Monitoring Road Safety with Alcohol Biomarkers: 
Types, Measurement, Interpretation, and Recommendations¹

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Abstract

Context
Technologies for detecting alcohol use among drivers have advanced rapidly in recent years. Private sector businesses that supply alcohol monitoring products, such as portable breath testers, ignition interlocks or transdermal monitoring bracelets, are motivated to educate government authorities about their wares. The authorities may know about some alcohol biomarkers GGT, AST, MCV, ALT or CDT, but less often about PEth, EtG (hair or urine), or FAEE (hair), all of which have been studied as decision aides in forensic situations. There is no sole outside supplier of alcohol biomarkers measurements. Most test availability and biomarker measurement expertise germane to road safety is dispersed throughout academic research communities.

Objective
The objective is to conduct a preliminary assessment of prospective end users of this information. Recommendations are warranted for those interested in collection of bodily specimens (hair, blood or urine) for the purpose of estimating exposure to alcohol by using biomarkers. There is still no central information clearinghouse for helping with driver-related monitoring decisions. This pilot project distinguishes markers by consumption levels reflecting “excessive” alcohol use using published evidence and cutoffs that can support decisions. These initial recommendations will be revised with more input.

Outcomes
Information and classifications include: test availability, estimated costs, specimen types, direct or indirect markers, surveillance windows, sensitivity, and specificity for detecting alcohol consumption in the past days to past months. Evidential bases are from DUI populations, clinical populations, self-report assessments, diagnostic assessments, and interlock or driver data.

Discussion and Conclusions
While several nations have long used alcohol biomarkers to support driver fitness decisions, it is still uncommon in North America. This study provides a set of reference materials for those with responsibilities for making judgments about driver fitness and might want a primer on the types, virtues and liabilities of alcohol biomarkers.

Background

Brief Overview of Alcohol Markers

Medical research distinguishes state and trait alcohol biomarkers. Trait markers, indicators of alcoholism susceptibility, are nor the subject of this project. Here, alcohol biomarkers refer

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exclusively to the chronic or acute biological indicators of alcohol use: State markers. Published research on alcohol biomarkers derive from two main sources: the biochemists and toxicologists, who develop and improve these measurement methodologies, and the clinical alcoholism researchers who use alcohol biomarker measurements as adjunctive decision aids for evaluating excessive use, dependence, or abstinence among patients under their medical care. Beyond the biomedical literature, studies are also coming from the forensic sciences and criminal justice professionals who use alcohol biomarker results as decision criteria. The extra-clinical exploitation of alcohol markers migrating into safety science is still in an early stage. More developments will follow more usage and will yield more outcome data. Recommendations can nonetheless be made for estimating driver exposure to alcohol by using biomarkers.

**Direct or Indirect Alcohol Biomarkers**

Alcohol biomarkers can be indirect or direct indicators of drinking. They can be indirectly elevated because of the way the bodies respond to chronic, toxic or repeated exposure to alcohol, or markers can be directly elevated because of an acute response to alcohol exposure.

Selecting the best marker or panel of markers depends on the questions to be answered. For example, it is possible with high accuracy to mark the cutoff levels of most consumption (direct) biomarkers that denote alcohol abstinence, (or at least levels that denote low or non-problematic drinking levels). But beyond non-drinking drivers, the problem of discriminating normal/modal social levels of drinking from excessive consumption is more nuanced. How can we distinguish consumption levels thought to reflect excessive alcohol use or levels of drinking that are potentially hazardous to the driving public? Recognized published cutoffs from clinical science can support those decisions. Questions about drinking in the past day or two will require a different marker than questions about a pattern of drinking in the past week, month, or months. In all cases, the information will be more dependable than self-reported consumption.

Indirect alcohol markers were the first type studied forensically. These are most often used as possible indicators of alcohol disease to aid in the diagnosis of alcohol dependence, alcoholism, or relapse. Five indirect markers are most widely used. The first three, shown below, are insensitive, non-specific, or both. The last two are more informative. Indirect markers include:

- alanine aminotransferase (ALT), a liver enzyme measured in blood serum,
- aspartate aminotransferase (AST), a liver enzyme measured in blood serum,
- macrocytic volume (MCV), an estimate of the size (volume) of red blood cells,
- gamma glutamyltransferase (GGT), a liver enzyme measured in blood serum,
- carbohydrate deficient transferrin (CDT), an abnormal form of an iron transport protein measured in blood serum
- Other indirect markers are under active study and include, among others: 5-hydroxytryptophol, sialic acid index of plasma apolipoprotein J, beta hexosaminidase.

