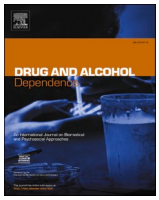
Drug and Alcohol Dependence 257 (2024) 111128

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep



# Comparative analysis between CDT in serum and Ethyl glucuronide in hair to define the best reliable tool for the diagnosis of alcohol abuse

Alice Cerioni [a], Erika Buratti [a,*], Gianmario Mietti [a], Isabella Lima Arrais Ribeiro [b], Marta Cippitelli [a], Giovanna Tassoni [a], Mariano Cingolani [a], Rino Froldi [a], Roberto Scendoni [a]

[a] Forensic Medicine and Laboratory (For. Med. Lab), Institute of Legal Medicine, University of Macerata, Via Don Minzoni 9, Macerata 62100, Italy
[b] Postgraduate Program in Dentistry, Federal University of Paraíba, Campus I, João Pessoa 58051-900, Brazil

ARTICLE INFO

*Keywords:*
Alcohol abuse
Ethyl glucuronide
CDT
Biomarkers

ABSTRACT

The increase in alcohol consumption in society has not only led to a number of medical issues but has also become a matter of considerable legal importance. Thus, there is both scientific interest and the necessity to diagnose alcohol abuse in the application of the provisions of the law through laboratory tests that ensure maximum objectivity. The purpose of this work is to study and compare the diagnostic performance of two of the main markers of alcohol abuse, serum carbohydrate-deficient transferrin (CDT) and Ethyl glucuronide (EtG) in a group of 336 driving under the influence (DUI) of alcohol offenders. Thus, it is possible to establish the best marker of alcohol consumption in order to assess the fitness to drive of DUI subjects.EtG was detected in 55 hair samples, while CDT was detected in 5 blood samples. Of the EtG-positive subjects 96,4% had CDT values below the cut-off. While CDT refers to an alcohol consumption of approximately the previous 10 days, EtG allows to detect an excessive alcohol consumption of the last few months. Because of these two different time-windows, EtG proves to be more reliable, since it is more difficult for subjects to change their drinking practice to test negative to toxicological analysis. The determination of Ethyl glucuronide on hair matrix is a valuable tool for the diagnosis of alcohol abuse, with high sensitivity and specificity and certainly greater reliability than traditional markers such as CDT, being a direct marker of alcohol consumption.

## 1. Introduction

Chronic and excessive alcohol consumption is recognized as a major public health issue (Oppolzer et al., 2017). In the western world there has been a strong increase in health issuesrelated to excessive alcohol consumption (Torrente et al., 2012). Annual per capita alcohol consumption has also increased. As shown by the most recent data published by the World Health Organization (WHO) the total per capita consumption of alcohol in the world population over 15 years of age is 6.4 liters per year, which corresponds to 13.9 g of alcohol per day (WHO, 2018). The consequences of alcohol consumption affect families and the community in general due to the deterioration of personal and work relationships, criminal behavior (e.g. vandalism and violence), road safety hazard, loss of productivity and the cost of health care. The present study focused on the topic of road safety related to driving under the influence of alcohol.

From ISTAT-ACI data it emerges that, in Italy, out of a total of 52,459 accidents with injuries detected by the General Command of the Carabinieri and the Road Police Service of the Ministry of the Interior in 2021, 5085 were those where at least one of the drivers of the vehicles involved was in a state of alcohol intoxication. From the data communicated by the Municipal or Local Police it appears that 36% of the penalties for drunk driving arose out of a road accident. For drunk driving, according to the checks carried out by the Municipal Police, the percentage of drivers testing positive to the breathalyzer was 7.3% (8.3% in 2020 and 7.4% in 2019) (ACI and ISTAT, 2022).

As regards fitness to drive, Legislative Decree N. 59/2011 states that driving licenses must not be issued or renewed to drivers who are addicted to alcohol (D.Lgs 59/2011). Alcohol consumption is in fact a significant danger for road safety (Snenghi et al., 2015), owing to the depressive action that it causes at the level of the central nervous system. For this reason, it is necessary to research and develop diagnostic methods able to early and objectively detect excessive alcohol consumption. The use of ethanol induces biochemical changes in the body

* Correspondence to: Via Giovanni XXII, 45, Macerata 62100, Italy.
*E-mail addresses:* alice.cerioni@libero.it (A. Cerioni), e.buratti1@unimc.it (E. Buratti), giovanna.tassoni@unimc.it (G. Tassoni), mariano.cingolani@unimc.it (M. Cingolani), rino.froldi@unimc.it (R. Froldi), r.scendoni@unimc.it (R. Scendoni).

