
Ethyl glucuronide—the direct ethanol metabolite on the threshold from science to routine use

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ABSTRACT

Aims Current biological state markers remain suboptimal with regard to sensitivity and specificity for monitoring recent alcohol consumption in various settings. Furthermore, these biomarkers can be influenced by age, gender and a variety of substances and non-alcohol-associated diseases and do not cover fully the time axis for alcohol intake. Ethyl glucuronide (EtG) is a non-volatile, water-soluble, stable, direct metabolite of ethanol that can be detected in various body fluids, tissues and hair. Shortly after the consumption even of small amounts of ethanol, EtG becomes positive. It can detect ethanol intake up to 80 hours after the complete elimination of alcohol from the body, covering a unique and important time spectrum for recent alcohol use. EtG seems to meet the need for a sensitive and specific marker to elucidate alcohol use not detected by standard testing.

Design, setting, participants, methods and findings The literature was reviewed with a focus on possible diagnostic and therapeutic applications, currently available methods and future perspectives. To date, more than 4000 samples of body fluids, tissues and hair from approximately 1500 individuals have been assessed.

Conclusions The data suggest that EtG is a useful tool in numerous settings, including alcohol and drug treatment (to detect lapse/relapse and for motivational feedback), in safety sensitive work settings where use is dangerous or in other settings where alcohol use may be risky (e.g. such as driving, work-place, pregnancy or monitoring physicians or other professionals who are in recovery and working) or for resolving forensic questions. If the question of recent alcohol consumption has to be answered in a binary way (yes/no), such as for determining lapses, the use of EtG in urine is among the preferred tests. The use of this marker alone and complementary with other biological state markers and self-reports is expected to lead to significant improvement in treatment outcome, therapy efficacy and cost reduction.

KEYWORDS Alcohol drinking, biomarker, cost reduction, ethyl glucuronide, glucuronates, therapy efficacy.

INTRODUCTION

The global burden of disease from alcohol exceeds that of tobacco and is on a par with the burden attributable to unsafe sex practices world-wide (WHO Global Status Report on Alcohol 1999). To prevent and reduce health

and social problems for the individual and society related to alcohol use, biological state markers and marker combinations capable of monitoring alcohol consumption with a high sensitivity and specificity, providing information on the drinking pattern, are required.

Whereas the traditional state markers of alcohol consumption such as gamma glutamyltransferase (GGT), mean corpuscular volume (MCV) or carbohydrate deficient transferrin (CDT) reflect persistent consumption of higher amounts of alcohol (>2 weeks, >1000 g of ethanol in 2 weeks) it is, for many of the reasons noted above, important to detect even single-drink or short-term consumption events although ethanol itself has been eliminated from the body. This would include alcohol consumption in social drinkers and monitoring alcohol consumption in alcoholics or polydrug abusers in abstinence-orientated treatment programmes.

Among the biological state markers of alcohol intake, major shortcomings limit their value to include: (1) the time spectrum of detection they reflect (e.g. serum ethanol detects only recent use within hours); (2) the amount of alcohol that must be consumed before the markers are elevated (e.g. >1000 g of ethanol within 2 weeks for CDT); (3) the availability and practicability of the test (e.g. special laboratories being required for testing phosphatidyl ethanol, fatty acid ethyl esters, dolichol, acetaldehyde, etc.); and influences of age, gender and a variety of substances and non-alcohol-associated diseases (Gilg & Soyka 1997; Laposata 1999).

To help fill the gap on the time axis with regard to recent alcohol consumption occurring between 1 day to 1 week, state markers capable of monitoring alcohol consumption within this intermediate period are desirable. (Figure 1 gives a synopsis of the intervals of alcohol intake necessary before exceeding reference values in different markers and the possible time spectrum of detection after ethanol has left the body.) Ethyl glucuronide, which appears to meet this need, has been studied together with other non-oxidative direct ethanol metabolites, including fatty acid ethyl esters (FAEE) and phosphatidyl ethanol (PEth), each offering a characteristic

time spectrum of possible detection of ethanol consumption. These time windows are for: (a) FAEEs up to 24 hours (Diczfalusy *et al.* 1999, 2001; Laposata 1999); (b) EtG up to 5 days (Schmitt *et al.* 1995, 1997, 1998; Wurst *et al.* 1995, 1996, 1997, 1999a,b,c, 2000a,b,c, 2001, 2002a,b, 2003; Alt *et al.* 1997, 1999; Seidl *et al.* 1998, 2001; Nishikawa *et al.* 1999; Dahl *et al.* 2002; Stephanson *et al.* 2002); and (c) PEth up to 2 weeks (Alling *et al.* 1983; Hansson *et al.* 1997; Gunnarsson *et al.* 1998; Varga *et al.* 1998; Wurst *et al.* 2003).

