ASSESSMENT AND DETECTION
Ethanol Concentrations in Antemortem Blood Samples Under Controlled Conditions

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Abstract — Aim: The change of antemortem blood alcohol concentration (BAC) in inadequately processed samples was examined.

Methods: The study was performed on nine healthy, sober volunteers after overnight fasting. Blood samples were divided into groups and stored, with or without NaF, for varying time periods (12, 24 and 48 h) and at different temperatures (4°C and 20°C). BAC analysis was performed by the gas-chromatography method.

Result: All groups showed very low values of BAC. Conclusion: Surprisingly, this study showed no ethanol production in improperly processed antemortem blood samples in healthy and sober individuals who were subjected to overnight fasting.

INTRODUCTION
Measurement of blood alcohol concentration (BAC) in living individuals is often performed in forensic practice (e.g. after traffic accidents). In such situations the offenders/perpetrators are taken by police officers to the nearest health centre, where blood samples are properly collected for the analysis. The collected samples from the South Bačka region of Serbia (Južno Bački okrug) are then transported to and processed at the Institute of Forensic Medicine, Clinical Centre of Vojvodina.

It is known that alcohol can be produced in blood after sampling. The amount of generated ethanol depends on the species of microorganisms present, the available substrates, the temperature and time of storage and the presence of preservatives added to the specimens (Blume and Lakatuš, 1973; Clark and Jones, 1982). Glycolysis is considered to be the principal process for ethanol production, but some other metabolic processes are also possible—usage of substrates such as glucose, lactate, ribose and amino acids (Blackmore, 1968; Bogusz et al., 1970; Corry, 1978). The possibility of alcohol production in sampled blood calls for appropriate removal and preservation of specimens. Thus, police officers are equipped with appropriate—chemically clean and sterile tubes with added preservative and a plastic cap, and they are trained to fill the container with blood up to the top, close tightly and refrigerate (at 4°C) prior to delivery to the Institute. Unfortunately, this is not always carried out in everyday practice; samples are sometimes too small (<3 mL), they are stored at inappropriate (room) temperatures and for prolonged periods of time, etc. Later, these factors can influence interpretation of results by the authorities since the levels of alcohol measured may not reflect the real values at the moment of sampling. Thus, the goal of this study was to check the range of BAC changes in inadequately processed samples (presence/absence of preservative, duration of storage at different temperatures).

MATERIAL AND METHODS
The study was performed at the Institute of Forensic Medicine, Clinical Centre of Vojvodina, Novi Sad, Serbia, on nine healthy volunteers of both sexes, aged 25–37, who previously signed informed consent about participation in this study, approved by the Ethics Committee of Faculty of Medicine at the University of Novi Sad. The participants had not consumed alcoholic beverages for at least 24 h before the examination. The blood was taken from cubital vein in the early morning hours after overnight fasting. Blood sampling equipment was chemically clean and sterile, while the disinfection of the skin before venepuncture was performed by ethanol (70%) and then by distilled water. The amount of blood taken was 3 mL for each sample (total amount per volunteer—15 mL); the tube was filled up to the top, closed tightly with plastic cap and was shaken gently where it was necessary (samples with preservative—1% NaF).

Blood specimens were divided into a control and two experimental groups: one with and one without preservative. The control group was analysed immediately after the sampling. Each experimental group was divided into two subgroups, based on storage temperature (4°C and 20°C in a refrigerator and 20°C in a water bath—Memmert GmbH, Germany). Each of these was then subdivided into three groups, which were stored for 12, 24 and 48 h prior to testing. These time periods were chosen since, in our practice, the usual interval between blood sampling and BAC measurement varies between 12 and 48 h.

Preparation of the blood samples for analysis was carried out by measuring 0.1 mL of blood in the glass container vial (volume 20 mL), and then by adding 0.75 g NaCl and 0.1 mL 0.2% solution of n-propanol (propyl alcohol pro analysi, Merck KGaA, Germany) as the internal standard.

