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THE EFFECT OF PRESERVATIVE AND TEMPERATURE ON BLOOD ALCOHOL CONCENTRATION DURING STORAGE PERIOD

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ABSTRACT

Determination of blood alcohol concentration (BAC) on ante mortem and post mortem samples is often performed in forensic laboratory. BAC determination has been common task in forensic laboratory due to rising accident cases due to driving under influence of alcohol. Earlier researchers have proven that factors such as preservative, storage temperature and storage period affect the blood alcohol stability and BAC determination. Apparently, no regulation governs these factors in BAC determination. Therefore, this study is performed to address the effect of preservative, temperature and storage days on BAC level. A blood specimen of ante and post mortem samples with preservative and non preservative in two different conditions (chilled temperature, 4-7^{o}C and room temperature, 24-26.9^{o}C) has been examined. The analysis was carried out on 1st day, 7th day and 14th day of blood sample received. The blood samples have been diluted with an aqueous internal standard solution of *n*-propanol and analyzed using gas chromatography with flame ionization detection (GC-FID). The analysis showed that the temperature and preservative have no effect on BAC of ante mortem and post mortem blood samples and blood alcohol analysis can be performed within 21 days.

ABSTRAK

Penentuan kepekatan alkohol dalam darah individu hidup dan mati sering dilakukan dalam makmal forensik. Contohnya, bagi kes kemalangan jalan raya dan memandu di bawah pengaruh alkohol. Sejak kebelakangan ini, analisis alkohol telah menjadi analisis rutin di makmal forensik disebabkan oleh kes kemalangan yang meningkat akibat memandu di bawah pengaruh alkohol. Kajian sebelum ini membuktikan bahawa faktor-faktor seperti pengawet, suhu penyimpanan dan tempoh penyimpanan menjejaskan kestabilan darah dalam penentuan kandungan alkohol. Pada masa yang sama, tiada peraturan yang setara dalam mengawal faktor-faktor tersebut dalam penentuan alkohol dalam darah. Oleh yang demikian, perubahan kandungan alkohol dalam darah orang hidup dan mati dikaji berdasarkan faktor pengawet dan pada suhu yang berbeza iaitu 4-7^{o}C dan suhu bilik 24-26.9^{o}C. Sampel darah dicairkan dengan larutan standard n-propanol dan dianalisa menggunakan peralatan saintifik, kromatografi gas-pengesanan pengionan nyala. Hasil analisis menunjukkan suhu dan bahan pengawet tidak memberi kesan ke atas kandungan alkohol dalam sampel darah individu hidup ataupun mati sehingga 21 hari.

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LIST OF ABBREVIATIONS

ADH	-	Alcohol dehydrogenase enzyme
BAC	-	Blood Alcohol Concentration
BAL	-	Blood Alcohol Level
FID	-	Flame Ionization Detector
GC	-	Gas Chromatography
g/100 mL	-	gram per 100 milliliter
MIROS	-	Malaysian Institute of Road Safety Research
mg/100 mL	-	milligram per 100 milliliter
ND	-	Not detected
NS	-	Not significant
oz.	-	Fluid ounce
QC	-	Quality Control
S	-	Significant
SOP	-	Standard operating procedure
STD. DEV	-	Standard Deviation
temp	-	Temperature
UAL	-	Urine Alcohol Level
w/out	-	without
WHO	-	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Alcohol is the most popular drug misused by young people according to Polymerous (2007). Apart from that, alcohol also one of the most widely used psychoactive drugs known to man and it has been with us since the dawn of civilization (Canadian Government's Commission Inquiry, 1971). On top of that, alcohol is known to be the most intoxicating substance by Bobo (2003) in American Prosecutors Research Institute Report.

Driving and drinking of alcohol is a relative recent phenomenon not older than 60 years which concern many people over the world. Alcohol has created problems in traffic offences which warrant serious consideration. Without much debate, alcoholism on the road is the main contributing factor to the causes of road accidents which often resulted in tragedies related to loss of life and property (Bobo, 2003).