Ethyl glucuronide (EtG) is a non-volatile, water-soluble, stable, direct metabolite of ethanol that can be detected in various body fluids, tissues and hair, having the potential to detect alcohol intake shortly after its beginning and—dose-dependently—up to 80 hours after the complete elimination of alcohol from the body. EtG thus spans a unique and important time spectrum. It seems to satisfy the need for a sensitive and specific marker to detect alcohol use not detected by standard testing.

In 1901 Neubauer described the detoxifying pathway of alcohol elimination via conjugation with activated glucuronic acid (uridine-5'-diphospho- β -glucuronic acid). In 1952, EtG was isolated by Kamil *et al.* as triacetyl methyl ester from the urine of rabbits (Kamil *et al.* 1952). Later, this conjugate was determined in human urine (Jaakonmaki *et al.* 1967; Koza 1973; Besserer & Schmidt 1983).

Conjugation of ethanol with activated glucuronic acid in the presence of membrane-bound mitochondrial uridine diphosphate (UDP) glucuronyl transferase represents a minor detoxifying pathway for ethanol. Only about 0.02–0.06% of the dose of ethanol administered is recovered as EtG in urine in humans (Dahl *et al.* 2002; Goll *et al.* 2002). The molecular formula of EtG is $C_8H_{14}O_7$, the molecular weight is 222 g/mol and the melting point (decomposition temperature) is about 150°C.

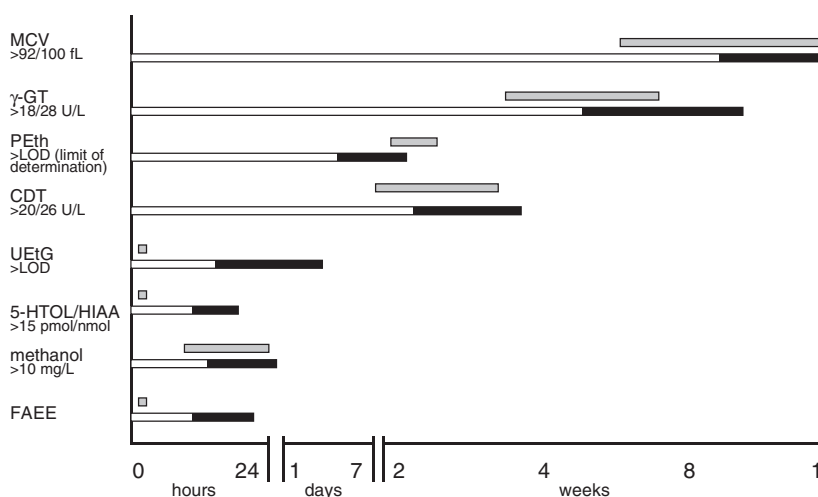


Figure 1 Intervals of intake necessary before exceeding reference values (□) and time spectrum of detection after ethanol has left the body (■); possible variation

THE LAST DECADE

Since 1993 various research teams have attempted to enhance knowledge on this promising new marker. In 1995 a five-step synthesis, analytical data and determination of EtG in serum and urine were reported by Schmitt *et al.* (1995). Using a gas chromatography-mass spectrometry (GC-MS) method, with EtG as standard substance and acetylation for derivatization, they found EtG concentrations of between 3 and 130 mg/l in urine samples from 12 drunken drivers. Alt and Wurst achieved better results by using silyl derivatives rather than acetyl derivatives (Wurst *et al.* 1995, 1996; Alt *et al.* 1997). With this method they reported a detection time of up to 75 hours in the urine of 23 alcohol withdrawal patients, all of whom had detectable alcohol levels at hospitalization. In a drinking experiment with a healthy volunteer (60 g of ethanol had been consumed over 2 hours), no EtG was determined initially in the urine. The EtG curve had a phase delay in comparison to the urine ethanol curve, but EtG was detected for a much longer period of time than ethanol. In 1997, Schmitt *et al.* (1997) reported on ethyl glucuronide concentrations in the serum of human volunteers, teetotallers and suspected drinking drivers. They found a maximum EtG of 3.7 mg/l in drinking experiments with up to 90 g ethanol, a half-life of EtG of 2–3 hours, and were able to determine EtG in serum up to 8 hours beyond the time that ethanol was no longer detectable. With a serum ethanol concentration of less than 1 g/l, at a serum EtG concentration significantly higher than 5 mg/l, they adjudged alcohol use to be problematic.

METHODOLOGICAL MILESTONES

After these initial studies, further progress was made by new techniques including the development of an internal standard, a robust LC/MS method and an enzyme-linked immunosorbent assay (ELISA), as follows.

