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The Effects of Long Term Storage
on Blood Alcohol Levels

presented by

Mark Joseph Milford

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of the requirements for

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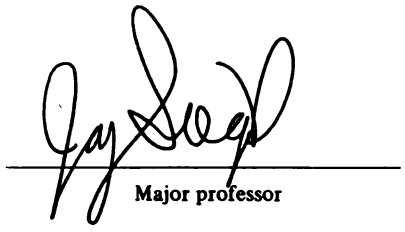

Major professor

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THE EFFECTS OF LONG TERM STORAGE ON BLOOD ALCOHOL LEVELS

By

Mark Joseph Milford

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ABSTRACT

THE EFFECTS OF LONG TERM STORAGE ON BLOOD ALCOHOL LEVELS

By

Mark Joseph Milford

The preservation of evidence is of utmost importance in cases involving blood samples contained in Driving Under the Influence of Alcohol (DUI) investigations. In the state of Illinois the prosecution is responsible for maintaining an untested vial of blood, one from the two originally submitted, should the defense request it. Therefore, this thesis was undertaken to test the effects that freezing blood samples for a period of one year at -11° C has on initial blood alcohol levels. Samples were tested on a Hewlett Packard 5890 gas chromatograph. The resulting paired sample t-test revealed that there was a significant decrease in the blood alcohol levels but that decrease was not as prevalent in the range where the original court decision possibly could be overturned.

DEDICATION

This Thesis is dedicated to the people who have shown me the meaning of patience and understanding. Thank you, Minerva, Mom and Dad, and Michael Milford!

ACKNOWLEDGMENTS

I would like to extend my thanks to the Northern Illinois Police Crime Laboratory and Dr. Jane Homeyer for allowing me the opportunity to complete the experimental research on blood alcohol levels needed for this thesis project!

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INTRODUCTION

Ethanol detection in blood samples is a common procedure done at the Northern Illinois Police Crime Laboratory in Highland Park, Illinois. On the average, 350 cases a year are received at the lab where blood alcohol examinations are done by gas-liquid chromatography with a flame ionization detector.

The cases are submitted in standard DUI kits supplied by the State and consist of two gray top tubes of blood. The gray top tubes are indicative of containing sodium fluoride, which acts as a preservative and potassium oxalate which serves as an anticoagulant. Upon arrival, one tube is tested by the laboratory and the other is retained at the laboratory for a period of one year according to Section 510.110 of the Illinois Department of Public Health "Standards and Procedures for Testing for Alcohol and/or Other Drugs".

Retaining the samples for up to a period of one year brings up a very important issue. If these samples are going to be held at the laboratory they must be stored properly in the event that the defense wants to retest the blood.

It is standard procedure to preserve all blood specimens taken from live subjects suspected of drunken driving offenses. When dissolved in 4 ml of blood, concentrations of

approximately 2.5 mg/ml sodium fluoride and 2 mg/ml potassium oxalate are obtained. Solutions of the additives are evaporated to dryness in the vials before use and two 3 mm diameter glass beads are present to facilitate mixing.

Once the blood specimens are collected, two per DUI case, they are brought to the laboratory for blood alcohol analysis. One sample is subsequently tested and the sample not tested is put into the laboratory freezer where it is kept for a period of one year at -11° C.

This research project looks at the effects that long term storage has on blood alcohol levels by comparing pre- and post storage blood alcohol levels by means of the paired sample t-test. From the results obtained it is hoped that it will be determined whether or not the freezing procedure is sufficient for the preservation of the blood samples. Should they show that it is not, other forms of preservation must be examined.

LITERATURE REVIEW

Shajani, Image and Chu (1989) ran a dual experiment. In the first experiment, paired samples, one stored at room temperature and the other stored at 4° C were compared over a period of 32 weeks. The samples stored at room temperature gave lower blood ethanol level results and were significantly different at a 95 % confidence level. In the second experiment forensic samples were analyzed and then stored over a period of at least one year at 4° C. The samples analyzed one year later also gave lower blood ethanol level results and were significantly different at a 95 % confidence level.

Brown, Neglan, Reynolds and Smalldon (1973) ran a 2^5 factorial experiment. The five factors were as follows:

Time of storage: 4 weeks and 8 weeks
Fluoride concentration: none and 1 % (W/V) NaF
Alcohol level: 110 mg % and 220 mg %
Temperature of storage: 4° C and 17° C
Container: Polypropylene cups and sealed, ring-snap, glass ampoules

It was found that temperature was the most important

factor because the growth of micro-organisms and alcohol oxidation were strongly temperature-dependent. The fluoride concentration was important because it inhibited catastrophic losses due to the growth of micro-organisms. The presence of fluoride was more important at room temperature than under refrigerated conditions where microorganism growth is unlikely even if contamination had occurred. The time of storage was obviously important for all three mechanisms of ethanol loss which were highly temperature-dependent, ethanol oxidation which was independent of the ethanol concentration over a wide range, destruction of ethanol by the action of micro-organisms in the absence of a preservative, which could be inhibited by a 0.5 % (W/V) sodium fluoride and diffusion which was found to occur from 5.6 % of the polypropylene containers used in Britain for the purpose of the Road Traffic Act 1972.