https://doi.org/10.1016/j.drugalcdep.2024.111128
Received 18 August 2023; Received in revised form 30 January 2024; Accepted 8 February 2024
Available online 14 February 2024

that can be highlighted through appropriate laboratory investigations. Carbohydrate-deficient transferrin (CDT) is one of the most popular indirect and specific alcohol biomarkers to detect chronic alcohol consumption, particularly in subjects attending the Local Medical Commission for the regranting of licenses (Bianchi et al., 2015).

Transferrin is a serum glycoprotein (β1-globulin) synthesized in the liver and in small amounts from the reticuloendothelial system and endocrine glands with a half-life of about 7–10 days (Gruppo di lavoro SIBioC et al., 2010). It consists of a polypeptide chain of 679 amino acids (Bortolotti et al., 2006), separated in two globular domains, N-terminal (aa 1–336) and C terminal (aa 337–679), each of which can bind one $Fe^{3+}$ ion. At the C-terminal domain two glucidic chains are bound at the nitrogen of asparagine 413 and 611 (Golka and Wiese, 2004). The heterogeneity of transferrin is due to the glycanic chains, consisting of N-acetylglycosamine, mannose and galactose; each chain can have two, three or four antennas, each of which ends with a molecule of sialic acid. Thus, there can be transferrins with residues of sialic acid from 0 to 8 (Arndt, 2001). Deficient transferrin carbohydrate glycophores are collectively referred to as CDT. Following the intake of high amounts of alcohol, there is a reduction in the glycosylation of transferrin, producing an increase in CDT.

In recent years, traditional biomarkers have been joined by determination in both conventional (blood, urine) and alternative (meconium, hair) matrices of minor products of the non-oxidative metabolism of alcohol, such as Ethyl glucuronide (EtG). EtG is a stable, non-volatile, polar molecule (Vezzoli et al., 2015), formed by the conjugation of ethanol with glucuronic acid by the mediation of UDP-glucuronyl transferase (UGT) (Foti and Fisher, 2005) and therefore represents a direct non-oxidative marker of ethanol. For this reason it is considered highly specific for the evaluation of alcohol misuse (Helander, 2003). The Society of Hair Testing (SoHT) issued in 2019 a document of consent for the use of alcohol misuse markers by proposing, for 3–6 cm long hair samples in the proximal segment, the cut-off of 5 pg/mg of Ethyl glucuronide to discriminate repeated alcohol consumption with respect to abstinence or occasional consumption, and a cut-off of 30 pg/mg of Ethyl glucuronide to identify chronic excessive consumption of alcohol (General Assembly of Society of Hair Testing (SoHT), 2019).

The purpose of this study is to compare CDT on blood and EtG in hair to assess which is more reliable and accurate for the diagnosis of alcohol misuse.

## 2. Materials and methods

### 2.1. Subjects and samples

The participants in this study were subjects applying for the regranting of driver's licenses following suspension or revocation due to driving under the influence of alcohol. We performed this comparative study covering the period from October 2018 to January 2023. From each subject, a sample of blood and a sample of hair were taken. Blood samples were collected from peripheral veins, typically the antecubital veins, for CDT analysis. Hair samples for EtG analysis were collected by cutting with scissors as close as possible to the scalp from the posterior vertex. The 3 cm proximal hair segment, representing approximately 3 months of growth, was used for EtG determination. The analyses of Ethyl glucuronide were performed at the laboratory For.Med.Lab. The blood samples for the analysis of the CDT were collected and analyzed at the Hospital of Ascoli Piceno with the technique of capillary electrophoresis. CDT amounts were considered indicative of a high risk of excessive alcohol consumption (>60 g/die) when above the reference laboratory value of 2.0%. All data were coded in order to ensure anonymity.

### 2.2. Standards and reagents

Ethyl glucuronide and its internal standard, Ethyl glucuronide-d5 (EtG-d5), were acquired from Sigma Aldrich (Italy). Methanol for analysis, water for analysis, methanol for UHPLC, ultrapure water for UHPLC, acetonitrile for UHPLC and formic acid were obtained from Carlo Erba (Italy). All reagents were of analytical grade and stored according to the manufacturer's instructions.

