

FORENSIC AND CLINICAL ISSUES IN LIQUOR ANALYSIS: AN UPDATE¹*Gaurav Kumar Singh, ²Shivam Gujarathi B., ²Spandita Hati and ²Rahul Verma¹Ph.D. Scholar at Dept. of Forensic Science, Chandigarh University.²M.Sc. Forensic Science Student – Dept. of Forensic Science, Chandigarh University.***Corresponding Author: Gaurav Kumar Singh**

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ABSTRACT

The alcohol and psychotropic substance are widely consumed all over the globe. Among them alcohol is one of the preferred drinks over western countries and even in India it is manufactured on large scale. The alcohol is administered by varying age groups but, according a survey 18 to 30 years of peoples are highly addicted. This paper precisely discusses the forensic and clinical issues which are encountered by forensic professionals. The alcohol analysis for antemortem and postmortem cases are possessing significantly different aspects. Here we have discussed the issues occurring in sample collection, its analysis and interpretation of results. Some important aspects are focused on sampling, the interference of medications on results and use of biochemical science in postmortem cases. Also, the clinical professional should give importance to medication history, specimen type, its advantages and disadvantages while performing the Ethanol Testing on subject.

KEYWORDS: Alcohol analysis, postmortem, BAC, metabolism, urinalysis, correlation coefficient, specimens, vitreous humor, forensic science.

INTRODUCTION

Forensic science always offers a novel way to investigate the crime. Nowadays, the alcohol testing is a choice of interest for forensic toxicologists as it focuses in alcohol intoxication and to estimate the ethanol content in blood, breath and urine. The forensic issues mainly emphasize on sampling and collection of blood and urine samples. Along with them, handling and preservation also contribute equally in alcohol analysis. The preservation of blood for alcohol analysis in appropriate and a suitable solvent is necessity for alcohol analysis. More specifically, preservation of sample of a diabetic person is quite difficult job as due to presence of glucose there are high chances of microbial fermentation which alters the alcohol concentration (usually high). On an average, the person eliminates only 1 to 2 percent of ethanol through urine and can be determined between 1 to 2 hours. Thus, the time of sample collection is important fact for test. The clinical issues involve the impairment in testing due to the altered pharmacokinetics (ADME) which may vary results. Some complications come across in way of investigation in post-mortem cases because of synthesis or diffusion of alcohol, poor sampling sites in deceased person and limited blood specimen. The recent advancement in clinical alcohol analysis is the use of biomarkers for alcohol consumption. It is not only useful in clinical aspect but also in forensic context (e.g., in child custody proceedings or as documentation of alcohol abstinence after temporary confiscation of a driver's license). They

are useful in the field in clinical medicine and for verification of abstinence or to check out the harmful use of alcohol.

WHAT IS ALCOHOL?

Alcohol is the drug of choice for addiction. It is a substance which acts as drug and poison, depending in concentration. It shows effect on Central Nervous System (CNS) of human body. The FDA and WHO is considering it as Central Nervous System Depressant. Moreover, it may act as stimulant, helps in focusing on work and maintenance of normal human body temperature when consumed in lower concentration. It shows major effects as dose increases. It leads to slurred speech, unsteady movements, disturbed perceptions and delaying to react quickly. Its adverse effects are respiratory depressions (where breathing becomes slow, shallow and stops completely). The Blood Alcohol Concentration (BAC) over time differentiates acute and lethal effects.

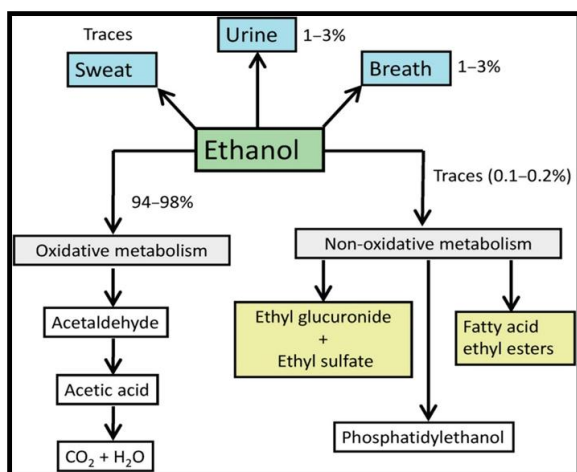
Non-Alcoholic Beverages are alcohol-free or non-alcoholic drink, also known as a temperance drink. It is basically an alcoholic drink made without alcohol, or with the alcohol removed or reduced to almost zero. Alcoholic Beverages are those alcoholic drinks which content the varying amount of alcohol. The Table 1 shows the alcoholic content of some alcoholic beverages.

