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Change in blood alcohol concentration with time in samples collected from living

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Abstract
Blood alcohol level estimation is usually done in various circumstances where the blood collected from the accused/victim is then sent to the FSL for alcohol level estimation. In India there may be delay in this process, the delay may be due to the medical examiner or police to send the sample or by the FSL to analyze the same. The present study was done to find out the differences in the blood alcohol level due to the above said delays. The blood alcohol estimation was done using gas chromatography, and the test was done on 4 known samples with a time gap of 24 hours, 7 days and 14 days.

Keywords: Blood alcohol estimation, forensic science lab, delay, gas chromatography

Introduction
Alcohol consumption and related crimes are on the rise. Most common crime related to alcohol can be drinking and driving. Traffic accidents and fatalities due to driving under the influence of alcohol are on the rise in India. Accurate determination of blood alcohol concentration is of great importance Medico legally. Generally in police checking breathalyzers are used to estimate the alcohol concentration in the offender's body. But in cases where a medical officers help is taken to examine and issue a drunkenness certificate, and also in road traffic accident cases where autopsy is warranted, blood is collected in vacutainers or plastic bottles with added preservatives and sent to Forensic Science Laboratory for estimation of blood alcohol concentration. In Medico legal cases, it is important to provide an intact chain of possession from the person who is drawing the blood to the analyst [1]. Blood is collected by the Medical officer, handed over to the investigating officer who in turn hands over the sample to the Forensic Science laboratory. Gas chromatograph is used to estimate the concentration of blood in such set up. The blood in such cases is collected, stored in various ways and most of the times the blood may not be analysed immediately. The reasons may be backlog in the end of Forensic Science Laboratories which most of times are over burdened. The reason for delay sometimes may be due to delay in collection of the collected sample from the mortuary/hospital by the police or delay in handing over the sample to the Forensic Science Laboratory. The condition and time for which the sample is stored may thus vary. This may lead to change in alcohol concentration in the collected sample. In such cases there can be loss of alcohol from the sample due to evaporation [2], oxidation [3] or adsorption into the rubber stopper or there can be increase in alcohol concentration due to *in vitro* synthesis of alcohol due to bacterial action [2].
Some studies have indicated that ethanol in blood for alcohol estimation remains stable when Sodium Fluoride is used as preservative and blood is refrigerated [4].
In this study an attempt was made to assess the change in blood alcohol concentration with time.

Materials and Methods
The objective of this study was to study the change in blood alcohol concentration with time in samples obtained from living human subjects during the year 2013. Sample size of the present study was 4 volunteers. 12 vacutainers, sodium fluoride, potassium oxalate, four 10 ml syringes were kept ready (Picture 1). Written informed consent was taken from the volunteers. 3 subjects were asked to consume alcohol. 3 volunteers consumed Vodka with 38% concentration.

One volunteer did not consume any alcohol and was taken as control. The volume of alcohol consumed was not regulated. The volunteers were designated as W, X, Y and Control. 30 minutes after the last drink, 10 ml of venous blood was collected from each volunteer. Each sample was divided into three parts and stored in vacutainers (Picture 2) in room temperature to be examined on days 1, 7 and 14 days after collection. On the day of analysis, the samples were subjected to steam distillation. 'Chemito GC 1000' gas chromatograph with Flame ionization detector was used. Standard solution of ethanol was injected. Standard calibration curve was drawn for standard solution (Picture 3).

2 micro liters of each study sample distillate was injected to gas chromatograph on day 1, 7 and 14 and results curve were obtained for analysis. (Picture 4) (Picture 5) (Picture 6)

Observations & Results

The blood alcohol concentration of sample W on day 1 was 47.46mg%, on day 7 the concentration was 42.28% and on day 14 was 42.1mg%. Percentage decrease on day 7 compared to day 1 was 6.98% and percentage decrease on day 14 compared to day 1 was 9.64%. (Table 1)

The blood alcohol concentration of sample X on day 1 was 9.75mg%, on day 7 the concentration was 9.07% and on day 14 was 8.81mg%. Percentage decrease on day 7 compared to day 1 was 10.91% and percentage decrease on day 14 compared to day 1 was 11.29%. (Table 1)

The blood alcohol concentration of sample Y on day 1 was 16.33mg%, on day 7 the concentration was 12.45% and on day 14 was 11.87mg%. Percentage decrease on day 7 compared to day 1 was 23.75% and percentage decrease on day 14 compared to day 1 was 27.31%. (Table 1)

The control sample did not show any blood alcohol on days 1, 7 and 14.

Discussion

The present study was a case control study.

A study done by Tracey Winek *et al.* Showed 10-19% loss in blood alcohol concentration when stored in temperatures between 26.7-37.8 Celsius in 35 days. The finding is similar to the present study [5].

A study on effect of storage conditions on blood alcohol concentration done by a group or researchers from Karnataka showed blood alcohol concentration in blood with preservative and unrefrigerated samples showed significant fall with time. Similar finding was observed in the present study [6].

