

The Effects of Storage on the Accuracy of Blood Alcohol Readings.

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Background

Currently, in New South Wales, Australia, blood samples are taken from all drivers and pedestrians (over 15 years of age) who are injured in a motor vehicle collision and taken to hospital. Once the blood is extracted, the sample is divided and placed into two screw topped glass vials containing sodium fluoride (1% w/v) as an enzyme inhibitor and potassium oxalate (0.2% w/v) as an anticoagulant. One sample is retained by Police for analysis at the Division of Analytical Laboratories (DAL), and the other is presented to the driver for independent analysis, if required. Due to concern in relation to the possibility of needlestick injuries and infectious diseases, it was proposed that the current screw top glass vials be replaced by Monovette (Sarstedt) plastic containers with a vacuum collection system.

All blood samples taken by Police for alcohol determination are analysed at DAL by gas-liquid chromatography using the direct injection technique. The 99% confidence limits vary from ± 0.001 at readings from 0.020 - 0.049 g/100 ml to ± 0.004 at readings > 0.250 g/100 ml. Around 18,000-19,000 such samples are analysed by the Laboratory each year, of which about 10% are positive for alcohol.

Blood samples taken for alcohol analysis in New South Wales can be subjected to wide variations in both storage conditions and transit times to the Laboratory. In most cases, the time from withdrawal of the blood sample to analysis is 7 days. Depending on the time of year and the location, ambient temperatures can vary from below 0°C to over 40°C. Once a blood sample has been received by the Laboratory, it is stored at 4°C prior to analysis.

A number of studies have demonstrated both generation and loss of alcohol in stored blood samples. For example, it has been shown that in *post-mortem* blood samples, both high storage temperatures (Christopoulos *et al.*, 1973) and an insufficient enzyme inhibitor concentration (Plueckahn & Ballard, 1968) can result in alcohol generation, presumably as a result of bacterial fermentation. Furthermore, in alcohol-spiked blood samples, it was noted (Brown *et al.*, 1973) that, irrespective of the type of container used, blood alcohol concentrations progressively decreased as the storage temperature and storage time increased. However, Moynham *et al.* (1983) found that in blood taken from living subjects, there was no alcohol generation regardless of varying storage temperatures, times and the

presence or absence of an enzyme inhibitor, but there was some alcohol depletion after longer storage times. Similarly, Peel *et al.* (1988), found no alcohol generation, but losses of varying magnitude occurred with longer storage time, and there was a tendency for higher alcohol losses when sodium fluoride was present.

Forensically, each step in the sequence, from the taking of the blood sample, to the storage and transport in the vial, to the final analysis, must be taken into account in authenticating the reliability of the final blood alcohol concentration which is reported.

Objectives

To determine whether alcohol was generated or lost in blood samples placed in vacuum collection containers subjected to different storage periods and temperatures, in the presence or absence of sodium fluoride as a preservative.

Methodology

A unit (~500 ml) of expired whole blood containing an approved anticoagulant-preservative solution was obtained from the Red Cross Blood Bank. Three baseline samples were taken to exclude prior contamination of the blood with alcohol.

Twenty control 8.5 ml blood samples were collected, ten were placed into unpreserved, and ten into preserved Monovette vacuum containers. Absolute ethanol was then added to the remaining blood to achieve a target blood alcohol concentration of 0.135 g/100 ml. The blood was thoroughly mixed and the sampling process repeated.

The samples were then stored at either 4°C or 25°C for 1, 2, 4, 8 or 42 days.

Samples were delivered to DAL, where they were stored at 4°C prior to analysis. Each sample was analysed twice and the mean reading determined. The significance of differences among the sample means was determined using 2-tailed t-tests.

Results

No alcohol was found in the three baseline samples.

There was no generation of alcohol in the control samples under any temperature or storage time condition, with or without preservative (Table 1).

Table 1 – The effects of storage on blood samples [unpreserved (U) and preserved (P)] containing no alcohol.

Storage (days)	g alcohol/100ml	Temperature (°C)
1	0 (U)	4
	0 (P)	25
2	0 (U)	4
	0 (P)	25
4	0 (U)	4
	0 (P)	25
8	0 (U)	4
	0 (P)	25
42	0 (U)	4
	0 (P)	25

In samples containing alcohol, there was no generation of alcohol. At a storage temperature of 4°C, virtually no alcohol was lost up to 42 days (Figure 1), whereas at 25°C, loss was minimal up to 8 days, then more extensive at 42 days (Figure 2). Blood alcohol concentration readings were slightly but consistently significantly lower ($p=0.0006$ at 4°C; $p=0.013$ at 25°C) in the presence of preservative (Figures 1 and 2). Differences between the alcohol content of samples stored at 4° and 25°C were not statistically significant.

Figure 1 – The effects of storage at 4°C on blood samples containing alcohol.