Table 1: Alcohol content.

Type of Beverage	Percentage of Alcohol
Beer	1-5 %
Cider	3-7 %
Wine	9-21 %
Tequila	40 %
Rum	42 % or more
Brandy	42 % or more
Gin	41-48 %
Whisky	41-52 %
Vodka	41-52 %
Liqueurs	20-60 %

PHARMACOLOGY OF ETHANOL

The pharmacology of ethanol is quite easy and straight forward. After consumption of alcohol, it is absorbed in proximal small intestine within 30 to 90 minutes, i.e. it gets absorbed too rapidly.^[1,2] Due to its hydrophilic nature, its volume of distribution is high, i.e. the volume of distribution of alcohol is 0.6 to 0.7 L/Kg. The presence of food interferes and delays its absorption. Metabolism of alcohol is carried out by group of enzymes, known as microsomal mixed function oxidase which is majorly found in the liver. More specifically, the ethanol is oxidized by Alcohol Dehydrogenase (ADH) to acetaldehyde and acetic acid. The Alcohol Dehydrogenase (ADH) is also found in small quantity in gastric mucosa. On comparison of available data, the women have higher ethanol peak than men due to increased bioavailability as the Gastric Alcohol Dehydrogenase (ADH) is less active in women.^[3]

**Fig. 1: Metabolism of Alcohol.**

Above 20 mg/dl plasma level of alcohol, it follows zero order kinetics (i.e., the clearance or half-life of ethanol changes with a change in concentration). The metabolic rate of alcohol is 10 to 30 mg/dL per hour which may vary from person to person because of genetic polymorphism variants of ADH.^[4] However, it is reported as children have high metabolic rate i.e. about 28 mg/dL per hour.^[5] Common drugs such as aspirin and histamine blockers inhibit the gastric ADH, which may show

increased ethanol blood level.^[6] There are many elimination pathways as renal (primary), faeces, lungs and sweat.^[7]

PHARMACOKINETICS OF ETHANOL

Absorption: Ethanol is mainly absorbed from Gastrointestinal Tract (GIT). Because of its high hydrophilicity and lower molecular weight (MV=46), less than 20 % of ethanol is absorbed by passive diffusion in blood from stomach, the major portion is absorbed from proximal small intestine i.e. jejunum due to its high surface area. There are various factors such as gastric emptying time, presence of food, type of alcoholic beverage, GI motility and supply of blood flow.^[8]

Distribution: Distribution of alcohol in body depends on miscibility rate. As ethanol is hydrophilic in nature, it is miscible in water and insoluble in fat. The volume of distribution (V_d) of ethanol is approximated as to lean body. The ethanol is least distributed in adipose tissue as it contains little amount of water. Women comprises of more adipose tissue than men, hence their volume of distribution (V_d) is smaller. Therefore, we can say that the volume of distribution (V_d) of ethanol is directly proportional to Total Body Water (TBW). Because ethanol distributes majorly in Total Body Water (TBW) compartment, V_d of ethanol is affected fat & lean body mass. In 1930s, Widmark calculated the mean values for volume of distribution (V_d) for males and females separately as, for male it is 0.7 L/Kg and for females 0.6 L/Kg, which is later utilized for estimation of Blood Alcohol Concentration (BAC) in legal issue (equation 1). In post-mortem cases, this BAC equation is required to standardize according to BAC units, because in post-mortem cases, the tissue is used as sample instead of whole blood.^[8]

BLOOD ALCOHOL CONCENTRATION

In a drink of any alcoholic beverage, the BAC will be 0.02 percent in a male of 200lb (90 Kg) and 0.04 percent in a female of 125lb (57 Kg). This BAC will increase simultaneously as we increase twice or thrice the drink. According to this, we can conclude that number of drinks is directly proportional to increase in BAC.