Penta deuterium-labelled EtG (d_5 -EtG) as an internal standard

Deuterium-labelled EtG (d_5 -EtG), a gold standard for the determination of EtG because of physical and chemical properties identical to those of EtG with the exception of its mass, has been available commercially since January 1997 (Medichem Inc., Steinenbronn, Germany) and since then has been employed by one German group; in the last year it has also been used by a Swedish group with excellent results (Alt *et al.* 1997, 1999; Seidl *et al.* 1998; Dahl *et al.* 2002; Stephanson *et al.* 2002; Wurst *et al.* 1995, 1996, 1997, 1999a,b,c, 2000a,b,c, 2001, 2002a,b, 2003).

Electrospray ionisation/liquid chromatography (LC/ESI-MS/MS) and tandem MS methods

Marked methodological improvement and cost reduction resulted from the development of a LC/MS-MS method with the advantage of no derivatization being required. Reports on the determination of urine samples (Wurst *et al.* 1999a,b) were followed quickly by the application for serum samples (Wurst *et al.* 1999b, 2000a), post-mortem body fluids and tissues (Wurst *et al.* 1999b) and hair (Janda *et al.* 2002). Also single MS methods (Nishikawa *et al.* 1999; Dahl *et al.* 2002) have been reported to be robust and reliable.

Future perspective: ELISA

An ELISA procedure based on a polyclonal antibody yielded 23% false positives and 24% false negatives in urine as contrasted with a GC-MS method (Schmitt *et al.* 1998; Zimmer *et al.* 2002). ELISA based on a monoclonal antibody is under development at Mediagnost Inc., Germany. However, because the EtG is small (molecular weight of 222 g/mol) it is difficult to create an antibody. Nevertheless, the first immunization of two BALB/c mice with one lipopeptide conjugate—first fusion (after 7 weeks) of the spleen of one immunized mouse—produced one positive, a second fusion (after 15 weeks) of the second mouse resulted in 10 positive clones (Wurst *et al.* 2001).

Applications

The test for EtG has been applied for concerns such as: (a) legal medicine (e.g. driving while intoxicated (DUI), expert assessment of judging driving ability, autopsy), (b) alcohol treatment and practice monitoring abstinence in substance abuse disorders and (c) basic science such as determining the stability of EtG and the possibility of artificial formation, storage and distribution in the body.

In a clinical study, 33 alcoholics with a mean BAC of 183 mg/dl at admission to the hospital were included and 181 urine samples were taken every 4–12 hours during detoxification. Ethyl glucuronide concentrations ranging between 3.6 and 710 mg/l and with detection times of up to 80 hours were found (Wurst *et al.* 1999a). After detoxification, no EtG could be found in urine. No correlation between the concentration of EtG in urine at hospitalization and the blood ethanol concentration, or the time-frame of detection, or the total amount of clomethiazole required for the treatment of withdrawal symptoms were able to be established (Wurst *et al.* 1999a). Furthermore, the stability of EtG in urine was investigated by measuring the ethanol metabolite in an autosampler at room temperature every hour for 140 hours. The results of the internal ratio EtG/ d_5 EtG fall within a very narrow range,

proving the analyte's stability (Wurst *et al.* 1999a). This is of importance if the EtG test becomes widespread.

In order to make EtG values and their correlation to other parameters comparable, the impact of very different levels of intoxication (0.195–1.8 g/l) at admission to a detoxification unit was eliminated by standardizing EtG values on the time-points when breath ethanol concentration was zero, followed by testing for EtG 24 and 48 hours thereafter. Under such standard conditions a strong correlation was found between EtG values at the time-point when BAC was zero and EtG values 24 and 48 hours afterwards (Spearman's rank correlation r_s up to 0.872; $P < 0.01$) as well as for the time-frame of detection (hours) ($r_s = 0.762$, $P < 0.01$) (Wurst *et al.* 2002b).

In drivers suspected of driving while intoxicated (DUI) serum ethanol concentration (SEC) of 0.1–3.9 g/l, serum EtG (SEtG) 3.2–13.7 mg/l, urinary ethanol (UEC) 0.1–2.0 g/l and urinary EtG (UEtG) 3.0–130 mg/l have been found (Schmitt *et al.* 1995). A second study showed 37 of 50 drivers to have positive SEtG (Schmitt *et al.* 1997).

EtG also demonstrated utility in the expert assessment for judging driving ability (Seidl *et al.* 1998). Thirteen of 151 cases have been found to be positive for UEtG. None of the traditional markers including GGT, MCV or CDT had evidenced for alcohol consumption in these cases.