According to Cambs. Institute of Alcohol Studies (2010), alcohol has been a major cause of accidents and accidental injury. Alcohol consumption shall be regulated since the presence of alcohol in the body has shown to increase the severity of injuries from accidents. Alcohol has range of psychomotor and cognitive effects that increase accident risk.

According to World Health Organization (WHO) report, until the year 2020, the rate of injury and death due to road accident will increase about 65% if there are no appropriate enforcements (Canadian Government's Commission of Inquiry, 1971).

In Malaysia, the Ministry of Transport and the Malaysian Institute of Road Safety Research (MIROS) have documented road accidents statistics. Malaysia recorded the highest number of road accidents per number of vehicles where in terms of per 10 thousands registered vehicles in 1990 to 8.2 per 10 thousand vehicles in 1996 (Annual Report, Ministry of Transport, Malaysia, 2010). Apart from that, Road Transport Department's statistics (2010), up to year 2004 also shows that about 10–15% of the 6.3 million drivers were reported to be under the influence of alcohol and drugs. The most recent statistics from Annual Report, Road Transport Department of Malaysia shows that the number of road accidents and death due to alcohol consumption had been on increasing since 2001 until 2010. Total of 414, 421 road accidents and 5, 264 number of deaths were reported in 2010.

According to Norlen *et al.,* (2012), alcohol is widely used by drivers involved in road accidents compared to use of other drugs. Comparing by types of substance use, alcohol use was higher among single vehicle accidents (35.9%) compared to multiple vehicle accidents cases (8.5%).

The most obvious and specific test for heavy drinking is the measurement of alcohol in breath, urine and blood. Beginning July 1, 1995, motor vehicles rules were enforced in Klang Valley. It was an offense to drive if one's blood alcohol level is equal or above 80 mg/DL (0.08%) under this law (Norlen *et al.*, 2012).

Figure 1.1 and Table 1.1 extracted from Malaysian Institute of Road Safety Research report, (2012), shows the distribution of blood alcohol level among cases positive for alcohol. The mean and standard deviation of blood alcohol concentration (BAC) was 176.56 and 77.7. The breakdown of cases by different levels of BAC revealed that 82% of fatal accident cases occurred with BAC levels of 80 mg/100 mL and above. 11% of fatal cases occurred at BAC level between $<50<x\leq80$ and 6.6%

occurred at BAC levels of 50 mg/mL and below. This indicates that 17.6% of road traffic deaths related to under the influence of alcohol occurred at BAC level of 80 mg/100 mL and below.

