Alcohol Biomarkers

BMF 77 - Alcohol Biomarkers

Self-reporting of alcohol consumption is objective and inherently unreliable. This has led to an intensive search for a reliable marker of chronic, excessive drinking. The detection of ethanol in biological specimens indicates recent alcohol consumption or exposure but the detection window is very short. Alcohol biomarkers can be used to extend the time window for detection and can persist long after complete elimination of ethanol. In this way they are useful relapse or abstinence markers.

> "Alcohol biomarkers are physiological indicators of alcohol exposure or ingestion and may reflect the presence of an alcohol use disorder"
>
> *Substance Abuse Treatment Advisory. Sept 2006, Vol 5, Issue 4*

Alcohol biomarkers can be divided into 2 types:

1) **Indirect** – Detect toxic effect of heavy alcohol use on organ systems and body chemistry, e.g.
   - Gamma Glutamyl Transferase (GGT)
   - Aspartate Amino Transferase (AST) or Alanine Amino Transferase (ASL)
   - Mean Corpuscular Volume (MCV)
   - Carbohydrate Deficient Transferrin (CDT)
   - 5-Hydroxytryptophol (5HTOL)

2) **Direct** – Measure alcohol exposure or use (Analytes of alcohol metabolism)
   - Ethyl Glucuronide (EtG)
   - Ethyl Sulfate (EtS)
   - Phosphatidyl Ethanol (PEth)
   - Fatty Acid Ethyl Esters (FAEEs)

Traditional, indirect markers of alcohol consumption are non-specific in that they are also influenced by age, gender, medication and non-alcohol related conditions. For this reason there has been increased interest in direct markers in recent years.

Alcohol biomarkers may be applied in a number of clinical and forensic settings (see table 1) and are now in routine use.

Clinical Settings	Forensic Settings
• Screening for alcohol problems • Documenting abstinence • Identifying relapse to drinking • Motivating change in drinking behavior • Evaluating interventions for alcohol problems • Conditional liver transplantation	• Differentiation of anti-mortem consumption and post- mortem production of ethanol • Establishing alcohol use after clearance • Child custody cases • Driving offences/Reinstating of driving licenses • Conditional probation – threat of return to jail • Loss of employment

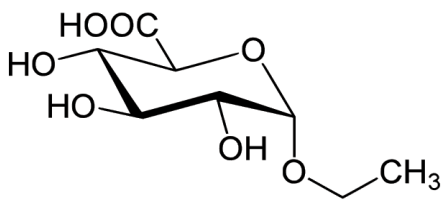
Table 1: The application of alcohol biomarkers in clinical and forensic settings.

Alcohol biomarker monitoring should not be used in isolation, but instead should be considered complimentary to clinical assessment and expert interpretation and opinion. Due to the relative strengths and weaknesses of the different biomarkers they are often used in combination. Combining biomarkers which are formed by different metabolic pathways with differing limitations increases the sensitivity and reliability.

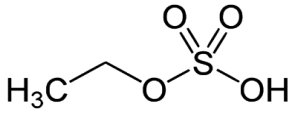
Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS)

EtG and EtS are water soluble, stable, non-volatile, direct metabolites of ethanol. Metabolism occurs as soon as alcohol enters the blood stream. More than 90% of alcohol consumed is metabolised in the liver, via the action of the enzyme alcohol dehydrogenase (ADH). ADH catalyses the reaction in which alcohol is oxidised to acetaldehyde. This is then metabolised to acetate by aldehyde dehydrogenase and then to carbon dioxide. Between 5-8% of unchanged alcohol is excreted in the urine, sweat and breath. The elimination of ethanol by enzymatic conjugation with glucuronic acid or sulfate represents approximately <0.1% of the total ethanol elimination. Whilst the detection period for alcohol is relatively short, EtG can be detected for up to 80 hours and peaks at approximately 4 hours after alcohol consumption. EtS has a similar window of detection. EtG and EtS are direct, specific and sensitive markers of alcohol consumption, being present only if ethanol is consumed. They are not influenced by age, gender, medication or non-alcohol related disease and are not dependant on chronic alcohol consumption.