Smalldon and Brown (1973) were concerned with the mechanism of ethanol oxidation of blood samples stored at room temperature. Results showed that the oxidizing activity was shown to arise from an oxyhaemoglobin intermediate and to be inhibited by compounds which destroyed oxyhaemoglobin. The reaction was found to be first order with respect to the oxyhaemoglobin concentration and zero order with respect to the ethanol concentration.

Chang, Smith, Walkin and Reynolds (1984) reanalyzed blood samples, stored in B-D Vacutainer tubes #4741, at room temperature after 3 years and then after 6.75 years. All

samples exhibited a decline in ethanol concentration, with most losses falling in the 20 to 40 mg % range.

Manno and Manno (1978) spiked heparinized blood with anhydrous ethyl alcohol to provide three concentrations, approximately 50, 100 and 150 mg %. The blood samples were then stored at room temperature (21 - 24° C), in the refrigerator (2° C) and frozen (-15° C). Following a 12 day period it was determined that the blood specimens, whether stored at room temperature, under refrigeration or in the freezer, did not change more than +/- 7 to 10 %.

Bradford (1966) determined that a screw cap vial with a resilient liner faced with teflon or polyethylene made a very satisfactory container. It was also determined that mercuric chloride was a practical and effective inhibitor of reactions leading to the disappearance of alcohol in blood. Samples with a mercuric chloride concentration of 1:10,000 were found to be stable at room temperature for periods of six months and longer.

Kaye (1980) found that 1 % sodium fluoride is the most satisfactory preservative. Amounts less than that allowed micro-organisms to grow and could inhibit glycolysis and thus provide glucose for the unkilled micro-organisms to ferment into ethanol.

It was also found that blood without sodium fluoride was reliable at: room temperature (25° C) for about two days; refrigeration (5° C) for about two weeks and the freezer (-15° C) for about four weeks. With a sodium fluoride preservative

of at least 1 %, the blood was found to be reliably retested within +/- 0.02 g/dl at various conditions: room temperature (25° C) for about two weeks; refrigeration (5° C) for about three months and the freezer (-15° C) for about six months (plus). These approximations were based on work from 172 autopsies and partly on unpublished research.

Glendening and Waugh (1965) did a study on the stability of blood samples kept in rubber stoppered tubes for alcohol analysis under varying conditions. Groups of ten samples were held at room temperature for different intervals of time and all showed an average decrease in alcohol content from .009 % at each of the one-week, two-week and one-month periods to .045 % at two years. The changes were not statistically significant at the two-month period or below, but all groups at the six-month or longer storage time showed a significant loss of alcohol. Significant changes in alcohol content were not found for samples stored as long as ten months in the refrigerator or nine months in the deep freeze. Samples stored in an incubator at 94° F showed a significant loss of alcohol at the two-week and one-month period as did those left in an automobile trunk for approximately one week during hot weather. It was also found that samples preserved with sodium fluoride and potassium oxalate gave similar decreases over a two-three month period.

Meyer, Monge and Sakshaug (1979) tested fresh blood samples for alcohol content and then froze them at -20° C for a period of 6 months at which time they were tested again.

The samples were tested two more times, once after 14 days and the final time 28 days later. The results showed that there were only insignificant differences between the first analyses of the samples and the three later analyses.

They also ran another experiment where blood samples were stored at -20° C for about 6 months and then for another 5 months at 3° C before reanalysis. The results showed that the samples tested after 11 months were still comparable with the values for alcohol content in the fresh samples.

Hayden, Layden and Hickey (1977) ran a series of four experiments. In the Series 1 Experiment, an initial analysis was performed on blood samples for alcohol content. The samples were then stored at 4° C for a period of 30 to 50 days at which time they were reanalyzed. A chi-square test was applied and it was found that there was not a significant difference between the original samples and the ones retested 30 to 50 days later.

In the Series 2 Experiment blood samples were stored for a period of 1 year at 4° C before reanalysis. Results showed that there was a significant decrease in the retested samples at a 95 % confidence level.

In the Series 3 Experiment blood samples were stored for a period of 3 to 6 weeks at room temperature before reanalysis. Results showed that there was not a significant difference in the retested samples.

In the Series 4 Experiment was a practical application of the above studies in which blood samples were stored for a

period of 23 days at 4° C before reanalysis. Results showed that there was not a significant difference in the retested samples.

Winek and Paul (1983) examined the effects of time, temperature and a preservative (sodium fluoride) on ethanol concentrations in stored samples of whole blood from living human specimens. They measured the ethanol in the first, second, seventh and 14th day of storage by gas chromatography. Samples were stored at 0 - 3° C and at 22 - 29° C, with and without preservative. None of these showed significant gains or losses in concentration. The average differences between ethanol as measured on the day of collection and after storage were all within the range of experimental error of the method (+/- 5 %).

Moynham, Beverstock, Perl and Starmer (1985) ran three experiments. In Experiment I the samples were stored at 4° C and 25° C and were assayed on the day of collection, every day thereafter for 5 days and after 6 weeks storage at 4° C. Assays were carried out on Days 0, 1, 2, 3 and 5 (Experiment II) and on Days 0 and 42 (Experiment III). Temperature variation was less than 1° C. The data in Experiment I indicated that no generation of alcohol occurred in the control samples under either storage condition whether sodium fluoride was present or not. No consistent changes occurred in the alcohol content of the other samples over time and, in most cases, the changes which did occur were within the 95 % confidence limits of the assay. No effect could be attributed

either to the storage temperature or the presence or absence of enzyme inhibitor (sodium fluoride).