ETHYL GLUCURONIDE IN VARIOUS BODY FLUIDS AND TISSUES

Subsequently, a LC/MS-MS procedure for the analysis of tissue samples was developed to specify distribution and storage of EtG in various body fluids and tissues. Analyses of samples from five corpses indicate that it is possible to assess EtG in serum, urine, cerebrospinal fluid and tissues including liver, fat and brain as well as in hair, even post-mortem (Wurst *et al.* 1999b). It is unclear, however, why EtG was not found in the liver of one alcoholic and in the gluteal fat of another alcoholic who died intoxicated. These results indicating distribution and storage of EtG in the body are of importance with regard to the possibility of redistribution of EtG from various body compartments into the blood and urine and, thereby, influencing EtG levels.

ETHYL GLUCURONIDE IN HAIR SAMPLES

Despite preliminary reports on hair analysis for ethyl glucuronide (e.g. Sachs 1997) in the 1990s, analysis of this material is still not common and it was not until 2000 that systematic experiments with larger sample numbers were performed (Alt *et al.* 2000; Pragst *et al.* 2000; Skopp

et al. 2000). In this year, Pragst and colleagues reported being unable to discover EtG in hair samples of three heavy alcoholics. In contrast, two other groups were successful in the same year: Skopp and colleagues reported on analyses of a total of 14 hair samples (Skopp *et al.* 2000) and Alt and colleagues published data from analyses of 31 hair samples (Alt *et al.* 2000). Alt *et al.* detected EtG in 14 hair samples, taken at autopsy from 16 people with a known history of alcoholism, and found EtG concentrations of between 218 and 4025 pg/mg hair. In all four hair samples from alcohol withdrawal patients, EtG concentrations between 119 and 388 pg/mg hair were detected. However, measurable concentrations of EtG were not observed in hair from six social drinkers with a self-reported daily ethanol consumption of up to 20 g or five hair samples from children.

In a recently published study (Janda *et al.* 2002) 97 hair samples of alcoholics, social drinkers and teetotallers were investigated. In 49 of 87 hair samples from individuals with a known history of frequent alcohol misuse, EtG was detected in concentrations up to 13 157 pg/mg, whereas in 38 of these cases EtG was not detected with a limit of determination of 51 pg/mg. Measurable concentrations of EtG were detected in hair of one of five social drinkers reporting daily consumption of up to 30 g ethyl alcohol. In hair samples of five children EtG was not detected. In addition, no correlation between the amount of ethanol consumed and the detected EtG concentration in hair has been found.

These results emphasize that the absence of EtG in hair does not indicate a person to be abstaining from alcohol. However, consumption of higher amounts of ethanol on a frequent basis clearly has to be suspected if EtG is detectable in hair samples.

USE OF ETHYL GLUCURONIDE IN FORENSIC PSYCHIATRIC PATIENTS WITH SUBSTANCE USE DISORDERS

EtG also proved its usefulness in the treatment of forensic psychiatric addicted in-patients. The patients were all sentenced according to §64 StGB (penal code), an option of German law if someone commits a substance-related offence (including murder, rape or robbing under the influence of psychotropic substances) and is deemed to have diminished or no legal responsibility (§20/21 StGB) if at the time of the crime the offender's capability of recognizing the wrongfulness of the action, or to act out of this understanding, is considerably impaired. The treatment of these patients is of particular interest to the public and therapists bear significant responsibility in this special setting. Furthermore, the closed ward offers the unique opportunity to monitor addicted patients under

well-controlled and well-defined conditions. In more than 200 urine samples from over 100 forensic psychiatric addicted in-patients in various hospitals, an additional detection, i.e. lapse rate of 5–10% at each single occasion, was found in cases where neither traditional markers nor the therapist's rating gave an indication for a slip (Wurst *et al.* 2000a, 2003).

The first study (Wurst *et al.* 2000a) was multi-site and performed on a single occasion. In addition, 35 forensic psychiatric in-patients in a closed ward who had committed a substance-related offence (§64 StGB, penal code) were followed during 12 months (Wurst *et al.* 2003). The complete time spectrum of possible alcohol consumption was covered by the complementary use of a marker battery including breath and urinary ethanol (hours), urinary EtG (days), %carbohydrate-deficient transferrin (CDT)/PEth (weeks) and gamma-glutamyltranspeptidase (GGT)/mean corpuscular volume (MCV) (weeks–months). Fourteen of the 146 urine samples examined were positive for EtG. In all cases positive for EtG, patients reported alcohol consumption of between 40 and 200 g of ethanol 12–60 hours prior to testing. Urinary and breath ethanol were positive in only a single case. In the blood samples PEth was not positive in any case and %CDT did not exceed the reference value. Isoelectric focusing showed no abnormal Tf-subtypes (Wurst *et al.* 2003). The findings emphasize the diagnostic and therapeutic usefulness of EtG as a marker of recent alcohol use by proving that ethyl glucuronide is capable of detecting alcohol consumption in cases where neither traditional biological state markers of alcohol intake nor clinical impression gave an indication for lapse or relapse. A summary of the results from studies in forensic patients is given in Table 1.