EtG and EtS are not without their limitations. Firstly there is no correlation between these biomarkers and blood alcohol concentration. Unlike urinary excretion of ethanol, EtG and EtS concentrations are highly influenced by diuresis and therefore standardisation against creatinine is recommended. EtG and EtS do not differentiate between alcohol exposure and consumption at lower levels. EtG can be bacterially produced if ethanol is present or produced in-vitro. Conversely bacterial β-glucuronidases can also breakdown of EtG. EtS can also be degraded although research to date suggests this is not prone to in-vitro synthesis in the same way as EtG. Production and hydrolysis can be reduced by correct storage and preservation. It should also be noted that alcohol can be found in a wide range of every day products including; foods, medicines, mouthwashes, perfumes, hygiene products, disinfectants, hand sanitisers and automotive fuel. Appropriate cut-offs should be employed to discriminate between consumption and incidental exposures.



Ethyl Glucuronide (EtG)



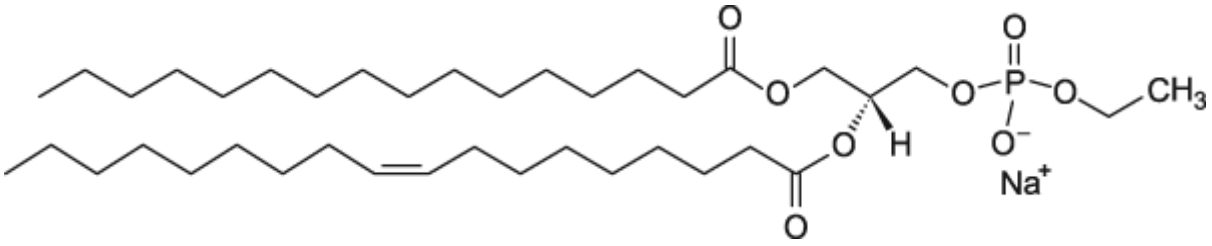
Ethyl Sulfate (EtS)

Fatty Acid Ethyl Esters (FAEE)

FAEE are minor, non-oxidative metabolites of ethanol metabolism. They may be formed spontaneously through conjugation of ethanol and free fatty acids, but this process is more often catalysed by a range of enzymes. FAEE are produced throughout the body and are found in the liver, pancreas, heart, brain, white blood cells and fat (i.e., adipose) tissues up to 24 hours after alcohol consumption. They preferentially accumulate in adipose and hair. FAEE persist in hair and are therefore useful long-term markers of alcohol abuse (See alcohol biomarkers in hair). They are deposited in hair mainly from sebum. FAEE are defined as ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate. For interpretation, the sum of the concentrations of these four ethyl esters should be used. FAEE in meconium have been shown to have high specificity for prenatal exposure to alcohol in newborns.

Phosphatidylethanol (PEth)

PEth is an abnormal cell membrane phospholipid with two fatty acid chains. It is formed in the presence of ethanol by conversion of phosphatidylcholine to phosphatidylethanol via the action of phospholipase D. The palmitoyl/oleoyl (16:0/18:1, POPE) isomer is the most abundant PEth homolog. Once formed, PEth incorporates into the phospholipid membranes of blood and tissue cells. It has a half-life of 4-5 days in blood, which means that repeated or chronic exposure leads to accumulation, giving rise to a wide window of detection. PEth is detectable in blood for 2-3 weeks after heavy prolonged drinking or periods of 'binge drinking'. It is a less sensitive marker of alcohol consumption that EtG and EtS. PEth concentration seems not to be influenced by age, gender, other ingested substances or other pathological conditions. PEth concentrations above 20ng/mL have been reported to correlate with moderate to heavy alcohol consumption, however further studies are needed before arriving at a consensus on the most appropriate cut-off for differentiating social alcohol use from heavy drinking.



Phosphatidylethanol (16:0/18:1)

Alcohol Biomarkers in Hair

It is not possible to measure ethanol in hair due to its volatility and potential contamination from external sources. Instead the direct alcohol biomarkers, EtG and FAEE's are used to corroborate or exclude alcohol exposure. EtG is the preferred marker for abstinence monitoring. A concentration of 7pg/mg is consistent with self-reported teetotalism. It is not recommended to use FAEE in isolation, but it is useful where a false negative EtG is suspected. Conversely either/or EtG and FAEE is considered appropriate for assessment of chronic excessive alcohol consumption. A concentration of >30 pg/mg EtG and/or 0.5 ng/mg (Total) FAEEs in the 0–3 cm proximal segment is considered strongly suggestive of chronic excessive alcohol consumption. There is reported benefit from combining measurements of both EtG and FAEE in hair. Cosmetic treatments, but not natural hair colour, can influence the detection of alcohol biomarkers in hair. The Society of Hair Testing (SoHT) have established a consensus for the use of alcohol markers in hair for assessment of both abstinence and chronic excessive alcohol consumption.