The results from Experiment II were essentially similar to those obtained previously. That is, none of the control samples were found to have generated alcohol during the 5 - day storage period and those which contained alcohol showed an insignificant trend towards loss, irrespective of the presence of sodium fluoride.

In Experiment III, the effects of storage over the 42 - day period were examined. Student's t-tests carried out on the means of the differences between the blood alcohol concentrations on Day 0 and Day 42 revealed a number of significant alcohol depletion effects. The addition of fluoride was associated with greater alcohol depletion than when no preservative was present.

Dick and Stone (1987) described the circumstances in which some drivers' blood specimens containing added sodium fluoride (1% w/v concentration) deteriorated as a result of microbial contamination, accompanied by a decrease of alcohol concentration. Strains of the bacteria Serratia marcescens and Psuedomonas sp. were isolated from the specimens and proven capable of growing at ambient temperature in blood containing sodium fluoride at 1 % w/v concentration. They were shown to be active in alcohol degradation in preserved blood, the activity being dependent on sodium fluoride concentration and storage temperature. Blood diluters were assumed to be the source of microbial cross contamination from

one blood specimen to the next. The authors recommended that postmortem blood specimens be analyzed in separate batches from drivers' specimens when automated blood diluters are used, that the content of fluoride ions be increased to an equivalent of 2 % w/v sodium fluoride and that the storage of specimens at temperatures above 4° C be minimized.

Chang and Kollman (1989) studied the effect of temperature on microbial fermentation. Specimens of human blood from a blood bank were inoculated with Candida albicans, an organism capable of causing fermentation. A preservative was added to a portion of the inoculated specimens. These inoculated specimens, as well as uninoculated blood, were stored under various temperature conditions (37° C, 22° C, and 6° C). Production of ethyl alcohol was monitored over a period of six months. Fermentation was found to be highly temperature dependent, with refrigeration proving to be most effective at inhibiting ethanol formation.

Based on this literature review, it was determined that only four studies have dealt with storage periods of one year or longer. These studies can be summarized as follows:

Shajani, Image and Chu -
Forensic samples were tested then retested one year later following storage at 4° C. Results significantly decreased.

Chang, Smith, Walkin and Reynolds -
Reanalyzed samples stored at room temperature after 3 years

and then after 6.75 years. Samples declined in the 20 to 40 mg % range.

Glendening and Waugh -
Blood samples held at room temperature for a period of two years showed a decrease of 0.045 %.

Hayden, Layden and Hickey -
Blood samples held one year at 4° C showed a significant decrease when reanalyzed.

Of these studies, none have tested the samples following a period in which they were frozen or have taken into consideration concentration differences which may have skewed their results. Because most of the studies in the literature review have shown that decreased temperature is a very important factor in enabling the blood samples to remain stable, an attempt should be made to test the affects of freezing the samples for a period of one year. Also concentration differences should be examined to determine whether high initial blood alcohol values could possibly have resulted in erroneous decreases causing the overall statistical conclusion to be affected. Therefore the purpose of this research has been established.

METHODS AND MATERIALS

The basic method presented here is the analysis of ethyl alcohol in the equilibrated headspace above liquid samples by means of automated gas chromatography with a flame ionization detector. The corresponding quantitation being calculated with the use of an internal standard.

Summary of Operation - The following procedure is performed twice, once when the samples originally come into the lab for analysis and once following the one year freezing period.

Liquid samples (100 µl) in two separate aliquots are placed into two separate glass septum vials after dilution in fixed proportion with the internal standard solution. Reference samples are similarly treated and all vials are sealed with polymeric septum stoppers and crimped aluminum caps and inserted into the thermostated instrument turntable which has a capacity of 24 vials. The headspace sample uses a valve and loop system to transfer an aliquot of headspace gas to the gas chromatograph. The transfer is done automatically by pressurizing the vial with carrier gas,

allowing the headspace gas to fill a sample loop of known volume, and then delivering that aliquot, through a heated transfer line, to the gas chromatograph port. The analysis then proceeds for an adjustable, preselected analysis time. The response of the flame ionization detector is recorded, as a function of time, on a potentiometric strip-chart recorder. Each sample is identified on the recorder chart by the relative length of an identification trace which precedes the respective chromatogram.

Apparatus

1. Hewlett Packard 5890 Gas Chromatograph
2. Hewlett Packard 19395A Autosampler
3. Hewlett Packard 3392A Integrator
4. Eppendorf Pippettor (10-100µl adjustable)
5. Compressed Air - Used for the flame ionization detector
6. Hydrogen - Used for the flame ionization detector
7. Helium - Used as the carrier gas

Reagents

1. Calibration Reference Materials (Calibrators)
    a. Ethanol 0.15 % W/V Aqueous Solution
       College of American Pathologists
    b. Ethanol 0.15 % W/V Aqueous Solution
       Ultra Chem

2. Control Reference Materials

   a. Ethanol 0.15 % W/V Aqueous Solution
      College of American Pathologists

   b. Ethanol 0.10 % W/V Aqueous Solution
      Eth-a-trol Alcohol Control-Level I

3. Internal Standard Solution
   Isopropyl Alcohol 0.3128 % V/V

Supplies

1. Glass vials, 10 ml, with polymer septum stoppers and aluminum seals

Instrument and Analysis Conditions

The following are typical instrument and analysis conditions which have been found satisfactory in the laboratory.