The Widmark and Watson equation for estimation of Blood Alcohol Concentration (BAC) is –

$$\text{BAC (g\%)} = \frac{(0.8 \text{ gm/ml. A. } 100\%)}{W. 1000. r}$$

where A equals the amount of ethanol consumed in milliliters, W equals the weight of the subject in kilograms, and r is the Widmark factor, a constant relating the distribution of water in the body in liters per kilogram (for M, $r = 0.68$ & F, $r = 0.55$).

Table 2: Stages of Acute Alcoholic Influence and Intoxication in Nontolerant Individuals.

BAC (% wt/vol)	Stages of Alcohol Influence	Clinical Sign or Symptom
0.01-0.05	Sobriety	No apparent influence Behaviour nearly normal by ordinary observation Slight changes detectable by special tests
0.03-0.12	Euphoria	Mild euphoria, social ability, talkativeness Increased self-confidence, decreased inhibition Diminution of attention, judgement and control Loss of efficiency in finer performance tests
0.09-0.25	Excitement	Emotional instability, decreased inhibitions Loss of critical judgement Impairment of memory and comprehension Decreased sensory response, increased reaction time Some muscular in coordination
0.18-0.30	Confusion	Disorientation, mental confusion, dizziness Exaggerated emotional states (e.g. fear, anger, grief) Disturbance of sensation (e.g. diplopia) and of perception of color form, motion, dimensions Decreased pain sense Impaired balance, muscular in coordination, staggering gait, slurred speech
0.27-0.40	Stupor	Apathy, general inertia, approaching paralysis Markedly decreased response to stimuli Marked muscular in coordination, inability to stand or walk Vomiting, incontinence of urine and faeces Impaired consciousness, sleep or stupor
0.35-0.50	Coma	Complete unconsciousness, coma, anaesthesia Depressed or abolished reflexes Subnormal temperature Incontinence of urine and feces Impairment of circulation and respiration Possible death
>0.45	Death	Death due to respiratory paralysis

The National Safety Council, American Medical Association, Surgeon General, Association for the Advancement of Automotive Medicine, and American College of Emergency Physicians support reduction of the "per se" (means itself) BAC limits.^[14-16] Legal intoxication limits are variable throughout the United States. As of 1997, in 15 states it was illegal per se to drive with a BAC of 80 mg/dL; 33 states and the District of Columbia set per se BAC levels at 0.10 (100 mg/dL), and 2 states had no per se limit. A huge amount of deaths (more than 675) can be saved if all regulating authorities in states amend BAC limit for adult as 0.08 and 0 to 0.02% for younger drivers (below 20 years).

ELIMINATION

Elimination is a process to remove out the substances which are once ingested in body. Alcohol is 98 to 95 percent metabolized in liver by enzyme ADH which further gets metabolized into water and CO₂. Water is major source of elimination and is excreted in form urine while CO₂ is exhaled through breath.^[8]

ALCOHOL INTOXICATION AND IMPAIRMENT

Alcohol intoxication or alcohol poisoning, is the false positive behaviour due to the recent drinking of alcohol. At lower doses person may experience mild sedation and

poor coordination. At higher doses, symptoms may be slurred speech, trouble walking, and vomiting. Extreme doses may lead to decreased effort to breathe (respiratory depression), coma, or death.

Ethanol Intoxication, in terms of clinical is abnormal working of Central Nervous System (CNS). Inebriation is basically in ability to perform day to day activities while impairments reflects towards inability to person to perform a particular task or is prone to automobile accidents.^[9]

Blood Alcohol Concentration (BAC) is expressed in many units but for a clear understanding a single unit is essential for estimation of alcohol in blood. BAC is expressed as percentage of alcohol by weight (no. of grams of alcohol in 100ml of blood). Most of the regional laws follows these BAC units and is considered as legal limits for in intoxication and impairment.

A clear understanding of the units used to express ethanol levels is essential. Blood alcohol concentration (BAC) is the ethanol concentration in whole blood. The BAC often is expressed as the percentage of alcohol by weight (i.e., grams of ethanol in 100 mL of blood),

because most regional laws consider this as a legal limit of impairment or intoxication.