### 2.3. Sample preparation and extraction

The analysis of EtG on hair matrix (hEtG) was carried out according to the method shown below, which has been internally validated by the Diagnostic Italian Center of Milan (CDI), following recommendations by the Society of Hair Testing (General Assembly of Society of Hair Testing (SoHT), 2019). Hair was first cut into small fragments of about 1–2 mm, resembling a powder, until 100 mg of hair sample was obtained. Then they were washed once with water and then with methanol. After removing all the methanol and drying the hair in an oven at 45 ºC for three minutes, the samples were extracted by the simultaneous addition of 600 µl of ultrapure water for LC-MS, 80 µl of ultrapure methanol for LC-MS and 20 µl of internal standard (EtG-d5, 1 ng/ µl). Samples were incubated at room temperature overnight, subjected to ultrasonic bath for one hour and then centrifuged at 13.000 rpm for 10 minutes. After centrifugation, 500 µl of the supernatant was placed in a 1,5 ml vial and dried using the evaporator Savant SPD121P by Thermo Scientific. Finally, samples were resuspended with 50 µl of water with 0.1% (v/v) of formic acid for injection in the Thermo Scientific Dionex Ultimate 3000 chromatographic system (UHPLC) coupled with Thermo Exactive Plus Orbitrap (HR-MS).

### 2.4. Analysis by UHPLC-HRMS

The column used was a Luna Omega 3µm Polar C18 (50 ×2.1 mm) by Phenomenex, kept at 25 °C.

Column flow was set at 0.4 ml/min of water with 0.1% (v/v) formic acid (Phase A) and acetonitrile with 0,1% (v/v) formic acid (Phase B), using the following elution gradient: from 0 to 1 min 99% of phase A and 1% of phase B was passed, changing to 5% of phase A and 95% of phase B from 1 to 4 min and mantained for 2 min, then returned to 99% of phase A and 1% of phase B in 1 min and mantained for 3 min.

For EtG and EtG-d5 identification, the exact mass (EM) and the masses of the ions produced by electronic impact fragmentation (PI) with an acceptability range of 5 ppm, were used. The values recorded for the analytes were: 221.06668 (EM), 75.00877, 85.02950, 113.02442 (PI) for Ethyl glucuronide and 226.09805 (EM) for Ethyl glucuronide-d5.

The cut-off for Ethyl glucuronide positivity was set at 30 pg/mg, as indicated by the SoHT.

### 2.5. CDT analysis

CDT analyses were carried out at the laboratory of the Hospital of Ascoli Piceno by using the capillary electrophoresis instrument Capillarys Sebia 2 Flex Piercing and the kit ready-to-use Capillarys CDT (2) on fresh undiluted serum samples, following the instructions provided by the kit manufacturer. This system subjects the sample to a high voltage (8200 V) zone electrophoresis in alkaline buffer (pH 8.8) to separate the five major CDT isoforms. The transferrin glycoforms were quantified by UV absorption at 200 nm wavelength. After calibration of the analysis system, carried out through the appropriate calibrators, the direct detection automatically provides the percentage of calibrated disialotransferrin (CDT-IFCC) calculated from the total amount of transferrin detected. The CAPILLARYS CDT-IFCC method complies with the recommendations of the IFCC working group on the standardization of the CDT.

### 2.6. Statistical analysis

The data were analyzed using the IBM SPSS software (version 26.0,

IBM Corporation, Armonk, New York, USA). The Fisher's Exact test and the Student's t test were applied to observe the association or the difference in distribution of interest variables into CDT and EtG classification. A logistic regression was used to evaluate the relation between the variables of interest. Significance level of 5% was adopted for all tests.

## 3. Results

The present study involved a total of 336 subjects, 306 male and 30 female. The age average is 42 years, with a minimum of 20 years and a maximum of 75 years. Positive CDT (>2.0%) was observed for 5 individuals (1.49%), all male, and positive EtG (>30 pg/mg) for 55 individuals (16.37%), with 53 male and 2 female. Only 2 samples tested positive for both biomarkers (0.59%), while 3 samples resulted positive only for CDT (0.89%) and 53 only for EtG (15.77%), with 51 male and 2 female. The interesting data on which we focused more was the large number of positive samples of only Ethyl glucuronide. Of these samples, the average of the EtG value was 275.51 pg/mg and the median was 155 pg/mg. The minimum value found was 60 pg/mg, while the maximum was 1500 pg/mg. High EtG values were found, much higher than the cut-off of 30 pg/mg for discrimination of alcohol abusers. 53 EtG positive samples were not identified as alcohol consumers through CDT analysis; of these, 49 subjects had low CDT levels (<1.1%).