Column:	Carbowax 1500 (0.3 %) on 80/100 mesh Carbopack A 7 ft. X 1/8 inch stainless steel column		
Carrier Gas:	Helium;	inlet pressure	60 psi
		column head pressure	50 psi
		flow rate	25 ml/min

Temperatures:	Heating bath	60° C
	Valve/Loop	65° C
	Injector	250° C
	Column oven	85° C
	Detector	250° C
FID:	Hydrogen; inlet pressure	25 psi
	Air; inlet pressure	47 psi
Program:	Equilibrium time	3 minutes
	Pressurization	20 seconds
	Vent	2 seconds
	Injection	20 seconds
	Analyses/vial	1
Integrator Settings:	Range	5
	Attenuation	X 5
	Chart speed	5 mm/min
	Peak width	0.4 minutes
	Threshold	5
	Area reject	0
	Stop time	2.50 minutes

EXPERIMENTAL RESULTS

Tables 1a and 1b list the results of the blood alcohol analysis before and after the one year freezing period at -11° C. Controls are listed for each of the 92 cases before and after the freezing period along with a calculated initial analysis mean and final analysis mean which will be used later in the statistical calculations.

Table 1a. Pre- and Post Freezing Blood Alcohol Results for Cases 1 - 46.

Case #	Initial Date	Initial Control	Run #1	Run #2	Initial Mean %	Final Date	Final Control	Run #1	Run #2	Final Mean %
1	6/15/92	0.149%	0.050%	0.046%	0.048%	7/21/93	0.155%	0.045%	0.045%	0.045%
2	6/21/92	0.149%	0.216%	0.223%	0.219%	7/21/93	0.155%	0.222%	0.209%	0.215%
3	6/10/92	0.148%	0.166%	0.167%	0.166%	7/21/93	0.152%	0.167%	0.172%	0.169%
4	6/2/92	0.149%	0.167%	0.166%	0.166%	7/21/93	0.152%	0.167%	0.167%	0.167%
5	6/16/92	0.150%	0.203%	0.208%	0.205%	7/21/93	0.152%	0.177%	0.176%	0.176%
6	6/30/92	0.149%	0.200%	0.203%	0.201%	7/21/93	0.151%	0.189%	0.192%	0.190%
7	6/15/92	0.147%	0.182%	0.182%	0.182%	7/21/93	0.151%	0.179%	0.179%	0.179%
8	6/8/92	0.151%	0.218%	0.223%	0.220%	7/21/93	0.151%	0.214%	0.215%	0.214%
9	6/18/92	0.151%	0.232%	0.230%	0.231%	7/21/93	0.151%	0.225%	0.225%	0.225%
10	6/10/92	0.149%	0.238%	0.238%	0.238%	7/21/93	0.151%	0.213%	0.213%	0.213%
11	6/18/92	0.154%	0.164%	0.169%	0.166%	7/22/93	0.151%	0.158%	0.157%	0.157%
12	6/25/92	0.148%	0.213%	0.219%	0.216%	7/22/93	0.151%	0.206%	0.204%	0.205%
13	6/12/92	0.151%	0.075%	0.074%	0.074%	7/22/93	0.150%	0.068%	0.069%	0.068%
14	6/22/92	0.153%	0.123%	0.127%	0.125%	7/22/93	0.150%	0.116%	0.120%	0.118%
15	6/5/92	0.153%	0.210%	0.213%	0.211%	7/22/93	0.150%	0.217%	0.214%	0.215%