The clinical laboratories report BAC in milligrams per 100 ml or dL or in millimoles /L. These values can be converted in mg/dL or percentage wt/vol by shifting decimal point 3 places to left. For example, 145mg/dL gets converted to 0.135% wt/vol. These are following conversions used in ethanol testing:

$$1.00 \text{ g/L} = 0.10 \text{ g/dL} = 0.10\% \text{ wt/vol} = 100 \text{ mg/dL} = 21.71 \text{ mmol/L}$$

Table 3:

Ability	Serum Ethanol Level
Impairment	<100 mg/dL
Disturbed Driving skill	<50 mg/dL
Avoid crash	<30 mg/dL
Tracking, divided attention, reaction time, information processing	20 – 30 mg/dL

Therefore, many countries such as Portugal, Australia, Norway, Yugoslavia, Netherlands, Japan, Finland's, Greece & Iceland validate limit of 50 mg/dL in case of driving. Some countries from 1921 often have enacted law of 20 mg/dL for driving. In India, BAC is permitted upto 0.03 %/100ml blood, i.e. 30mg/dL.^[13]

SPECIMEN & ITS HANDLING

Different specimens are utilized for alcohol analysis such as breath (exhaled air), serum, whole blood, saliva, plasma & urine. The collection of specimen depends on the antemortem and post-mortem cases. Medical professionals recommend to biologic fluids for analysis. While collecting the blood sample, the vein puncture sites should not be sterilized by ethanol swabs and pads, instead use povidone – iodine or benzalkonium chloride for disinfecting the skin.^[22]

Due to volatile nature of ethanol, the specimen or sample are stored in sealed packed containers. It is necessary to analyse plasma and serum within 4 hours without addition of preservatives and under sterile and aseptic condition. When the samples are forwarded to forensic analysis, potassium oxalate or sodium fluoride should be added in whole blood for prevention of thickening of blood sample. It acts as anticoagulant and also prevents the sudden increase or decrease of ethanol concentration (because of microbial fermentation).^[23,24] Before proceeding to analysis, various factors are considered as one of factor is water content. As ethanol is more soluble in water, its concentration varies from 98% to 86% in serum and whole blood. Ethanol has high volume of distribution (V_d) and is distributed primarily in aqueous phase instead of cellular phase of blood. Hence, the concentration of ethanol in serum is higher than whole blood.^[25,26]

It is found that ethanol concentration in arterial & capillary blood is 25 % more than venous blood after initial administration of alcoholic beverage. These

Other common conversion formulas for ethanol are as follows:

$$\text{mmol/L} - \text{mg/dL} \times 0.7171$$

$$\text{mg/dL} = \text{mmol/L} \times 4.61$$

According to information & survey, impairment and disturbed driving skill depends upon different serum ethanol levels which are listed in Table 3.^[10,11,12]

variables may need to be kept in consideration while analysis of ethanol in investigative purposes.

Table 4: Appropriate Relative Alcohol Concentrations in the Post absorptive State in Selected Body Fluids or Tissue.

Specimen	Ratio
Whole blood	1.0
Serum or Plasma	1.09 – 1.18
Adipose	0.02
Vitreous	0.73 – 1.13
Breath	2100 – 2300
Urine	1.2 – 1.35
Liver	0.6 – 0.91
Blood clot	0.77
Muscle	0.89 – 1.48
Saliva	1.0 – 1.3
Kidney	0.66
Bone Marrow	0.34 – 0.79
Cerebrospinal fluid	0.9 – 1.18
Brain	0.65 – 0.94

*Data from Basselt RC, Cravey RH eds. *Ethanol in Disposition of Toxic Drugs and Chemicals in Man*, 4th ed. Foster City, Calif: Chemical Toxicology Institute; 1995; 293-296; Coe JJ, Sherman RE. *Comparative study of post-mortem vitreous humor and blood alcohol*. *J Forensic Sci.* 1970; 15; 185 – 190; Freimuth HC *Forensic aspects of alcohol*. In: Spitz WU, ed. *Spitz and Fisher's Medico legal Investigation of Death*, 3rd ed. Springfield, III: Charles C. Thomas; 1993:767-775; Garriott JC, *Skeletal muscle as an alternative specimen for alcohol and drug analysis* *J Forensic Sci.* 1991;36:60-69. All ratios expressed relative to whole blood. Post-mortem values; therefore; redistribution in the tissues may have occurred.