WHO/ISBRA STUDY ON STATE AND TRAIT MARKERS OF ALCOHOL USE AND DEPENDENCE

The aim of the work as part of the WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence was to elucidate further the usefulness of EtG in comparison to other biological state markers and to demonstrate the robustness of the LC/MS-MS method in large numbers. Samples from $n = 304$ patients (288 male, 16 female) from Australia and Brazil were examined. The age was 37.58 (mean), body mass index (BMI) 25.51 (mean) and total grams of ethanol consumed last month ($n = 226$) was 2318.2 g (mean). An ESI-LC/MS-MS method with deuterium-labelled EtG as internal standard was employed.

Ethyl glucuronide concentrations of between 0 and 1038 mg/l were determined in urine samples from 304

patients. A significant ($P < 0.001$) Spearman's rank correlation was found between ethyl glucuronide and sobriety in days (-0.60), 5-hydroxytryptophol/5-hydroxyindoleacetic acid (HTOL/HIAA) ratio (0.58), total grams of ethanol consumed last month (0.47), CDT (0.46), EtOH level (0.43), GGT (0.43) and methanol (0.2).

The correlation ($P < 0.001$) between ethyl glucuronide and total grams of ethanol consumed last month ($r = 0.47$) is almost identical whether consumption is expressed in relation to body weight or body water, possibly indicating minor influence of body weight and body water on EtG values.

When comparing subjects who had measurable EtG levels and/or elevated (>15) HTOL/HIAA ratios ($n = 264$), 68.8% of those positive for EtG were not elevated for the HTOL/HIAA ratio and 31.2% were positive for both parameters. This might reflect the longer time spectrum of detection for EtG (up to 5 days) as contrasted to HTOL/HIAA ratio (<1 day).

Table 2 summarizes selected studies, contributing new knowledge on EtG.

DISCUSSION

Clearly, there is a need and a desire for a means to detect recent alcohol use. Biological tests can, in addition to self-reports, provide counsellors and programme evaluators with complementary information. Their role in alcoholism treatment has been recently discussed (Allen & Litten 2001) and besides other aspects the authors have pointed out their increasing importance as outcome measures in clinical trials. Furthermore, they suggested that informing the patient that blood will be tested periodically to evaluate treatment progress might, in fact, itself contribute to reduce lapse and relapse and thus could enhance treatment progress. Numerous tests and devices (breath ethanol analysis, blood and urine alcohol analysis, sweat patches, transdermal electronic alcohol sensors, tests for methanol, HTOL/HIAA ratio, apolipoprotein J, beta-hexosaminidase, etc.) have been developed and suggested to uncover recent alcohol consumption (Gilg & Soyka 1997; WHO/ISBRA 1997; Laposata 1999; Helander *et al.* 1992a,b, 2002).

Whereas the traditional state markers of alcohol consumption such as GGT, MCV or CDT reflect longer-lasting consumption of higher amounts of alcohol (>2 weeks, >1000 g of ethanol in 2 weeks), it is of interest to identify even single or short-term consumption.

EtG, a non-volatile, water-soluble, stable, direct metabolite of ethanol and a state marker for recent alcohol use, seems to meet this need. EtG also appears to be highly sensitive and specific. Shortly after the

Table 1 Synopsis of selected studies, contributing new aspects to the knowledge on EtG.

