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Decreases in blood ethanol concentrations during storage at 4 °C for 12 months were the same for specimens kept in glass or plastic tubes

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ABSTRACT

Background: The stability of ethanol was investigated in blood specimens in glass or plastic evacuated tubes after storage in a refrigerator at 4 °C for up to 12 months.
Methods: Sterile blood, from a local hospital, was divided into 50 mL portions and spiked with aqueous ethanol (10% w/v) to give target concentrations of 0.20, 1.00, 2.00 and 3.00 g/L. Ethanol was determined in blood by headspace gas chromatography (HS-GC) with an analytical imprecision of < 3% (coefficient of variation, CV%). Aliquots of blood were re-analysed after 2, 7, 14, 28, 91, 182 and 364 days of storage at 4 °C.
Results: The standard deviation (SD) of analysis by HS-GC was 0.0059 g/L at 0.20 g/L and 0.0342 g/L at 3.00 g/L, corresponding to CVs of 2.9% and 1.1%, respectively. The decreases in blood ethanol content were analytically significant after 14–28 days of storage for both glass and plastic tubes The mean (lowest and highest) loss of ethanol after 12 months storage was 0.111 g/L (0.084–0.129 g/L) for glass tubes and 0.112 g/L (0.088–0.140 g/L) for plastic tubes. The corresponding percentage losses of ethanol were 43–45% at a starting concentration of 0.20 g/L and 3.9–4.1% at 3.00 g/L.
Conclusion: The concentration of ethanol in blood gradually decreases during storage at 4 °C. After 12 months storage the absolute decrease in concentration was ~0.11 g/L when the starting concentration ranged from 0.20 to 3.0 g/L. Decreases in ethanol content were the same for specimens kept in glass or plastic evacuated tubes.

1. Introduction

The first evacuated tubes used to sample venous blood for clinical laboratory analysis appeared in the early 1950s [1]. These tubes were originally made of soda glass, although more recently plastic tubes have become available [2]. Tubes made of plastic are more robust and are less likely to break during transport, handling and storage of specimens or after cycles of freezing and thawing [3]. The switch from glass to plastic tubes has also occurred in connection with forensic analysis of ethanol and other drugs in blood, such as in traffic-law when impaired drivers are arrested [4]. This raises the question of the stability of blood-ethanol concentrations after various periods of storage in glass and plastic evacuated tubes.

For several decades the Swedish National Laboratory of Forensic Toxicology purchased fluoride-oxalate (grey stoppered)

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evacuated tubes from a Belgian company (Terumo Europe N.V.). These tubes were made of glass with a nominal volume of 10 mL and contained sodium fluoride (100 mg) and potassium oxalate (22.5 mg) as preservative and anticoagulant, respectively. Several years ago the manufacturer informed us that they intended to stop selling glass evacuated tubes, but were able to supply plastic tubes containing exactly the same chemical additives.

Venous blood samples from impaired drivers arrested throughout Sweden are sent for analysis of ethanol and other drugs to a central laboratory. The specimens are shipped by express mail and usually arrive within 1–2 days. After registration the blood samples are placed in racks and kept in a refrigerator at 4 °C pending analysis a few days later. In forensic casework, a request to re-analyse the blood specimen is not uncommon, such as if the accuracy of the result is challenged or if there is some doubt about integrity of the sample. This makes it important to know whether the ethanol concentrations in blood are stable during short- and long-term storage of specimens in a refrigerator [5].

The aim of this study was to compare and contrast the stability of ethanol in stored blood samples after storage in sealed evacuated tubes made of soda glass or plastic for up to 12 months.

2. Methods

2.1. Evacuated tubes

For purposes of the present study Terumo Europe N.V. (Leuven, Belgium) supplied us with VENOJECT glass evacuated tubes (product number VT–100SFX07) and VENOSAFE plastic tubes (product number VF–109SFX07). Both the glass and plastic tubes were fitted with grey stoppers and contained a mixture of sodium fluoride (100 mg) and potassium oxalate (12.5 mg) as preservative and anticoagulant, respectively [6].

2.2. Spiking of blood with ethanol

Blood (~500 mL) was purchased from the blood transfusion unit at the University Hospital in Linköping and was used to prepare a range of samples with known target concentrations of ethanol. The bag of blood was rotated several times to ensure mixing of plasma and erythrocytes before aliquots were removed for mixing with ethanol.

Spectroscopic grade ethanol (99.9% purity) was diluted with distilled water to make a 10% w/v solution by weighing on a sensitive balance. Known amounts of the 10% ethanol stock solution were weighed directly into 50 mL graduated flasks and immediately made up to the mark by carefully adding well-mixed blood. In this way target blood ethanol concentrations of 0.20 g/L, 1.00 g/L, 2.00 g/L and 3.00 g/L were prepared.

After spiking the blood with ethanol the target concentrations were verified by triplicate analysis by headspace gas chromatography (HS-GC), a method that was described in more detail elsewhere [7]. The mean concentration of ethanol from analysis of three glass and three plastic tubes of blood was taken as the initial (starting) concentration. Evacuated tubes (10 mL) made of glass or plastic were filled with ~9 mL of spiked blood and immediately made air-tight with the rubber stoppers supplied.

2.3. Gas chromatographic analysis

For routine forensic blood-alcohol analysis two HS-GC auto-sampler instruments (HS–101) were supplied by PerkinElmer Inc., (Waltham, MA, USA). The two instruments were fitted with capillary columns (BAC–1 and BAC–2) purchased from Restek Corporation (Bellefonte, PA, USA). Two laboratory technicians performed all blood–ethanol determinations and they worked independently with different GC instruments and diluters.

The glass and plastic tubes containing blood were placed in a rotamix device for a few minutes before the stoppers were removed and aliquots withdrawn for GC analysis. Dilution of the blood with internal standard was done with equipment purchased from Hamilton Inc., (Reno, NV, USA). The Microlab 503A diluter–dispenser was programmed so that 100 μL of blood was diluted 11 times (1+10) with aqueous n-propanol as the internal standard. The diluted blood samples were ejected into 22 mL glass vials, which were immediately made airtight with a butyl rubber stopper and crimped on aluminium cap. All tubes of blood were analysed in duplicate and the mean result of six determinations (three glass and three plastic tubes) was taken as the best estimate of blood–ethanol concentration after various periods of storage.

2.4. Experimental design

To simulate conditions that might operate during transport of specimens to the laboratory, for the first two days of storage one plastic and one glass tubes was in a horizontal position and two other tubes stood upright (vertically) in a rack. After the first 2 days of storage all tubes were positioned upright (vertically) in racks and kept in a refrigerator at 4 °C pending re-analysis. After designated storage times of 7, 14, 28, 91, 182 and 364 days the racks of blood were removed from the refrigerator and allowed to attain room temperature before aliquots were removed for GC analysis as described above.