Another study on blood ethanol stability in different storage periods, done by a group of workers in Turkey, showed decrease in plasma ethanol concentrations with time in all the samples [7]. The finding is similar in the present study.

Study done by Chistopher Scott Vance *et al.* on immediate and delayed testing for blood alcohol concentration in alcohol positive and negative samples showed that all ethanol negative samples remained negative throughout the duration of study [8]. The finding was similar in the present study.

A study done by Moynham *et al.* on blood taken from living subjects showed no alcohol generation with varying temperature and storage. The finding was similar to present study [9].

In a study done by Singh and Chandra in 1999 showed that on 14th day of analysis there was post mortem loss of alcohol [10].

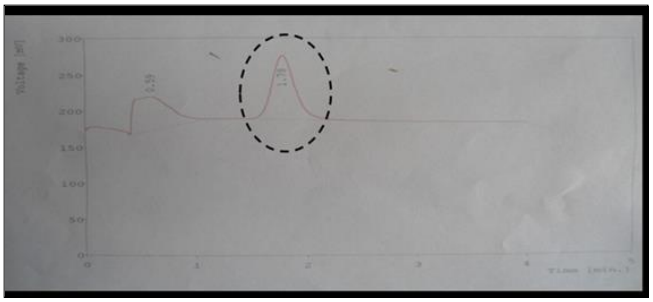
The limitations of the present study were its sample size, unmonitored storage conditions and unmonitored quantity of alcohol consumed.

Table 1: Showing changes in blood alcohol concentration with time

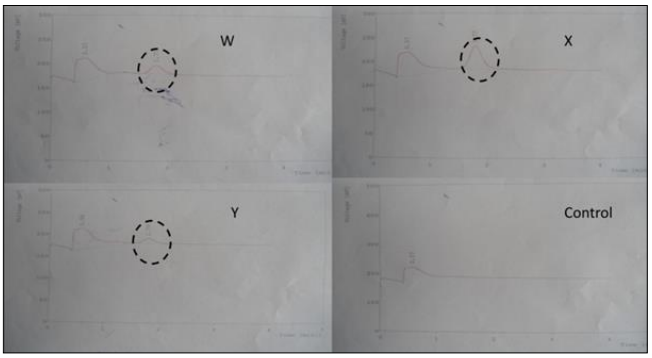
Sample	Day 1	Day 7	Day 14
W	9.75 mg%	9.07 mg%	8.81 mg%
X	47.46 mg%	42.28 mg%	42.1 mg%
Y	16.33 mg%	12.45 mg %	11.87 mg%
Control	nil	nil	nil

Picture 1: Showing materials used for sample collection

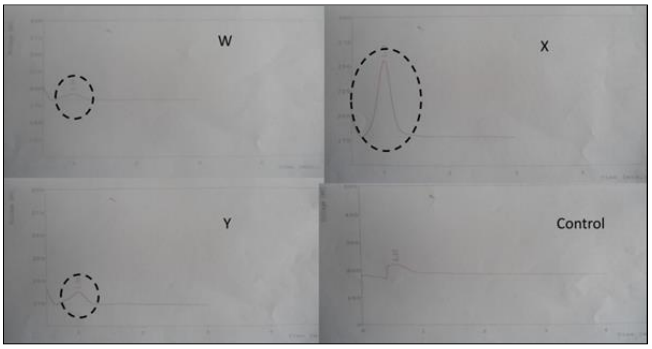
Picture 2: Showing collected sample for analysis



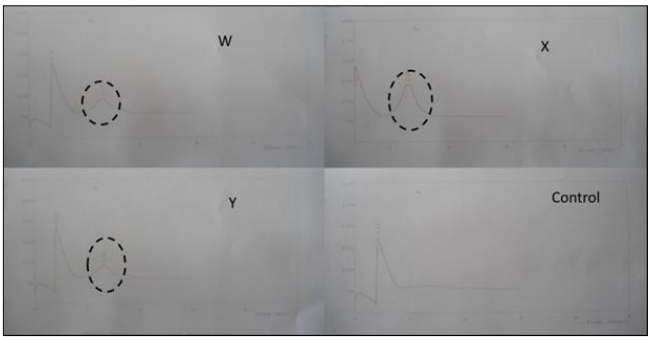
Picture 3: Standard chromatogram of standard ethanol sample.



Picture 4: Day 1 chromatograms showing peaks



Picture 4: Day 7 chromatograms showing peaks



Picture 4: Day 14 chromatograms showing peaks

Conclusion

Proper collection and storage of blood for alcohol estimation is very important. Immediate evaluation of blood samples will help in getting accurate results and proper blood alcohol values. Delays might result in loss of alcohol from the collected sample as shown in the present study. Steps must be taken to sensitize the investigating authorities regarding this particular aspect so that delays don't occur in collection and analysis of the sample. A further study with larger sample size must be conducted to properly generalise the present results.

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- Kruthika : Scientific officer

Conflict of interest: Nil

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