Commercial CDT measurement is widely available in most nations. GGT is the best of the liver enzyme markers with a sensitivity approaching CDT, but CDT elevation is more specific to alcohol than is a rise in GGT. None of the indirect markers have been found sensitive to episodic heavy drinking (SAMHSA, 2012) and are more attuned to heavy chronic drinking.
Direct alcohol markers directly reflect alcohol consumption and are usually products of alternative ethanol metabolic pathways that persist in circulation longer than ethanol or its metabolites. These are formed only in the presence of ethanol. The major direct markers with a good body of research that have also been studied for forensic purposes include:

- ethanol itself,
- ethylglucuronide (EtG), a minor metabolite of ethanol most commonly measured in urine, but can also be retrieved from blood, hair and oral fluid,
- ethyl sulfate (EtS) like EtG but with a slightly shorter half-life,
- phosphatidylethanol (PEth), an abnormal cell membrane phospholipid produced in the presence of ethanol. PEth occurs readily because a common enzyme, phospholipase D, that may be unrelated to alcohol use.
- fatty acid ethyl esters (FAEE), formed by condensing alcohol and an acid.

PEth and EtG are the most studied direct markers. In acute dosing, blood PEth zeroes out in a week, but very heavy drinkers’ levels take weeks of abstinence to reach zero (Wurst et al., 2012). However with significant acute dosing PEth elevates gradually and upon cessation of drinking has a half-life of about a week (Viel et al., 2012). Regular heavy drinkers have detectable PEth. EtG and FAEE can be measured in urine, blood, or hair. In urine, EtG extends detection of ethanol use by one to two days. In hair, the alcohol/EtG detection window is months as EtG is sequestered in the growing hair shaft. In hair samples, EtG or FAEE provide long term exposure indicators, and the only objective alternatives to confessional estimates of historical alcohol use.

Current Uses in Road Safety Decision Making

In addition to their clinical uses, licensing authorities in several nations, measure the liver enzymes GGT, CDT, AST and ALT, and MCV as part of a biomarker panel to aid in decisions about driver license restitution for DUI driving. AST, ALT and MCV are too insensitive to detect binge drinkers or the high end of normal, especially among youth who represent a significant portion of alcohol road risk.

While not specific to alcohol, GGT is often elevated after chronic alcohol consumption. It was one of the earliest used and is still presently a useful marker, receiving considerable study among driver license applicants in the Netherlands, Sweden, Switzerland, and Norway, to name a few. GGT is best used in combination with other markers since it is affected by diseases that may be unrelated to alcohol use (e.g. biliary or hepatic diseases). CDT is a specific indicator of high levels of alcohol use. The carbohydrate deficiency of transferrin occurs because the iron transport protein loses some of its carbohydrate containing sialic acid end groups as a result of regular ethanol exposure. It is not clear why ethanol does this, but the amount of carbohydrate deficiency strongly reflects the degree of ethanol exposure. Transferrin becomes more deficient after regular consumption of about 60 g ethanol per day (about 5 standard drinks). The relative percent of carbohydrate deficiency (%CDT) reflects the growth of these deficient isoforms relative to the total CDT found in an individual, and today %CDT, not total CDT, is the recommended method for reporting. The percent of total deficiency improves the value of the marker by overcoming some age and gender-related variation in total transferrin. In the past few years, toxicology researchers who study CDT have focused on the “disialo” isoforms of %CDT. Targeting these more specifically deficient isoforms has caused some change in the recommended cutoff levels for heavy drinking from 2.6% to about 1.8% of total. The type of %CDT under study should be noted to understand the reported levels.
GGT and CDT levels can be combined. Berner et al., (2006) in a large German study of drivers found distinct advantages to combining the GGT and %CDT markers to improve sensitivity, especially for women. Marques et al., (2011) found the combined marker, γ%CDT, had better accuracy for detecting the highest risk group in an interlock DUI population (based on failed BAC tests) than did either marker alone.

The **direct markers** are more sensitive and more specific. Road safety studies with EtG, PEth, and FAEE are now widely available. EtG measured in urine and hair, are the primary biomarkers now in Germany for assessing drinking prior to making driver fitness judgments. In urine, EtG and EtS, can be measured for about 1-1½ days after blood ethanol goes to zero. External exposure slightly raises urine EtG, but above 500 ng/ml precludes most incidental exposure.