URINE SAMPLES

Urinalysis is useful for determination of alcohol in urine. According to table 4, the ratio of urine/blood ethanol is 1.3; although it is valid only during elimination phase. If this ratio is more than 1.3, then the complete alcohol is absorbed and postabsorptive state is experienced by person. This ratio cannot be applied to individuals who have very low ethanol levels and same for postmortem cases.^[33,34]

Urine ethanol level can be used to estimate alcohol intoxication. On basis of available data, it is found that urine ethanol level does not correspond with blood alcohol concentration. Another study shows, the urine/blood ratio varies from 1.10 – 2.44 and 0.21 – 2.66.^[22,35]

Some medical officers consider this ratio appropriate for calculation of BAC in medical issue. But in case of forensic analysis, it should not be used because the collection time of sample plays an important role in increment or decrement of ethanol level in urine as urine is continuously pooled in bladder. Only it can be done as correlating the ethanol levels to average BAC for such specimen.

One alternative to get better result is collection of two samples. The individual should empty bladder completely and this sample is first which is of no use. The second sample collection should be between 30 to 60 minutes. Now, if the second sample holds the ethanol, then it must contain alcohol in blood during the time period of collection.^[26,33] Therefore, the obtained results are used to calculate BAC by taking ratio 1.3.

There are some factors which interfere in result of urinalysis. After consumption of alcohol, there is increase in volume of production of urine (known as diuresis), although it causes no effect on urine/blood ratio.^[27] Another factor is that, some bacteria or yeast causes fermentation which causes increase in ethanol concentration in samples of person which are diabetic or having any Urinary Tract Infection (UTI).

SALIVA SAMPLES

The concentration of alcohol in saliva & BAC is having a statistical relationship, i.e. it is 8 – 9 percent higher than BAC also the equilibrium reaches in 30 minutes. The average saliva/blood ratio is 1.10 (according to table 4).^[29, 30] The intra individual ratio, however, seems constant.

For ethanol analysis, saliva specimen should be collected with appropriate handling so as to gain reliable result. As the saliva flow in alcoholic person is low, it is important to collect saliva in proper manner. To avoid fluctuation in results, the saliva sample should not be collected after administration of alcoholic products as syrup, mouthwash, breath spray and cough syrup. Also, after consumption of any alcoholic beverage, upto 10 to 20

minutes the sample should not be collected.^[30] Saliva specimen is useful for those unconscious patients who are unable to perform breath analysis.

BREATH SAMPLES

The results of breath alcohol concentration are reliable & is widely applied in legal enforcement laws. It has also gain attention in medical emergencies. The ratio of breath/ blood alcohol concentration is 2100 (table 4) & this ratio is used as conversion factor in many countries. However, in post absorptive phase, this ratio varies from 900 to 3400.^[32] Contamination is a factor which misleads the results. The regurgitation of stomach contents or elimination of alcoholic gas and residues of alcohol in mouth interferes in results. To overcome this problem, the person should be kept under observation for at least 15 minutes to minimise this artefact.

METHODOLOGY

Alcohol is the most ordered, drug test in both clinical and forensic toxicology division. While in forensic toxicology, the accurate estimation is necessary as to assist the case. In clinical alcohol testing, the patients facing overdose of ethanolic concentration or intoxication is carried out.^[38] On analysing, alcohol concentration in biological specimen, it is needed to identify whether the alcohol is present as such or produced by any microbial or enzymatic reaction. In driving cases, the breath analyser is used to detect the Blood Alcohol Concentration (BAC) in breath. The breath analyser working principle is based on either series of reaction or on infra-red spectroscopy, i.e. based on absorption of infra rays. However, in clinical estimation, the samples are blood and urine. While in forensic toxicology, variety of biological samples are analysed to estimate the accurate concentration of alcohol. According to one study performed in Center for Advanced Laboratory Medicine, University of California, stated that there are variable differences in the alcohol concentration performed at hospital (clinical) and Forensic Laboratory, at San Diego County Medical Examiner's Office (SDCMEO). Clinical Laboratories uses enzymatic methods for estimation while forensic toxicology utilizes the chromatographic methods for alcohol quantitation. The study was performed commonly on 39 positive alcohol samples. The result states that there is a difference of 0.10 g/dL in both clinical and forensic laboratory, estimated concentrations of alcohol.^[39]