Ethanol analysis was performed by the gas-chromatography method after the calibrations had been done. Calibration solutions were prepared in a matrix of certified-negative human blood as dilutions of ethanol and n-propanol purchased from Merck, GC purity. The concentration of six calibrators ranged from 0.156 g/kg to 5 g/kg for ethanol and 2 g/kg for n-propanol. The coefficient of correlation for those values was \( r = 0.999 \). The estimated limit of detection (LOD) was 0.01 g/kg and the limit of quantification (LOQ) was 0.03 g/kg.

Vials containing blood samples were warmed at 70°C for 60 min in a headspace sampler 19395 A (Hewlett-Packard). After incubation, the samples were injected into gas chromatograph...
5790 A series (Hewlett-Packard) with a flame ionization detector (FID). Separation was achieved utilizing capillary column HP-20M Carbovax 20M (capillary length 25 m, ID 320 \( \mu \)m, film 0.25 \( \mu \)m) at a constant column temperature of 60°C, and the signals were processed by HP GC ChemStation, with Windows REV. A.09.01 (1206) system software. The carrier gas was laboratory nitrogen, GC purity, flowing at 30 mL/min. The GC parameters setting were for an injector temperature of 200°C, and a detector temperature of 250°C. The retention times of analytes were as follows: 2.92 for ethanol and 3.05 for n-propanol.

All experimental results are presented as mean values and ranges.

RESULTS

The results obtained in control and experimental groups are presented in Tables 1 and 2. Irrespective of the fact that all BACs are considerably below the detection limit cited in forensic literature, 0.1 g/kg (O’Neal and Poklis, 1996), and despite the previously mentioned LOD and LOQ in our laboratory, we presented the exact values measured. These low BAC values have no practical importance on legal driving limit (in our legislation 0.5 g/kg).

DISCUSSION

Ethanol is most commonly ingested through alcoholic beverages, but it can also be produced during metabolic processes in the body. The term ‘endogenous alcohol’ applies to spontaneous ethanol production in the body via different metabolic pathways. These values are extremely low, close to detection threshold of modern analytical equipment (~0.001 g/kg). However, some individuals of far eastern origin, particularly Japanese, can produce significant amounts of endogenous alcohol due to fermentation of carbohydrates caused by Candida albicans present in the normal gut microflora. The ethanol is then absorbed into the blood—a syndrome known as ‘Auto-Brewery Syndrome’ (Logan and Jones, 2000). The possibility that endogenous alcohol modifies the measured values of ethanol in the blood cannot, therefore, be excluded.

Our study showed a surprising lack of alcohol production in samples without preservative, stored at room temperature for 48 h. It may be that the aforementioned conditions were not enough for ethanol formation in vitro, primarily because of the lack of substrate (glucose) and suitable microbial flora (contamination, proliferation). The latter can be explained by the fact that blood has been taken after overnight fasting, in an aseptic way, with the samples being collected/stored in chemically clean and sterile tubes.

In a former study (Petković et al., 2005) that involved incubation of postmortem blood samples without preservative at 20°C for 48 h, there was an increase in BAC of 0.1 g/kg when compared with the initial measurements. This would confirm our view that the absence of preservative and prolonged storage at higher temperatures are not necessarily sufficient for alcohol production in antemortem blood samples. Furthermore, in postmortem specimens the glycaemia at the time of death was unknown and bacterial contamination was more likely.

CONCLUSION

On the basis of this study, the authors are somewhat surprised that no ethanol production occurs in everyday practice in improperly processed antemortem blood samples. This implies that measured BACs reflect concordance with the ethanol levels at the time of sampling. However, our research involved healthy and sober individuals who fasted overnight. It would, therefore, be of interest to extend the scope of the study—to include a different profile of volunteers (e.g. diabetic and febrile patients, those who have previously consumed alcohol and/or food), as well as to expand the range of temperatures and storage times.

REFERENCES


