K18 The Impact of Storage Temperature, Glucose, and Microorganisms on Blood Alcohol Concentration in Non-Decomposed Whole Blood

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After attending this presentation, attendees will have a better understanding of how storage temperature, time, and the presence of excess glucose, bacteria, fungi, and yeast have an impact on Blood Alcohol Content (BAC) in non-decomposed whole blood.

This presentation will impact the forensic science community by illustrating the effects of glucose and microorganism contamination on BAC over a six-month storage period.

The presence of microorganisms has been studied in biological matrices, particularly in urine, but to date there are no comprehensive, longitudinal studies that demonstrate the impact of microorganisms and excess glucose in whole blood. Current research has revealed that some bacteria and yeasts, particularly *Candida albicans*, can produce ethanol in blood samples through a fermentation pathway. Additionally, storage of samples in warmer temperatures can increase the bacteria's ethanol production.[1] Conversely, degradation of ethanol in blood alcohol samples can be caused by storage temperature, time of storage, and sample volume.[2] Currently, an international standard for the collection, handling, storage, and testing of blood alcohol samples has not been established. If samples need to be re-analyzed or significant time elapses between evidence receipt and analysis, the samples must be stored correctly to ensure accurate results.[3] This study illustrates the effects of sample volume, storage temperature, and presence or absence of excess glucose and microorganisms on BAC over a six-month period.

Two sets of stock solutions of seven different ethanol concentrations were prepared in defibrinated sheep's blood: 0g/dL, 0.05g/dL, 0.08g/dL, 0.10g/dL, 0.15g/dL, 0.20g/Dl, and 0.30g/dL. D-glucose was added to one set of stock solutions in sufficient quantity to result in a blood glucose measurement of at least 240mg/dL. The appropriate blood was then added to 10mL gray-stoppered BD Vacutainer™ blood collection tubes in varying amounts (2.5mL, 5mL, 7.5mL, and 10mL) by removing the stopper and adding the blood via a syringe. For each BAC, four groups of eight samples were prepared. Group 1 included tubes of each volume as described, with and without excess glucose stored at room temperature (25°C). Group 2 was stored refrigerated at 4°C. Groups 3 and 4 were inoculated with a mixture of *Saccharomyces cerevisiae, Candida albicans, Acinetobacter johnsonii, Fusarium oxysporum,* and *Staphylococcus aureus* to simulate microbe contamination by improper collection (these strains were chosen as some of the most abundant on skin and/or having been indicated as common sources of contamination).[4] All four sets were made at each BAC for monthly analysis (months 0-6), for a total of 1,568 tubes. Each month, samples were analyzed in duplicate with an internal standard of 0.005% 2-butanone in water by an Agilent® 7820AGC and 5977EMS with headspace after instrument calibration, with 0.10g/dL standards run every 24 vials. Additionally, samples from each BAC level were streaked on blood agar plates and incubated to determine viability of the bacteria, yeasts, and fungus. If excess glucose was present, tubes inoculated with the microorganism mixture produced ethanol. Refrigerated samples experienced less degradation than samples stored at room temperature. Sample volume affected the rate of decomposition; smaller sample volumes experienced greater amounts of sample degradation. At BAC greater than 0.20g/dL, the microorganism survival rate was lower.

The results of this study indicate that the presence of microorganisms, particularly in the presence of excess glucose, can negatively affect the accuracy of ethanol analysis and that storage conditions and sample collection conditions, if known, should be considered when analyzing BAC data obtained from casework.

Reference(s):

1. Petkovic et al. Ethanol concentrations in antemortem blood samples under controlled conditions. *Alcohol and Alcoholism.* 2008;43:658-660.
2. Ferrari et al. Kinetics of ethanol degradation in forensic blood samples. *Forensic Sci Int.* 2006;161:144-150.
3. 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.
4. Brocher et al. Bacterial Contamination of Blood Components. *Clinical Microbiology Reviews.* 2005;18(1):195-204.

BAC, Contamination, Longitudinal

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