In post-mortem cases not only BAC plays an important role but also UAC (Urine Alcohol Concentration). Various biological specimens are analysed for appropriate alcohol concentration such as blood, urine and vitreous humour and synovial fluid. Chemical, biochemical and sophisticated chromatographic techniques are used. Since alcohol is rapidly eliminated, chemical methods are not reliable for accuracy. Hence, chromatographic methods such as head space gas chromatography (HC-GC) is used. The correlation

coefficient is the factor that establishes the strength of relationship between two variables and accuracy of physical process. A comparative study on 592 autopsies stated that the correlation coefficient was $r = 0.936$ found between vitreous humour and blood. Another study was performed on cerebrospinal fluid and blood, the correlation coefficient factor was $r = 0.943$. In both studies' chromatographic methods such as gas chromatography was utilized.^[37] Recent advancement is to differentiate between ante-mortem alcohol intake and post-mortem formation due to putrefactive processes. Here, alcohol biomarkers are used such as ethyl glucuronide (EtG) and carbohydrate deficient transferrin (CDT). Method of estimation was GC-FID.^[40] There is difference between estimation of alcohol concentration in clinical and forensic toxicology and the reason might be as they lack to maintain chain of custody, proper handling of specimen and using advanced sophisticated techniques.

MEDICATIONS THAT INTERFERE IN INTERPRETATION OF RESULT

As the instant way to detect BAC is Breath analyser, there are some complications which tends to interfere with result. There are many reasons which interfere in interpretation of results. One may be the reason as using uncalibrated machine and another may be avoiding use to standard operating procedure (SOP). Some medications are listed below:

- Asthma medications: Albuterol, salmeterol, budesonide, and similar drugs interfere in test of breath analyser. As these medications are to be administered by inhalation, they get accumulated in air passage and remain there for longer duration.
- Over-the-counter medications: Nyquil, Vicks products, cough syrups comprise of alcohol in their formulations. These generate the false positive result. If the subject has administered large dose of cough drops or syrup before analysis of breath alcohol, it can make sudden change in result.
- Oral gels: oral gels are used to treat mouth aches and snores. There many medications for treatment. One of medication contains anbesol, which gives false positive results.
- Mouthwashes and breath sprays: Generally, the alcoholic content in mouthwashes and breath sprays is high and because of this it affects the results if the subject has used it.

POSTMORTEM ETHANOL ANALYSIS

Ethanol estimation in postmortem cases are simple and accurate both in terms of qualitative and quantitative parameters. However, some important elements such as nature of specimen, the time period between death and autopsy, the condition of body, and environmental conditions i.e. temperature and humidity are observed.

However, it is quite easy job to perform to perform alcohol analysis on a living subject, it becomes complicated to cadavers. The body starts decomposing

and there is substantial increase in blood ethanol concentration because of overgrowth of population of microbial flora and their fermentation. Vitreous humour is sterile and is used to estimate the ethanol concentration in postmortem cases. It is gives reliable result which is used in comparison of antemortem consumption and postmortem production of alcohol.

In postmortem cases, it is beneficial to collect more than one sample from different body compartments for laboratory analysis. It is so done because it gives more results to compare and facilitate optimal interpretation.^[37] While collecting samples in postmortem cases, always two blood samples should be collected, from heart central region and peripheral vasculature. As discussed previously, the venous BAC is too lower than arterial BAC in postabsorptive phase. The intravascular blood may have high or low ethanol concentration than bloody fluid.^[36]