Chiron offer a growing range of reference materials for direct alcohol biomarkers:

Chiron No.	Description (Synonym(s))	CAS No.	Concentration	Solvent	Vol.
1626.8-100-ME	Ethyl-ß-D-6-glucuronide (EtG)	17685-04-0	100µg base/mL	methanol	1mL
1626.8-K-ME	Ethyl-ß-D-6-glucuronide (EtG)	17685-04-0	1000µg base/mL	methanol	1mL
1626.8-10MG	Ethyl-ß-D-6-glucuronide (EtG)	17685-04-0	neat	neat	10mg
1627.8-100-ME	Ethyl-ß-D-6-glucuronide-d5 (EtG-d5)	1135070-98-2	100µg base/mL	methanol	1mL
1627.8-K-ME	Ethyl-ß-D-6-glucuronide-d5 (EtG-d5)	1135070-98-2	1000µg base/mL	methanol	1mL
1627.8-10MG	Ethyl-ß-D-6-glucuronide-d5 (EtG-d5)	1135070-98-2	neat	neat	10mg
10889.2-K-ME	Ethyl sulfate sodium salt (EtS)	546-74-7	1000µg EtS/mL	methanol	1mL
10889.2-10MG	Ethyl sulfate sodium salt (EtS)	546-74-7	neat	neat	10mg
10910.39-100-CF	Phosphatidylethanol PEth (16:0/18:1) 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol	765260-45-5	100µg acid/mL	chloroform	1mL
10910.39-1mM-CF	Phosphatidylethanol PEth (16:0/18:1) 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol	765260-45-5	1millimol (723.5µg/mL)	chloroform	1mL
10580.39-100-CF	Phosphatidylethanol PEth (16:0/18:1) 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol	322647-55-2	100µg free acid/mL	chloroform	1mL
10580.39-1mM-CF	Phosphatidylethanol PEth (16:0/18:1) 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol	322647-55-2	1millimol free acid (723.5µg/mL)	chloroform	1mL
10943.39-100-CF	PEth (16:0/18:1)-d5 ammonium salt 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol-d5 ammonium salt	N/A	100µg free acid/mL	chloroform	1mL
10943.39-K-CF	PEth (16:0/18:1)-d5 ammonium salt 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol-d5 ammonium salt	N/A	1000µg free acid/mL	chloroform	1mL
10671.20-K-AN	Ethyl oleate C18:1, Ethyl cis-9-octadecenoate	111-62-6	1000µg/mL	acetonitrile	1mL
10902.20-K-AN	Ethyl oleate-d5 C18:1, Ethyl cis-9-octadecenoate-d5	111-62-6 (unlabelled)	1000µg/mL	acetonitrile	1mL
3185.16-K-AN	Ethyl myristate C14:0, Ethyl tetradecanoate	124-06-1	1000µg/mL	acetonitrile	1mL
3185.16-K-IO	Ethyl myristate C14:0, Ethyl tetradecanoate	124-06-1	1000µg/mL	isooctane	1mL

Chiron No.	Description (Synonym(s))	CAS No.	Concentration	Solvent	Vol.
10903.16-K-AN	Ethyl myristate-d5 C14:0, Ethyl tetradecanoate-d5	1217033-63-0	1000µg/mL	acetonitrile	1mL
3187.18-K-AN	Ethyl palmitate C16:0, Ethyl hexadecanoate	628-97-7	1000µg/mL	acetonitrile	1mL
3187.18-K-IO	Ethyl palmitate C16:0, Ethyl hexadecanoate	628-97-7	1000µg/mL	isooctane	1mL
10904.18-K-AN	Ethyl palmitate-d5 C16:0, Ethyl hexadecanoate-d5	628-97-7 (unlabelled)	1000µg/mL	acetonitrile	1mL
3189.20-K-AN	Ethyl stearate C18:0, Ethyl octadecanoate	111-61-5	1000µg/mL	acetonitrile	1mL
3189.20-K-IO	Ethyl stearate C18:0, Ethyl octadecanoate	111-61-5	1000µg/mL	isooctane	1mL
10905.20-K-AN	Ethyl stearate-d5 C18:0, Ethyl octadecanoate-d5	111-61-5 (unlabelled)	1000µg/mL	acetonitrile	1mL

For products, pack sizes and presentations not listed, please contact sales@chiron.no

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