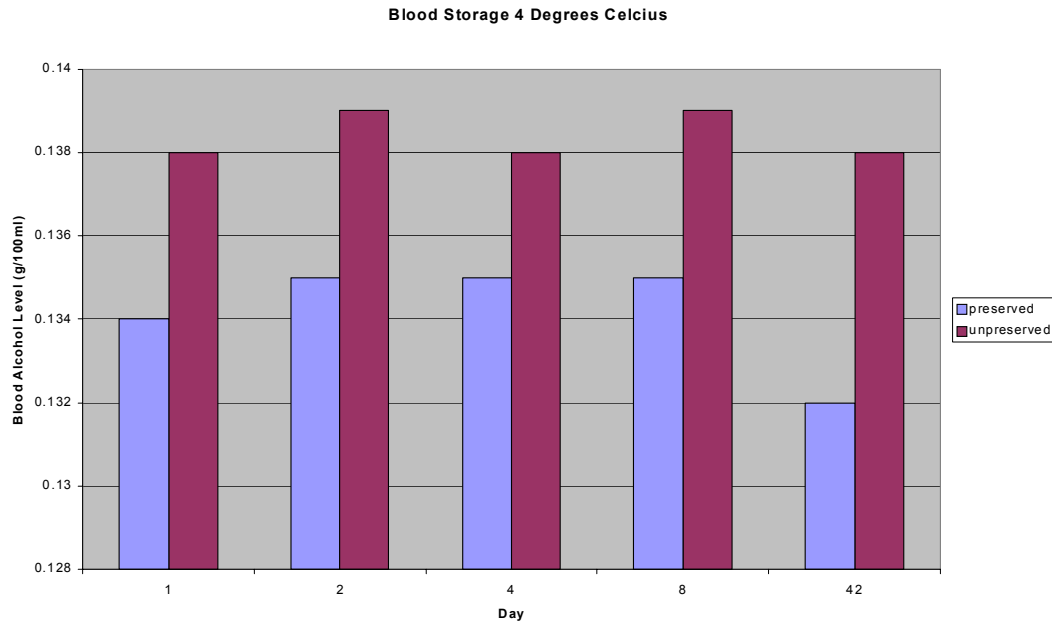
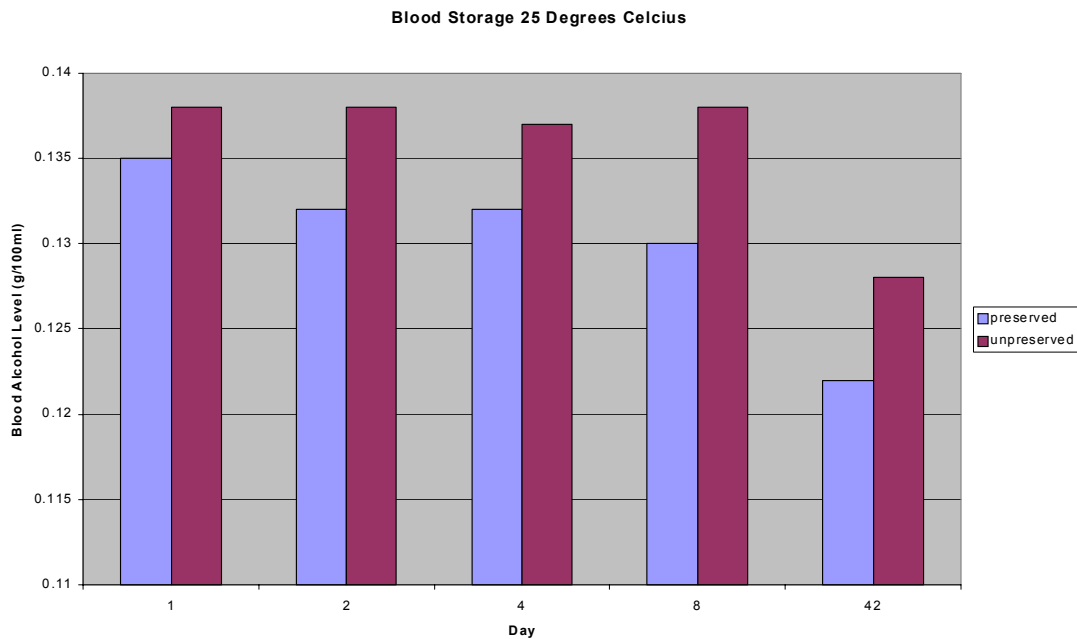


Figure 2 – The effects of storage at 25°C on blood samples containing alcohol.



Discussion

This study was carried out to determine the influence of storage variables on blood alcohol concentrations of samples placed in vacuum collection containers.

No alcohol was generated in any of the control samples over the 42 day storage period, whether or not preservative was present. This is in agreement with the findings of both Moynham *et al.* (1983) and Peel *et al.* (1988).

Loss of alcohol from the samples was minimal, apart from that after 42 days storage at 25°C in both preserved and unpreserved samples. This was most likely to have been due to escape of alcohol vapour from around the seal of the container and/or haemoglobin-catalysed oxidation of alcohol.

The consistently higher rates of alcohol depletion in the preserved samples might reflect a salting-out effect and/or some reaction between alcohol and sodium fluoride.

Conclusion

We have demonstrated that, in blood samples gathered in Monovette vacuum collection containers, no alcohol was generated and, if blood samples are analysed at around 7 days after collection, there is minimal loss of alcohol.

We thus conclude that these containers are appropriate for the collection of blood samples for forensic alcohol analyses.

References

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