16	6/23/92	0.150%	0.082%	0.082%	0.082%	7/22/93	0.150%	0.057%	0.058%	0.057%
17	6/23/92	0.151%	0.190%	0.193%	0.191%	7/22/93	0.150%	0.176%	0.175%	0.175%
18	6/10/92	0.150%	0.256%	0.257%	0.256%	7/22/93	0.149%	0.251%	0.251%	0.251%
19	6/18/92	0.151%	0.217%	0.215%	0.216%	7/22/93	0.149%	0.189%	0.187%	0.188%
20	7/15/92	0.154%	0.270%	0.271%	0.270%	8/2/93	0.150%	0.257%	0.259%	0.258%
21	7/27/92	0.148%	0.129%	0.130%	0.129%	8/2/93	0.150%	0.132%	0.132%	0.132%
22	7/15/92	0.157%	0.156%	0.158%	0.157%	8/2/93	0.150%	0.163%	0.164%	0.163%
23	7/22/92	0.151%	0.164%	0.156%	0.160%	8/2/93	0.150%	0.161%	0.166%	0.163%
24	7/6/92	0.148%	0.182%	0.184%	0.183%	8/2/93	0.150%	0.175%	0.178%	0.176%
25	7/28/92	0.150%	0.189%	0.195%	0.192%	8/2/93	0.151%	0.181%	0.187%	0.184%
26	7/15/92	0.154%	0.111%	0.112%	0.111%	8/2/93	0.151%	0.104%	0.107%	0.105%
27	7/22/92	0.148%	0.170%	0.167%	0.168%	8/2/93	0.150%	0.170%	0.172%	0.171%
28	7/30/92	0.149%	0.125%	0.122%	0.123%	8/2/93	0.150%	0.117%	0.117%	0.117%
29	7/28/92	0.149%	0.150%	0.145%	0.147%	8/2/93	0.150%	0.143%	0.149%	0.146%
30	7/20/92	0.152%	0.021%	0.020%	0.020%	8/2/93	0.152%	0.020%	0.021%	0.020%
31	7/20/92	0.152%	0.224%	0.225%	0.224%	8/2/93	0.152%	0.210%	0.213%	0.211%
32	7/27/92	0.148%	0.145%	0.145%	0.145%	8/2/93	0.148%	0.141%	0.141%	0.141%
33	7/22/92	0.149%	0.054%	0.055%	0.054%	8/2/93	0.148%	0.035%	0.036%	0.035%
34	7/9/92	0.151%	0.218%	0.219%	0.218%	8/2/93	0.148%	0.217%	0.218%	0.217%
35	7/22/92	0.150%	0.182%	0.181%	0.181%	8/2/93	0.151%	0.166%	0.173%	0.169%
36	8/18/92	0.151%	0.150%	0.150%	0.150%	9/1/93	0.148%	0.143%	0.143%	0.143%
37	8/18/92	0.148%	0.141%	0.143%	0.142%	9/1/93	0.148%	0.140%	0.142%	0.141%
38	8/24/92	0.154%	0.190%	0.196%	0.193%	9/1/93	0.148%	0.185%	0.190%	0.187%
39	8/18/92	0.148%	0.242%	0.248%	0.245%	9/1/93	0.148%	0.228%	0.233%	0.230%
40	8/18/92	0.151%	0.021%	0.021%	0.021%	9/1/93	0.154%	0.019%	0.019%	0.019%
41	8/11/92	0.148%	0.233%	0.234%	0.233%	9/1/93	0.156%	0.217%	0.222%	0.219%
42	8/10/92	0.155%	0.142%	0.144%	0.143%	9/1/93	0.156%	0.138%	0.140%	0.139%
43	8/31/92	0.152%	0.050%	0.052%	0.051%	9/1/93	0.153%	0.050%	0.049%	0.049%
44	8/14/92	0.148%	0.096%	0.096%	0.096%	9/1/93	0.153%	0.082%	0.075%	0.078%
45	8/31/92	0.152%	0.024%	0.022%	0.023%	9/1/93	0.153%	0.020%	0.019%	0.019%
46	8/11/92	0.148%	0.164%	0.165%	0.164%	9/1/93	0.151%	0.162%	0.164%	0.163%