Individuals and numbers	Major results	References
Drivers suspected of DWI	Synthesis of EtG as external standard SEC 0.1–3.9 g/l; SEtG 3.2–13.7 mg/l UEC 0.1–2.0 g/l; UEtG 3.0–130 mg/l	Schmitt et al. 1995
Drivers suspected of DWI (n = 50)	37 were positive for SEtG Half-life of EtG: 2–3 hours Teetotallers: negative for EtG	Schmitt et al. 1997
Male alcohol withdrawal patients (n = 33), 181 urine samples d5-EtG as an internal standard	UEC max. 0.40 per ml UEtG 3.6–710 mg/l Detectable up to 75 hours Phase delay: UEC/UEtG	Alt et al. 1997
Expert assessments for judging driving ability (n = 151)	13 cases positive UEtG 0.1–31.9 mg/l	Seidl et al. 1998
Alcohol withdrawal patients (n = 33)	UEtG 3.6–710 mg/l, Detection 57.7 ± 16.9 hours No correlation of UEtG with SEC, GGT, MCV, time-frame of detection or total amount of clomethiazole required Stability over 140 hours at room temperature	Wurst et al. 1999a
Detoxified alcoholics (n = 30)	Four cases UEtG 4.2–196.6 mg/l, one case SEtG 4.8 mg/l	
Neurorehabilitating patients (n = 43)	Eight urine samples from seven patients positive UEtG 2.9–23.49 mg/l	
Body fluids and tissues 27 samples from five subjects (corpses)	Detection of EtG in gluteal and abdominal fat, liver, brain, serum, urine and CSF	Wurst et al. 1999b
Detoxified polydrug-abusing patients (n = 9)	Four cases positive	Wurst et al. 2000
Detoxified alcoholics (n = 24)	UEtG 0.29–1.03 mg/l	
Forensic psychiatric in-patients §64 StGB (penal code) (n = 44)	Three cases positive at a single occasion (= morning) UEtG 0.1, 0.3 and 18 mg/l Therapists' rating: abstaining Patients self-report: lapsing Other biomarkers positive: none	
Hair samples from 16 alcoholics (postmortem)	14 of 16 post-mortem hair samples of alcoholics: 218–4025 pg EtG/mg	Alt et al. 2000
Four alcohol withdrawal patients	4/4 hair samples of alcohol withdrawal patients:	
Six social drinkers	119–388 pg EtG/mg;	
Five children	No EtG in hair of social drinkers and children	
Patients from the WHO/ISBRA study (n = 304)	Comparison of EtG with other biomarkers including HTOL/HIAA ratio, methanol, GGT, CDT Robustness of LC/MS-MS method demonstrated	Wurst et al. 2002b
Healthy volunteers (n = 7)	0.04% (SD 0.02%) of dose administered dose EtOH excreted in urine as UEtG	Goll et al. 2002
Healthy volunteers (n = 7)	0.02% of administered dose of EtOH on a molar basis recovered as UEtG Stability at room temperature over 4 days No <i>in vitro</i> formation of UEtG on storage of urine samples fortified with 1% ethanol	Dahl et al. 2002
Patients from an out-patient programme (n = 252)	Demonstration of the robustness of a LC/MS method with a porous graphite column 63% of the samples positive for EtG	Stephanson et al. 2002
Forensic psychiatric in-patients (n = 35) with substance dependence	During 1 year: 14 of 146 urine samples positive for EtG Other biomarkers giving an indication for lapse/relapse (breath ethanol, CDT, GGT, MCV, phosphatidyl ethanol): one case positive for breath ethanol patients' self-reports: lapsing with 40–200 g of ethanol 12–60 hours prior to testing	Wurst et al. 2003

Table 2 Comparison of UEtG, therapists rating, self reports and biomarkers of ethanol intake in forensic psychiatric in-patients.

Urine samples drawn	UEtG positive	Range (mg/l)	Therapists' rating	Self-report	Consumption reported	Breath ethanol concentration	Urinary ethanol concentration
223	18	0.1–46.9	Abstaining in all cases	Lapsing	40–200 g of ethanol	17 cases neg. One case 0.05 g/l	17 cases neg. One case 0.4 g/l

UEtG: EtG in urine.

consumption of even minor amounts of alcohol, such as 10 g (half a bottle of beer) (Kintz *et al.* 2002; Stephanson *et al.* 2002), EtG becomes positive and is also dose-dependently detectable for 4 days following complete elimination of alcohol from the body (Schmitt *et al.* 1995, 1997, 1998; Wurst *et al.* 1995, 1996, 1997, 1999a,b,c, 2000a,b,c, 2001, 2002a,b, 2003; Alt *et al.* 1997, 1999; Seidl *et al.* 1998; Seidl *et al.* 1998, 2001; Nishikawa *et al.* 1999; Droenner *et al.* 2002). With its specific time-frame of detection intermediate between short- and long-term markers and with its high sensitivity and specificity, ethyl glucuronide is a promising marker of alcohol consumption in general and a marker for lapse and relapse control.

Because EtG has a longer half-life than ethanol, an accumulation of EtG must be assumed and was confirmed in several studies (Alt *et al.* 1997; Schmitt *et al.* 1995, 1997; Wurst *et al.* 1999a,b,c, 2000a,b,c). Data from post-mortem body fluids and tissues indicate that it may be possible to explore storage and distribution mechanisms and that an EtG transporter may exist. No measurable ethyl glucuronide concentrations are observed in serum or urine of non-relapsing patients, non-drinking drivers or teetotallers (Schmitt *et al.* 1995, 1997; Wurst *et al.* 1995, 1999a,b,c, 2000a, 2002a,b, 2003; Alt *et al.* 1997; Seidl *et al.* 1998, 2001).