After heavy drinking if a person dies, there are high chances of occurring diffusion of alcohol from stomach to the peripheral blood sampling sites.^[37] While collection of blood specimen some of key element should be kept in consideration which are totally against to sampling techniques in cases of antemortem. Diffusion occurs continuously between the time period of death and autopsy. The diffusion mostly occurs from oesophagus and stomach to peripheral blood supply and pericardial cavity of heart. So, it is necessary to have autopsy within 48 hours of death. In cases of traumatic hypovolemia i.e. empty heart sign, the subclavian venous sites and femoral blood sites are preferred over central blood supply. But it may cause difficulty for analysis because of its small amount of volume obtained.^[36]

For preservation of blood specimen obtained after autopsy, the special type of tube are used which contains potassium or sodium fluoride as preservative which makes up the final concentration upto 1-2 % w/v. Fluoride plays an key role as enzyme inhibitor, which prevent the change in concentration of ethanol during the autopsy, dispatchment and transportation for further forensic analysis. If analysis is done simultaneously during autopsy, then addition of preservative is not recommended.^[37]

Another approach is testing the vitreous humour as it is watery and sterile in nature. It is so because, it has not any bloods supply directly from gut, it is not contaminated by microbial flora. Because of its intact and sterile nature, it is preferred in post-mortem cases to collect sample of vitreous humour. It is a proving means for quantitative comparison of ethanol with central or peripheral blood samples. As vitreous alcohol content (VAC) is higher than BAC, it can be used to know whether the deceased was in post absorptive or elimination phase.^[36]

Table 5: Examples of body fluids and tissues suitable for determination of ethanol in living and dead subjects.^[37]

Living Subjects	Dead Subjects
Venous Blood	Femoral Blood
Capillary Blood	Heart Blood
Plasma/Serum	Blood Clot
Urine (fresh void)	Bladder Urine
Tear Fluid	Vitreous Humour
Cerebrospinal fluid (lumber fluid)	Cerebrospinal Fluid (cisternal)
Saliva	Bile
Perspiration/Sweat	Synovial Fluid
Breath	Brain, Skeletal muscle, Liver

In severe cases or in trauma, if the vitreous humour is not obtainable, other body fluids and body organs are utilized from analysis. Some body fluids are urine, stomach contents, bile, synovial fluid, bone marrow and cerebrospinal fluid.^[36] The body organs are cardiac, spleen, smooth and skeletal muscle, intracerebral and paradural hematomas, lung, kidney and brain.

Method of choice over globe for quantitative and qualitative alcohol determination is Gas chromatography. In gas chromatography, the flame ionization detector is used mostly along with Head-spacing sampling. Head-space sampling is preferred for volatile substances. By using GC-HS, the chromatographic column is protected by the blood constituents which are non-volatile in nature. It also removes the vapour phase equilibrium with biological sample, which is stored at 50-60 degrees in airtight vials.

BIOCHEMICAL MARKERS FOR POSTMORTEM SYNTHESIS

In post-mortem analysis of alcohol, it is necessary to differentiate whether the antemortem ingestion and post-mortem synthesis of ethanol. After the long research, forensic scientists have developed a practical and useful biochemical marker. There are many non-oxidative metabolites of ethanol; one of them is Ethyl Glucuronide (EtG). It is basically a minor metabolite and if this metabolite is present in body fluid then the metabolism of alcohol is already occurred. This signifies that the person has consumed the alcohol before death. Moreover, the EtG is not much reliable because the half-life of EtG is more than the ethanol as it does not give a clear idea that the deceased is died with elevated BAC.

CONCLUSION

Many clinical and forensic laboratories perform alcohol testing for either proceedings of medications or for legal purpose. While in testing alcohol in laboratory, the professional should go through all the legal process. The clinicians should have enough knowledge of potential medicolegal ramifications regarding advantages and disadvantages of specimen type along with its importance. When ethanol testing becomes a medicolegal issue, a proper chain of custody and special documentation should be managed. The Forensic as well as Clinical professional should have adequate knowledge of ethanol pharmacokinetics and pharmacodynamic for

performing ethanol testing. Various issues occurring while Ethanol Testing in antemortem and post-mortem cases. The detailed medical history and health of person should be kept beside to compare the results as the medication can create problem while interpretation of results. Various technological developments can contribute to help the professional for analysis. The molecular science, nanoscience and the use of biochemical markers for ethanol analysis in post-mortem cases have proved to be helpful to avoid issues and successfully interpret the results.

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