K23 The Impact of Storage Conditions, Sample Volume, and Collection Technique on Blood Alcohol Concentration (BAC) in Non-Decomposed Defibrinated Sheep's Blood

Christian T. Pascual, Emporia State University, Emporia, KS 66801; S. Sharee Lambert, BA, Emporia State University, Emporia, KS 66801; Carrie Hodges, MLS, Kansas Bureau of Investigation, Topeka, KS 66604; Melissa M. Bailey, PhD, Emporia State University, Emporia, KS 66801*

Learning Overview: After attending this presentation, attendees will have a better understanding of the effects of the collection technique, time, temperature, sample volume, and presence of excess glucose on the BAC in non-decomposed whole blood.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating how the percentage of ethanol is affected when the blood sample is collected and stored under various conditions over a five-month period. This study also investigated how the presence or absence of excess glucose (>240mg/dL) affects the BAC over time, given that blood is often collected from suspects or victims with diabetes or metabolic disorders. The results of this longitudinal study could springboard to policy reforms in terms of the process of collection, storage, handling, or testing of samples taken from suspects of Driving Under the Influence (DUI) cases.

The forensic science community worldwide has no standardized procedures for the collection, storage, handling, or testing of samples for blood alcohol analysis. Degradation of ethanol in blood alcohol samples can be caused by storage temperature, time of storage, and sample volume.[1] If samples need to be re-analyzed or if significant time elapses between evidence receipt and analysis, the samples must be stored correctly to ensure accurate results.[2]

Two sets of aliquots of seven different ethanol concentrations were prepared in defibrinated sheep blood (Hemostat Laboratories): 0g/dL, 0.05g/dL, 0.08g/dL, 0.10g/dL, 0.15g/dL, 0.20g/dL, and 0.30g/dL. D-glucose from Fisher Scientific was added to one set of aliquots in sufficient quantity to result in a blood glucose measurement of at least 240mg/dL. The appropriate sample from each of the aliquot was then added to 10mL gray-stoppered BD Vacutainer® blood collection tubes (GST) in varying amounts: 2.5mL, 5mL, 7.5mL, and 10mL, either by drawing under vacuum (method 1) or by removing the stopper and adding the blood via syringe (method 2). For each ethanol concentration, four groups of samples were made. Group 1 had tubes of each volume as described, with and without excess glucose, collected using method 1 and stored at room temperature (25ºC)—8 tubes in total. Group 2 had 8 tubes as in group 1 but were refrigerated at 4ºC. Group 3 had 8 tubes as in group 1 but collected using method 2 and stored at room temperature. Group 4 had 8 tubes as described in group 3 but refrigerated at 4ºC. All four groups were made at each BAC for monthly analysis (months 0–5), for a total of 1,344 tubes. All the tubes were aliquoted at the beginning of the study. Room temperature was chosen to study the effects of improper storage conditions that could be encountered if samples are not appropriately refrigerated in a timely manner. Each month, samples were analyzed in duplicate with an internal standard of 0.002% 1-propanol in water by an Agilent® 7820A GC and 5977E MS with headspace after instrument calibration; with 0.10g/dL standards run every 46 vials.

The data in the study were analyzed using Analysis of Variance (ANOVA) in which the level of significance was tested with the *p*-value of 0.05. The study discovered that over time, samples with lower volume had a greater ethanol loss than samples with higher volume (0.014g/dL vs 0.006g/dL). Additionally, the samples collected through method 1 had more ethanol loss than those collected through method 2 (0.01g/dL vs 0.008g/dL). Samples that were stored at room temperature had a higher percentage of ethanol loss as compared to those that were refrigerated (0.016g/dL vs 0.004g/dL). However, the presence of excess glucose does not significantly affect ethanol loss. These findings show that storage temperature, collection technique, and sample volume may affect ethanol concentration in blood samples and these factors should be considered when drawing conclusions about data obtained from case samples.

Reference(s):

1. Ferrari et al. Kinetics of Ethanol Degradation in Forensic Blood Samples. *Forensic Sci Int.* 2006;161:144-150.
2. Penetar at al. Comparison among Plasma, Serum, and Whole Blood Ethanol Concentrations: Impact of Storage Conditions and Collection Tubes. *J Anal Toxicol*. 2008;32(7):505-510.

BAC, Collection Technique, Storage Condition