Pirro et al. (2011) in a comparative detection study recently characterized hair EtG (hEtG) as the most accurate biomarker for the identification of chronic alcohol misuse relative to known heavy drinking. For assessing drinking drivers, Liniger et al. (2010) found hEtG to be the best choice for assessing fitness. The Society of Hair Testing regards 30 picograms of EtG per mg of hair as the best cutoff for excessive drinking. Dufaux et al. (2012) analyzed samples from German drivers seeking to reinstate license status and found that the use of EtG in hair, with its longer time horizon, detected 12.7% positives relative to 2.1% positives found with the measurement of EtG in urine. These are stable findings based on sample sizes of 4000 and 13,000 respectively.

Marques et al., (2010) reported PEth to be the one alcohol marker among 9 others to have the strongest correlation with other markers, the largest F ratio predicting 3 risk groups of drivers based on failed interlock BAC tests, and showed the strongest relationship with psychometric assessments. Viel et al., (2012) reviewed PEth studies and reported that social drinkers’ PEth is either undetectable or below 1 µmol/l. With PEth levels between social drinkers and dependent alcoholics, Marques et al., (2010) reported that the 20% highest risk subset of ignition interlock users (based on rates of failed BAC tests) averaged 1.5 µmol/l and over 5.0 at the highest level. Most PEth studies in the literature have used the HPLC ELSD (high performance liquid chromatography with an evaporative light scattering detector) methods developed by the Lund University Neurochemistry Department in Sweden. The Swedish lab uses 0.7 µmol/l as the limit of quantitation (LOQ). There is no international consensus cutoff of PEth that differentiates social use and problematic use. Many labs use PEth levels above the LOQ up to 1µmol/l. In addition to measuring total PEth, newer LCMSMS (tandem mass spectrometry) methods can detect a molecular subspecies of PEth (16.0/18/1) that has been referred to as POPE ((1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol) and represents about 45% of the total PEth. Several studies with POPE have found it to be more sensitive than total PEth at detecting low levels of drinking. With drivers, Marques et al., (2011) found that POPE was detected in lower risk DUI offenders for whom full PEth was essentially zero (<.22 µmol/l).

This review has been made available to a sample of hospital researchers, DUI experts, judges and licensing authorities. The purpose has been to evaluate its usefulness, make adjustments where warranted, and revise. Table 1 will be modified as more recommendations are received.

**Discussion and Recommendations**

Following DUI charges, most alcohol offenders participate in some form of intervention to reduce public risk exposure, whether treatment, education, interlock or any combination of those.
None of those interventions can provide definitive information about a driver’s continued proclivity toward alcohol use. However effective a DUI intervention initially – such as interlock – most studies find a full return to control levels of recidivism after interlock removal. These observations suggest the need for better knowledge about alcohol consumption levels prior to relicensing, even if the offender has already fulfilled a government requirement that seeks to control or rehabilitate the individual. A balanced approach to recommending alcohol biomarkers for road safety applications would combine the most widely used indirect alcohol biomarkers, CDT and GGT, with key direct markers anchoring the panel to document drinking within a more specifiable timeframe. The latter include hEtG, PEth, and uEtG respectively, for longer, midrange, and shorter duration measures of drinking in the recent past.

There is a tradeoff between state of the art and availability of laboratories that can make these measurements. Table 1 reviews test availability, marker type, duration, and evidence of sensitivity, specificity in detecting alcohol consumption. Estimates about practical availability of tests and laboratories will vary regionally but a rough estimate is proposed. Tabled classification criteria are based on published evidence from: DUI populations, clinical populations, self-report assessments, diagnostic assessments, and interlock or driver data.