Table 1b. Pre- and Post Freezing Blood Alcohol Results for Cases 47 - 92.

Case #	Initial Date	Initial Control	Run #1	Run #2	Initial Mean %	Final Date	Final Control	Run #1	Run #2	Final Mean %
47	8/14/92	0.150%	0.131%	0.133%	0.132%	9/1/93	0.151%	0.145%	0.136%	0.140%
48	8/28/92	0.150%	0.220%	0.223%	0.221%	9/1/93	0.150%	0.218%	0.220%	0.219%
49	8/11/92	0.149%	0.185%	0.187%	0.186%	9/1/93	0.150%	0.184%	0.187%	0.185%
50	8/10/82	0.151%	0.211%	0.211%	0.211%	9/1/93	0.150%	0.211%	0.202%	0.206%
51	8/12/92	0.155%	0.089%	0.090%	0.089%	9/1/93	0.154%	0.063%	0.068%	0.065%
52	8/31/92	0.152%	0.248%	0.249%	0.248%	9/1/93	0.154%	0.238%	0.237%	0.237%
53	8/31/92	0.151%	0.127%	0.129%	0.128%	9/1/93	0.154%	0.125%	0.128%	0.126%
54	8/24/92	0.152%	0.132%	0.135%	0.133%	9/1/93	0.154%	0.126%	0.129%	0.127%
55	8/26/92	0.150%	0.209%	0.208%	0.208%	9/2/93	0.149%	0.204%	0.202%	0.203%
56	8/11/92	0.149%	0.125%	0.125%	0.125%	9/2/93	0.149%	0.121%	0.123%	0.122%
57	3/8/93	0.148%	0.104%	0.104%	0.104%	4/25/94	0.108%	0.107%	0.107%	0.107%
58	3/8/93	0.147%	0.298%	0.298%	0.298%	4/25/94	0.108%	0.252%	0.254%	0.253%
59	3/22/93	0.148%	0.215%	0.209%	0.212%	4/25/94	0.103%	0.203%	0.198%	0.200%
60	3/5/93	0.153%	0.037%	0.036%	0.036%	4/25/94	0.103%	0.028%	0.029%	0.028%
61	3/2/93	0.148%	0.150%	0.154%	0.152%	4/25/94	0.103%	0.142%	0.139%	0.140%
62	3/12/93	0.150%	0.214%	0.217%	0.215%	4/25/94	0.103%	0.200%	0.204%	0.202%
63	3/2/93	0.146%	0.071%	0.072%	0.071%	4/25/94	0.103%	0.060%	0.056%	0.058%
64	3/2/93	0.148%	0.277%	0.278%	0.277%	4/25/94	0.107%	0.216%	0.220%	0.218%
65	3/17/93	0.149%	0.175%	0.174%	0.174%	4/25/94	0.107%	0.170%	0.173%	0.171%
66	3/17/93	0.151%	0.251%	0.250%	0.250%	4/25/94	0.107%	0.225%	0.228%	0.226%
67	3/22/93	0.148%	0.045%	0.044%	0.044%	4/25/94	0.104%	0.041%	0.041%	0.041%
68	3/23/93	0.146%	0.276%	0.272%	0.274%	4/25/94	0.104%	0.256%	0.265%	0.260%
69	3/22/93	0.148%	0.084%	0.082%	0.083%	4/25/94	0.105%	0.087%	0.087%	0.087%
70	3/26/93	0.149%	0.081%	0.081%	0.081%	4/25/94	0.105%	0.082%	0.083%	0.082%
71	3/22/93	0.148%	0.174%	0.173%	0.173%	4/25/94	0.105%	0.155%	0.155%	0.155%
72	3/30/93	0.148%	0.215%	0.210%	0.212%	4/25/94	0.100%	0.194%	0.198%	0.196%
73	3/22/93	0.150%	0.163%	0.163%	0.163%	4/25/94	0.103%	0.153%	0.154%	0.153%
74	4/5/93	0.153%	0.162%	0.163%	0.162%	5/1/94	0.104%	0.145%	0.151%	0.148%
75	4/2/93	0.153%	0.211%	0.205%	0.208%	5/1/94	0.104%	0.200%	0.207%	0.203%
76	4/5/93	0.153%	0.415%	0.418%	0.416%	5/1/94	0.103%	0.316%	0.323%	0.319%
77	4/8/93	0.154%	0.136%	0.135%	0.135%	5/1/94	0.103%	0.119%	0.122%	0.120%
78	4/8/93	0.151%	0.088%	0.084%	0.086%	5/1/94	0.103%	0.079%	0.079%	0.079%
79	4/5/93	0.152%	0.039%	0.039%	0.039%	5/1/94	0.103%	0.029%	0.031%	0.030%
80	4/13/93	0.153%	0.142%	0.134%	0.138%	5/1/94	0.103%	0.125%	0.125%	0.125%
81	4/27/93	0.154%	0.215%	0.213%	0.214%	5/1/94	0.102%	0.183%	0.181%	0.182%
82	4/13/93	0.152%	0.064%	0.063%	0.063%	5/1/94	0.102%	0.066%	0.063%	0.064%
83	4/13/93	0.152%	0.241%	0.241%	0.241%	5/1/94	0.102%	0.209%	0.209%	0.209%
84	4/13/93	0.152%	0.102%	0.099%	0.100%	5/1/94	0.098%	0.094%	0.094%	0.094%
85	4/22/93	0.151%	0.197%	0.197%	0.197%	5/1/94	0.098%	0.182%	0.179%	0.180%
86	4/19/93	0.150%	0.416%	0.421%	0.418%	5/1/94	0.100%	0.320%	0.330%	0.325%
87	4/19/93	0.151%	0.112%	0.111%	0.111%	5/1/94	0.100%	0.102%	0.103%	0.102%
88	4/27/93	0.154%	0.120%	0.126%	0.123%	5/1/94	0.100%	0.107%	0.111%	0.109%
89	4/19/93	0.152%	0.073%	0.075%	0.074%	5/1/94	0.100%	0.069%	0.069%	0.069%
90	4/27/93	0.153%	0.105%	0.110%	0.107%	5/1/94	0.100%	0.093%	0.094%	0.093%
91	4/19/93	0.150%	0.372%	0.373%	0.372%	5/1/94	0.099%	0.286%	0.293%	0.289%
92	4/8/93	0.151%	0.242%	0.245%	0.243%	5/1/94	0.099%	0.222%	0.231%	0.226%

STATISTICAL CALCULATIONS

Paired Sample t-test

The statistical test chosen to compare the values between the samples in the original analysis to the values after the one year frozen storage period was the paired sample t-test. The paired t-test determines whether significant differences exist for the same groups at two different times. The major advantage of using the paired sample t-test is that because there is so little difference in variability between two groups, when a paired t-test is used, the t-statistic provides a sensitive test of the difference between the two means. The means are the t-test's focal points in determining if differences do exist.

The t value was calculated from the data in Tables 2a and 2b. For the purpose of eliminating small decimal numbers, the data was converted from gram percent to milligram percent.

The calculated t value was determined to be 6.40 while the critical value was 1.990, therefore the null hypothesis can be rejected. There was a statistically significant decrease in the blood alcohol levels after the samples had been frozen for a period of one year. The decrease was significant at the $p = 0.05$ level on a two tailed test.

Table 2a. Statistical Calculation Results for Cases 1 - 46.