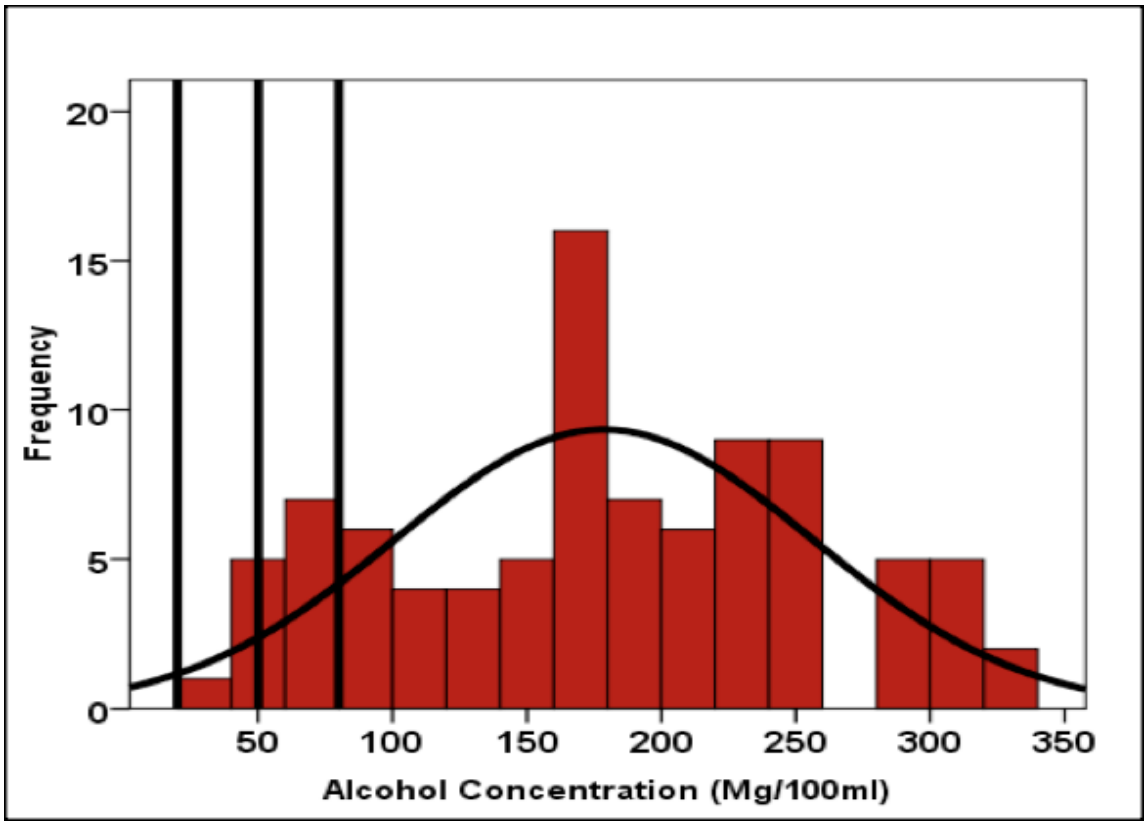

Figure 1.1: Blood Alcohol Concentration (BAC) versus Number of Death (Norlen *et al.*, 2012).

Table 1.1: Summary of blood alcohol concentration, number of death and percentage (Norlen *et al.*, 2012).

Alcohol Concentration (mg/100ml)	Number of death	Percentage (%)
< 20	0	0
20 < X < 50	6	6.6
50 < X < 80	10	11.0
X > 80	75	82.4

The statistics from Annual Report, Road Transport Department of Malaysia (2010) shows that road accidents in Malaysia have been increasing since 2001. The number of death cases also keeps on increasing year by year. Based on statistics, given by MIROS, it clearly shows that alcohol is being widely used during driving compared to other type of drugs. Therefore forensic toxicologists have an important role in the analysis of the alcohol level in the driver's body.

The measurement of BAC is important to determine whether the drivers are guilt or innocence. Therefore again the forensic department plays crucial role to ensure the accuracy of BAC measurement so that innocent people is not penalized. There are few factors that determine the accuracy of the BAC measurement such as equipment, methodologies as well as the blood samples (Pifeiffer *et al.,* 1992).

1.2 Statement of Problem

Most of the earlier studies had focused on the equipment and it's methodologies to ensure the BAC measurement. However, the importance of main component in the analysis which is blood sample is often neglected. The storage, temperature, handling and preservative of blood samples are important to ensure the accuracy of the measurement. This is because there is no law or regulations in Malaysia that spells out clearly on how to control the factors above. Therefore, this study is to focus on the effect of storage, preservative and temperature on blood samples and how it can affect the result of blood alcohol concentration (BAC).

1.3 Objectives of Study

The objectives of this study are:

a) To determine the BAC in blood samples.
b) To study the effect of preservative and temperature on BAC.

c) To study the effect of storage time (duration of storage) on BAC under controlled conditions.

1.4 Scope of Study

This study involves the analysis of BAC to determine the effect of preservative, storage and temperature of blood samples. The blood samples used are ante mortem and post mortem samples collected from hospital. The analysis was conducted by using Gas Chromatography with Flame Ionization Detector (GC-FID).

1.5 Significance of Study

Since this study is first of its kind in Malaysia, the results of the study would be useful as a guideline for both enforcement authorities and forensic chemist personnel to understand the characteristics of the blood samples taken from ante and post mortem blood specimen. This study also provides useful information on how the blood samples shall be handled and stored to preserve the blood alcohol concentration.

The data produced by this study also could be used as reference point to produce guidelines or standard operating procedure (SOP) for relevant parties to handle the blood samples taken from ante mortem and post mortem samples. Definitely this study will provide useful insights for other researchers to expand the current study in other dimension.

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