tokyo). 2005; 7: 299-305.
6. Musshoff F. Chromatographic methods for the determination of markers of chronic and acute alcohol consumption. J Chromatog. B Analyt Technol Biomed Life Sci 2002; 781: 457-480.
7. Liu DX, He JN. Effects of additives in blood collection tubes on testing the alcohol concentration in blood samples. 2014; 30:452-455.
8. Winek CL, Paul LJ. Effect of short-term storage conditions on alcohol concentrations in blood from living human subjects. Clin Chem 1983; 29: 1959-1960.
9. Baker SP, O'Neill B, Haddon W Jr, Long WB. The Injury Severity Score: a method for describing patients with multiple injuries and evaluating emergency care, J Trauma 1974; 14:187-196.
10. Tiscione N. The Impact of hemolysis on the accuracy of ethanol determinations. J Anal Toxicol 2015; 39: 672-3.
11. Lippi G, Salvagno GL, Montagnana M, Franchini M, Guidi GC. Phlebotomy issues and quality improvement in results of laboratory testing. Clin Lab. 2006; 52: 217–30.
12. Clinical Laboratory Standards Institute (CLSI). Blood alcohol testing in the clinical laboratory - Approved guideline. CLSI document C60. Wayne, USA: CLSI, 2014.
13. Toksikoloji Laboratuvar Kitabı. Güley M, Vural N. Ankara, A.Ü. Ecz. Fak. Yayınları, 1975; 37:1-5.
14. Kristoffersen L, Stormyhr LE, Smith-Kielland A. Headspace gas chromatographic determination of ethanol: the use of factorial design to study effects of blood storage and headspace conditions on ethanol stability and acetaldehyde formation in whole blood and plasma. Forensic Sci Int. 2006; 161:151-157.
15. Revision of the "Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations–Rili-BAEK" (unauthorized translation). J Lab Med 2015: 39; 26–69.
16. Martindale RA, Cembrowski GS, Journalt LJ, Crawford JL, Tran C, Hofer TL, et al. Validating New Reagents: Roadmaps Through the Wilderness. Laboratory Medicine 2006; 37: 347–51
17. Tiscione NB, Vacha RE, Alford I, Yeatman DT, Shan X. Long-Term Blood Alcohol Stability in Forensic Antemortem Whole Blood Samples. J Anal Toxicol 2015; 39: 419-425.
18. Jones AW. Are changes in blood-ethanol concentration during storage analytically significant? Importance of metod imprecision. Clin Chem Lab Med. 2007;45:1299-304.
19. Dubowski KM, Gadsden RH, Sr, Poklis A. The stability of ethanol in human whole blood controls: an interlaboratory evaluation. J. Anal. Toxicol. 1997; 21: 486–491.
20. David M. Penetar, Jane F. McNeil, Elizabeth T. Ryan, and Scott E. Lukas. Comparison among plasma, serum, and whole blood ethanol concentrations: Impact of Storage Conditions and Collection Tubes J Anal Toxicol. 2008; 32: 505–510.
21. WH Porter and TP Moyer. Clinical Toxicology. In Tietz Fundamentals of Clinical Chemistry, CA Burtis and ER Ashwood, Eds. WB Sounders, Philadelphia, PA, 2001, pp 636-679.
22. Bogusz M, Guminska M, Markiewicz J. Studies on the Formation of endogenous ethanol in blood putrefying in vitro, J Forensic Med, 1970;17:156-68.
23. NB Tiscione, I Alford, D T Yeatman, and X Shan. Ethanol analysis by headspace Gas chromatography with simultaneous flame-ionization and mass spectrometry detection. J Anal Toxicol. 2011: 35; 501-511.
24. Vassiliki A Boumba, Kourkoumelis N, Gousia P, Economou V, et al. Modeling microbial ethanol production by E. coli under aerobic/anaerobic conditions: Applicability to real postmortem cases and to postmortem blood-derived microbial cultures. Forensic Sci Int 2013; 232:191-8.
25. Quintas MJ, Costa P, Melo P, Castro A, Franco JM, Teixeira HM. Postmortem in vitro ethanol production-It could be more common than we think! Forensic Sci Int. 2017; 274:113-116.