Case #	Initial Mean mg % (A)	Final Mean mg % (B)	(A - B) = X	X^2
1	48	45	3	9
2	219	215	4	16
3	166	169	-3	9
4	166	167	-1	1
5	205	176	29	841
6	201	190	11	121
7	182	179	3	9
8	220	214	6	36
9	231	225	6	36
10	238	213	25	625
11	166	157	9	81
12	216	205	11	121
13	74	68	6	36
14	125	118	7	49
15	211	215	-4	16
16	82	57	25	625
17	191	175	16	256
18	256	251	5	25
19	216	188	28	784
20	270	258	12	144
21	129	132	-3	9
22	157	163	-6	36
23	160	163	-3	9
24	183	176	7	49
25	192	184	8	64
26	111	105	6	36
27	168	171	-3	9
28	123	117	6	36
29	147	146	1	1
30	20	20	0	0
31	224	211	13	169
32	145	141	4	16
33	54	35	19	361
34	218	217	1	1
35	181	169	12	144
36	150	143	7	49
37	142	141	1	1
38	193	187	6	36
39	245	230	15	225
40	21	19	2	4
41	233	219	14	196
42	143	139	4	16
43	51	49	2	4
44	96	78	18	324
45	23	19	4	16
46	164	163	1	1

Table 2b. Statistical Calculation Results for Cases 47 - 92.

Case #	Initial Mean mg % (A)	Final Mean mg % (B)	(A - B) = X	X^2
47	132	140	-8	64
48	221	219	2	4
49	186	185	1	1
50	211	206	5	25
51	89	65	24	576
52	248	237	11	121
53	128	126	2	4
54	133	127	6	36
55	208	203	5	25
56	125	122	3	9
57	104	107	-3	9
58	298	253	45	2025
59	212	200	12	144
60	36	28	8	64
61	152	140	12	144
62	215	202	13	169
63	71	58	13	169
64	277	218	59	3481
65	174	171	3	9
66	250	226	24	576
67	44	41	3	9
68	274	260	14	196
69	83	87	-4	16
70	81	82	-1	1
71	173	155	18	324
72	212	196	16	256
73	163	153	10	100
74	162	148	14	196
75	208	203	5	25
76	416	319	97	9409
77	135	120	15	225
78	86	79	7	49
79	39	30	9	81
80	138	125	13	169
81	214	182	32	1024
82	63	64	-1	1
83	241	209	32	1024
84	100	94	6	36
85	197	180	17	289
86	418	325	93	8649
87	111	102	9	81
88	123	109	14	196
89	74	69	5	25
90	107	93	14	196
91	372	289	83	6889
92	243	226	17	289
			$\Sigma x = 1108$	$43062 = \Sigma x^2$

It is seen from the calculated t-test that there was a statistically significant decrease in the blood alcohol level with the mean decrease being 0.012 % ($\overline{X}$ = 12.04). Therefore any sample with a blood alcohol level between 0.100 % and 0.111 % stands a chance of decreasing after the one year freezing affect to the point that the blood alcohol level would be below the legal cutoff level of 0.100 % causing the case to be possibly thrown out.

DISCUSSION

Statistical analysis revealed that there was a significant decrease in blood alcohol levels after the samples had been frozen and retested after a one year freezing period. It also disclosed that the decrease was 0.012 %. That seems like a very large amount which in turn could overturn an original guilty decision to one of not guilty if the sample was retested per request of the defense attorney.

Breaking the data into groups based on their original blood alcohol levels would allow a more in depth look at where the major decreases are occurring. For that purpose the data was divided up as follows:

Table 3 represents the mean decrease in blood alcohol levels between the pre- and post freezing period when the original blood alcohol level was between 0 and 99 mg %.

Table 4 represents the mean decrease in blood alcohol levels between the pre- and post freezing period when the original blood alcohol level was between 100 and 149 mg %.

Table 5 represents the mean decrease in blood alcohol levels between the pre- and post freezing period when the original blood alcohol level was between 150 and 199 mg %.

Table 6 represents the mean decrease in blood alcohol levels between the pre- and post freezing period when the original blood alcohol level was between 200 and 299 mg %.

Table 7 represents the mean decrease in blood alcohol levels between the pre- and post freezing period when the original blood alcohol level was above 300 mg %.

Table 8 summarizes Tables 3 through 7 and allows for easy comparison between the groups.

Table 8 reveals that the mean decrease is similar between the first three groups with a mean decrease of 0.007 %, 0.005 % and 0.006 % for (0 - 99 mg %), (100 - 149 mg %) and (150 - 199 mg %) respectively. It isn't until the original blood alcohol level gets above 200 mg % that a mean decrease jumps to 0.016 % and once the original level gets above 300 mg % the mean decrease escalates to an enormous 0.091 %.

Focusing on Table 4 which shows that the mean decrease of 0.005 % exists for the group with an original blood alcohol level between 100 and 149 mg % happens to be much lower than what was originally calculated 0.012 % ($\overline{X}$ = 12.04) for the entire groups combined. The mean decrease of 0.005 % is much

more acceptable to the criminal justice system and lowers the concern range from (0.100 % - 0.111 %) to (0.100 % - 0.104 %) where the retested sample after the one year freezing period could drop below the legal limit of 0.100 %.

Table 3. Initial Mean (A) Between 0 - 99 mg %.

Case #	Initial Mean mg % (A)	Final Mean mg % (B)	(A - B) = X
1	48	45	3
13	74	68	6
16	82	57	25
30	20	20	0
33	54	35	19
40	21	19	2
43	51	49	2
44	96	78	18
45	23	19	4
51	89	65	24
60	36	28	8
63	71	58	13
67	44	41	3
69	83	87	-4
70	81	82	-1
78	86	79	7
79	39	30	9
82	63	64	-1
89	74	69	5
		n = 19	Σx = 142

$$\bar{X} = \frac{\Sigma X}{n} = \frac{142}{19} = 7.47$$

Table 4. Initial Mean (A) Between 100 - 149 mg %.