References


Table 1: Aspects of Alcohol Biomarkers for Use in Monitoring DUI Drivers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Detect Heavy Drinking</th>
<th>Monitor Abstinence</th>
<th>Detect Past Drinking</th>
<th>Time to zero out after max</th>
<th>Sens.</th>
<th>Spec.</th>
<th>Methods</th>
<th>Availability Analysis</th>
<th>Comment</th>
<th>Road Research Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT</td>
<td>often</td>
<td>no</td>
<td>weeks</td>
<td>2-4 wks</td>
<td>med</td>
<td>low</td>
<td>common</td>
<td>high</td>
<td>liver diseases alter, but much safety research</td>
<td>yes</td>
</tr>
<tr>
<td>MCV</td>
<td>sometimes</td>
<td>no</td>
<td>month+</td>
<td>n/a</td>
<td>low</td>
<td>med</td>
<td>direct obs.</td>
<td>high</td>
<td>clinically useful</td>
<td>yes</td>
</tr>
<tr>
<td>%CDT</td>
<td>yes</td>
<td>no</td>
<td>weeks</td>
<td>2-3 wks</td>
<td>med</td>
<td>med</td>
<td>7</td>
<td>medium</td>
<td>best studied in road safety</td>
<td>yes</td>
</tr>
<tr>
<td>PEth whole blood</td>
<td>yes</td>
<td>somewhat</td>
<td>weeks</td>
<td>2-4 wks</td>
<td>med</td>
<td>high</td>
<td>*</td>
<td>low</td>
<td>special sample handling</td>
<td>yes</td>
</tr>
<tr>
<td>PEth-POPE</td>
<td>yes</td>
<td>better than whole PEth</td>
<td>weeks</td>
<td>2-4 wks</td>
<td>high</td>
<td>high</td>
<td>LCMSMS</td>
<td>medium</td>
<td>still new, need more studies</td>
<td>yes</td>
</tr>
<tr>
<td>EtG – urine</td>
<td>recent only</td>
<td>yes</td>
<td>2 days</td>
<td>1-3 days</td>
<td>high</td>
<td>high</td>
<td>LCMSMS</td>
<td>medium to high</td>
<td>levels &lt;500 ng/ml EtG pos. passive exposure</td>
<td>yes</td>
</tr>
<tr>
<td>EtG – hair</td>
<td>yes</td>
<td>long term only</td>
<td>1-9 mos.</td>
<td>3 cm=3 mos.</td>
<td>high</td>
<td>high</td>
<td>LCMSMS</td>
<td>low</td>
<td>best for extended term alcohol use proclivity</td>
<td>yes</td>
</tr>
<tr>
<td>EtG, EtS oral fluid</td>
<td>recent only</td>
<td>not very practical</td>
<td>1 day</td>
<td>hours</td>
<td>high</td>
<td>high</td>
<td>LCMSMS</td>
<td>low</td>
<td>not so useful (short half-life)</td>
<td>?</td>
</tr>
<tr>
<td>FAEE - hair</td>
<td>yes</td>
<td>long term only</td>
<td>1-9 mos.</td>
<td>3 cm=3 mos.</td>
<td>high</td>
<td>high</td>
<td>LCMSMS</td>
<td>low</td>
<td>good alternative if hEtG not available</td>
<td>yes</td>
</tr>
<tr>
<td>FAEE - blood</td>
<td>yes</td>
<td>yes</td>
<td>2 days</td>
<td>hours</td>
<td>high</td>
<td>high</td>
<td>LCMSMS</td>
<td>low</td>
<td>not so useful (short half-life)</td>
<td>yes</td>
</tr>
</tbody>
</table>

2 AST and ALT are not included since both sensitivity and specificity are too poor to recommend.
3 Implies good sensitivity, specificity and prompt elevation
4 Sensitivity of direct markers vary with source, but in general are very high; when from urine, blood or oral fluid they reflect recent consumption of alcohol. A marker sensitive for consumption may not be as useful as an alcohol disease/dependence/alcoholism indicator.
5 Measurement services are regionally availability and will differ – the estimate provided is general and approximate.
6 Percent disialo CDT is now becoming more widely used as the best isoform of %CDT to denote alcohol use. The expression of a % level of disialo CDT relative to total transferrin standardizes it by adjusting relative to total CDT levels.
7 Immunoassay, HPLC, capillary electrophoresis, others
8 Many: thin layer chromatography, HPLC with evaporative light scattering detector, gas chromatography, electrophoresis, immunoassay
9 Lund University Neurochemistry’s pioneered HPLC procedure with ELSD requires collection of whole heparinized blood in plastic tubes then transfer to glass for ultracold -80C storage.
10 POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol), also shown as PEth 16.0/18.1, is reportedly the most prevalent molecular species of phosphatidylethanol (PEth). It represents about 45% of PEth on a molar basis. Sample handling is similar to whole PEth.