Determination of creatinine values in urine can, as in illicit drug use testing, render it possible to detect manipulation of the sample. To make UEtG values inter- and intra-individually comparable, they can be standardized by expressing them as a ratio to creatinine (UEtG 100). Determining creatinine values, however, yields no additional information if the UEtG value is below determination limits. Therefore, for applications where a binary answer (pos./neg.) is sufficient, such as monitoring abstinence, determination of creatinine is not a necessity.

Determination of EtG in hair can be useful in forensic cases and for monitoring alcohol consumption over a longer time-frame. The current results corroborate the observation that the absence of EtG in hair does not prove that a person is abstinent from alcohol. However, consumption of higher amounts of ethanol on a frequent basis must be clearly suspected if EtG is detectable in hair samples. Although as yet no exact information exists

concerning the incorporation of EtG in the hair structure, several reasons may contribute to the missing correlation between alcohol consumption and EtG concentration in hair: EtG may be washed out due to its polarity during normal body care or hair washing. The colour of hair itself, the incorporation of EtG into hair through the bulb (hair root) and via sweat and sebaceous glands remains unexplored, as does the possibility of formation and/or degradation of EtG in the hair matrix cells. Finally, as occurs for several drugs, colourants may make a difference.

Although some methods, such as the indirect proof via acid and enzymatic hydrolysis, have failed (Ruß 1992; Weinmann 1995), or performed poorly in urine such as the ELISA based on a polyclonal antibody (Zimmer *et al.* 2002), there are several methods published from different groups, that produced positive results:

- GC/MS (Alt *et al.* 1997, 2000; Zimmer *et al.* 2002)
- LC/MS (Nishikawa *et al.* 1999; Dahl *et al.* 2002; Stephanson *et al.* 2002)
- LC/MS-MS (Wurst *et al.* 1999a, b, c, 2000a, b, 2003 Janda *et al.* 2002)

In that the chemical and physical parameters of d₃-EtG, with the exception of mass, are identical to those of ethyl glucuronide, deuterium-labelled ethyl glucuronide must be considered an excellent internal standard.

The somewhat divergent results of different research groups might be explained by (1) differences in the individuals tested for EtG, and their respective drinking patterns as well as (2) methodological differences.

Relevant issues among the latter might be:

- 1 using no internal standard (Schmitt *et al.* 1995, 1997, 1998; Nishikawa *et al.* 1999) or propyl glucuronide (Sticht *et al.* 1997), methyl glucuronide (Skopp *et al.* 2000) or t-butyl glucuronide (Wurst *et al.* 1995; Zimmer *et al.* 2002) as internal standard as well as;
- 2 acetylation (Schmitt *et al.* 1995, 1997, 1998; Sticht *et al.* 1997; Nishikawa *et al.* 1999; Zimmer *et al.* 2002) instead of silylation with BSTFA or MSTFA (Alt *et al.* 1997, 1999, 2000; Wurst *et al.* 1995, 1996, 1997, 1999a,b,c, 2000a, 2002a,b, 2003; Seidl *et al.* 1998, 1999; Skopp *et al.* 2000; Janda *et al.* 2002);
- 3 using different columns (for synopsis see: Musshoff 2002); and

4 employing a single MS (Nishikawa *et al.* 1999; Dahl *et al.* 2002; Stephanson *et al.* 2002) vs. a double MS (Wurst *et al.* 1999a,b,c,d, 2000a, 2002b, 2003).

A cut-off to distinguish between social/moderate and harmful/problematic drinking has been suggested for serum (Schmitt *et al.* 1997). One of the advantages of EtG, however, is that it can be detected in urine for a longer time and that drawing a urine sample is by far less invasive than taking a blood sample. There clearly is some potential to establish also a cut-off, which as a reasonable clinical guess could probably be set at a UEtG 100 of about 30–50 mg/l if BAC is low or zero.

UDP-glucuronosyltransferase (UGT), the microsomal enzyme responsible for glucuronidation reactions including the formation of EtG, exists as a superfamily of enzymes. Genetic polymorphism has been described for six of the 16 functional human UGT genes characterized to date (Mackenzie *et al.* 2000; Miners *et al.* 2002). Polymorphic variations in genes encoding UGT may have a significant impact on humans' capacity to form EtG and may contribute to interindividual differences in EtG values. Therefore, further studies on glucuronidation as well as on the influence of human and bacterial beta-glucuronidase activity, which also might influence EtG levels, are needed.

To foster more widespread use of tests for EtG, availability, practicability and reduction of test costs will be crucial. Development of immune-assay-based screening methodology would therefore be helpful, while GC/MS and LC/MS offer excellent validation methods.

According to forensic laboratory guidelines for validation analysis for a drug, there are at least three characteristic *m/z*-values for the compound identification for GC/MS in selected ion monitoring mode (SIM) (Aderjan *et al.* 2000) and for LC-MS/MS two MS/MS-transitions per analyte are required (Stolker *et al.* 2000). These requirements must also be fulfilled if a positive work-place (drug) test can lead to severe consequences, such as loss of employment. Therefore some analytical efforts still must be made, because none of the above-mentioned

LC-MS/MS, LC-MS or GC/MS-methods can fulfil these requirements.