Case #	Initial Mean mg % (A)	Final Mean mg % (B)	(A - B) = X
14	125	118	7
21	129	132	-3
26	111	105	6
28	123	117	6
29	147	146	1
32	145	141	4
37	142	141	1
42	143	139	4
47	132	140	-8
53	128	126	2
54	133	127	6
56	125	122	3
57	104	107	-3
77	135	120	15
80	138	125	13
84	100	94	6
87	111	102	9
88	123	109	14
90	107	93	14
		n = 19	$\Sigma X = 97$

$$\overline{X} = \frac{\Sigma X}{n} = \frac{97}{19} = 5.10$$

Table 5. Initial Mean (A) Between 150 - 199 mg %

Case #	Initial Mean mg % (A)	Final Mean mg % (B)	(A - B) = X
3	166	169	-3
4	166	167	-1
7	182	179	3
11	166	157	9
17	191	175	16
22	157	163	-6
23	160	163	-3
24	183	176	7
25	192	184	8
27	168	171	-3
35	181	169	12
36	150	143	7
38	193	187	6
46	164	163	1
49	186	185	1
61	152	140	12
65	174	171	3
71	173	155	18
73	163	153	10
74	162	148	14
85	197	180	17
		n = 21	Σx = 128

$$\overline{X} = \frac{\Sigma X}{n} = \frac{128}{21} = 6.09$$

Table 6. Initial Mean (A) Between 200 - 299 mg %

Case #	Initial Mean mg % (A)	Final Mean mg % (B)	(A - B) = X
2	219	215	4
5	205	176	29
6	201	190	11
8	220	214	6
9	231	225	6
10	238	213	25
12	216	205	11
15	211	215	-4
18	256	251	5
19	216	188	28
20	270	258	12
31	224	211	13
34	218	217	1
39	245	230	15
41	233	219	14
48	221	219	2
50	211	206	5
52	248	237	11
55	208	203	5
58	298	253	45
59	212	200	12
62	215	202	13
64	277	218	59
66	250	226	24
68	274	260	14
72	212	196	16
75	208	203	5
81	214	182	32
83	241	209	32
92	243	226	17
		n = 30	$\Sigma X = 468$

$$\overline{X} = \frac{\Sigma X}{n} = \frac{468}{30} = 15.60$$

Table 7. Initial Mean (A) Above 300 mg %.

Case #	Initial Mean mg % (A)	Final Mean mg % (B)	(A - B) = X
76	416	319	97
86	418	325	93
91	372	289	83
		n = 3	ΣX=273

$$\overline{X} = \frac{\Sigma X}{n} = \frac{273}{3} = 91.00$$

Table 8. Results Summary.

Initial Mean Blood Alcohol Range	Number of Cases	Mean Decrease in % After Freezing
0 - 0.099 %	19	0.007%
0.100 % - 0.149 %	19	0.005%
0.150 % - 0.199 %	21	0.006%
0.200 % - 0.299 %	30	0.016%
Above 0.300 %	3	0.091%

CONCLUSION

The purpose of this thesis experiment was to look at the effects that long term storage had on blood alcohol levels by comparing pre- and post storage blood alcohol levels by means of the paired sample t-test. Calculation of that test revealed that freezing the samples for a period of one year at -11° C did significantly decrease the blood alcohol levels but that decrease was not as prevalent in the range where the possibility existed that the original court decision could be overturned.

This decrease could have been caused by a number of factors. Firstly, one mechanism of ethanol loss, and probably the most likely, could be caused by physical loss from the containers due to the volatile nature of ethanol. Another reason for the decrease in blood alcohol levels could be attributed to a chemical mechanism in which erythrocyte - associated, temperature - dependent oxidation of ethanol to acetic acid via acetaldehyde occurred. The extent of alcohol loss is also affected by the amount of air held within the specimen container. Oxyhemoglobin could be formed from the oxygen in the air space of the container and subsequently utilized during ethanol oxidation.

The final mechanism in which ethanol loss could have occurred from is due to microbes. A wide variety of microbes are capable of utilizing ethanol as a source of carbon and energy. However, they are unlikely to have any great effect on ethanol levels unless they are present in relatively high numbers.

Looking at the data it can be noticed that some samples actually increased in blood alcohol concentration after the one year freezing period. This could be caused by chemical and biochemical mechanisms. There is the possibility that ethanol could have been produced from lactate in putrefying blood. Since blood often contains relatively high levels of lactate (240 to 350 mg/ml) this could be a common occurrence.

The research presented here demonstrates that the freezing of blood specimens for long term storage is a much more efficient way to preserve the samples, opposed to room temperature or refrigeration which was previously done in the literature. Also concentration differences should have been considered to determine that statistical analyses were skewed by large alcohol decreases from high initial blood alcohol levels.

In conclusion, the recommendations that can be made to decision makers in the crime laboratory is that the storage of blood specimens from Driving Under the Influence of Alcohol (DUI) cases should be in a frozen condition. Also they should emphasize to the courts that there will be an expected decrease in blood alcohol levels after the one year freezing

period and that decrease should not affect an earlier court decision. Incorporating these two suggestions into policy would result in a sufficient procedure for the preservation of evidence which could be upheld in a court of law.

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