From the data, which cumulatively include more than 4000 urine and serum samples of more than 1500 individuals, as well as on the basis of theoretical considerations and most prominently the fact that EtG is a direct metabolite of ethanol, the potential of EtG to demonstrate high sensitivity and specificity as a biological state marker of alcohol consumption seems clear. EtG is a useful tool to detect recent drinking and has proved superior to clinical impression and, in many respects, to other markers tested (Schmitt *et al.* 1995; Wurst *et al.* 1999a, 2002b, 2003; Dahl *et al.* 2002). This is of considerable interest in a variety of settings and situations. Figure 2 (data not published) gives an example of several markers taken from a single patient during detoxification.

The cumulative findings emphasize that there is potential to employ the test for ethyl glucuronide for screening, monitoring drug and alcohol treatment, motivational feedback, improvement of knowledge on drinking patterns, differentiation of moderate/social drinking from problematic/harmful drinking and differential diagnosis (e.g. elevated transaminases) elucidate the role of neuropsychological impairment following alcoholization (i.e. hangover state), and detection of recent drinking in social drinkers in safety-sensitive or risky situations [driving, work-places, pregnancy (FAS)] (Table 3).

EtG enriches the testing armamentarium for recent alcohol consumption. The time-frame of detection exceeds that of ethanol in serum and urine, HTOL/HIAA ratio and methanol. The complementary use of EtG, which has a specific time-frame of detection, with other biological state markers such as fatty acid ethyl esters, HTOL/HIAA ratio and phosphatidyl ethanol, can provide us with additional information, e.g. on drinking patterns.

If the question of recent alcohol consumption has to be answered in a binary way (yes/no), such as for the question of lapses, the use of EtG in urine can be suggested as a first-choice test. If specific questions have to be addressed, such as what kind of beverage was consumed,

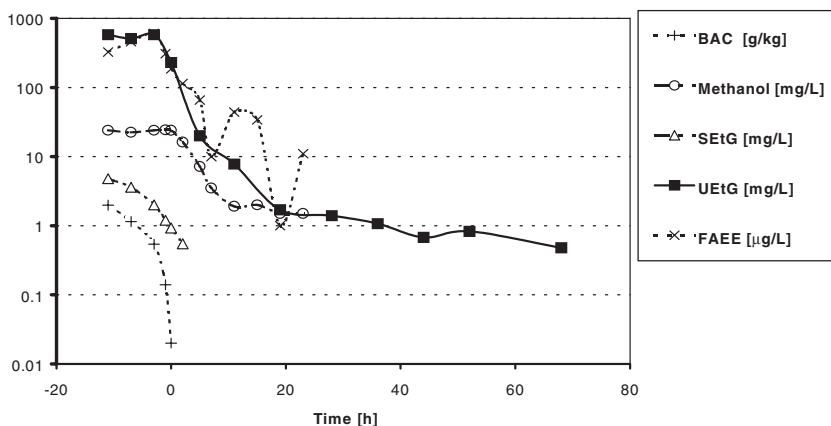


Figure 2 Time-course of different markers of alcohol consumption in serum and urine during detoxification of a patient, admitted to hospital with a BAC of 1.99 g/kg. BAC: blood alcohol concentration; SEtG: EtG concentration in serum; UEtG: EtG concentration in urine; FAEE: fatty acid ethyl esters in serum. The patient was admitted at -11 hours with a BAC of 1.99 g/kg; O is the time-point when BAC was < limit of determination (LOD)

Table 3 Possible applications of the test for EtG.

The cumulative findings emphasize, that altogether, the test for ethyl glucuronide can be used for:

- screening
- monitoring
- motivational feedback
- to improve knowledge on drinking patterns
- differentiate moderate/social drinking from problematic/harmful drinking
- differential diagnosis (e.g. elevated transaminases)
- evaluate treatment programmes and drug trials elucidate the role of neuropsychological impairment following alcoholization (i.e. hangover state) which plays a major role in accidents, disclose recent drinking in social drinkers in risky situations [driving, work-places, pregnancy (FAS)]

when exactly was the time of ethanol intake, drinking pattern, etc. additional parameters such as ethanol, HTOL/HIAA ratio, methanol and congeners, CDT, PEth and traditional markers such as GGT and MCV can—depending on the current need—be included.

The complementary use of this marker together with other biological state markers and self-reports is expected to lead to significant improvement in treatment outcome, therapy effectiveness and health, social and socio-economic benefits that will be hard to overestimate.

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