Considering EtG as the gold standard for alcohol abuse, a rate of 98.9% of true negatives and 1.1% of false positives was observed in classification from CDT compared with EtG. Additionally, a rate of 96.4% of false negatives and 3.6% of true positives was observed in classification from CDT compared with EtG. No associations were observed between sex and EtG classification, but an association between EtG classification and age was noted, since older individuals showed EtG positivity more frequently than the younger population (Table 1). The mean age of subjects who tested negative for EtG is 41.06, while the mean age in the group of alcohol consumers is 44.65.

## 4. Discussion

This study addresses the issue of comparing old and new markers of alcohol abuse, in particular CDT and EtG, in a real scenario consisting of 336 subjects who have applied for renewal or revision of their driving licence. The most interesting fact is that almost all the EtG positive samples (53/55) had a CDT value lower than the cut-off. Some factors that may influence CDT values have been found in literature, leading to a decrease in these values. It has recently been suggested that some drug therapies could reduce the CDT values (Vidali et al., 2014). It would also appear that high body mass index (BMI), high triglyceridemia and low levels of high-density lipoprotein cholesterol (HDL-C) are associated with a reduced sensitivity of CDT response to alcohol intake (Whitfield et al., 2008; Fagan et al., 2014). Finally, some genetic variants have been identified and they could interfere with the value of the disialous isoform, causing a decrease in values. In particular, the most common is variant C, with a prevalence >1%, followed by variants D and B. On the other hand, there have been also found some factors that interfere with CDT, leading to an increase of values. For example, CDT levels arise in subjects with end-stage severe liver disease or in patients with biliary cirrhosis, hepatitis B and C and hepatocarcinoma (Fleming et al., 2004). Despite this, some of these factors, such as genetic variants, have been identified, or they have a limited impact on CDT.

Another possible reason that explains the disagreement between the results obtained from the analysis of CDT and EtG can be found in their different kinetics. While the EtG, measured in hair samples of the length of 3 cm, refers to alcohol consumption during the last 3 months (being hair growth, on average, equal to one centimeter per month), CDT levels in the Caucasian population rise as a result of consumption of more than 60 g of alcohol per day for at least two weeks, with a half-life of about 7–10 days (Høiseth et al., 2009). In addition, if subjects are aware of the day the blood sample has to be collected, they may limit or stop alcohol consumption for a few weeks to normalize CDT values and, therefore, their classification as a chronic alcohol abuser may be in doubt. So, another advantage to take into account regarding the use of the hEtG as a marker of alcohol abuse, is that it is possible to evaluate long-term drinking pattern over several months. For this reason, EtG proves to be more reliable and useful, since it is more difficult for subjects to change their drinking habit for a long period to test negative to toxicological analysis. Moreover, thanks to the analysis of different hair segments it is possible to obtain information on the history of consumption. EtG is in fact stable up to 18 months in subjects who have not performed hair treatments (Crunelle et al.,2015). In addition, the analysis of EtG in hair matrix certainly has methodological and practical advantages, as the sampling is non-invasive and easy to perform, as well as its storage. Conversely, CDT has the advantage of having a lower analysis cost than EtG and it requires a less complex instrumentation.

By investigating whether certain parameters, such as age and sex, affected EtG concentration, a significant relationship was found between hEtG and age (p=0.041), with an increase in the amount of EtG with advancing age. This may be due to social factors, such as the fact that people tend to become a chronic user of alcohol as they get older, while binge drinking is more common in young people (Chung et al., 2018). Similar results emerged in other studies (Lendoiro et al., 2018). Cinquetti et al. (2023) found that older age was significantly linked with high EtG concentrations. In contrast, other works in literature have found different data than ours. For example, Hastedt et al. (2013) found a correlation coefficient below than 0.5 between age and EtG, so the bias from these parameters was unlikely. Regarding the association between sex and EtG, we found no correlation between these two variables. The same results have emerged in a study by Crunelle et al. (2014), who have established that gender has no impact on EtG incorporation in hair. However, it must be considered that the samples analysed are characterized by a small female population compared to the males. Conversely, Gareri et al. (2014) observed significantly lower EtG levels in female samples, suggesting lower EtG production or incorporation into female hair.

CDT is definitely one of the most studied markers and has been widely used by clinicians for the diagnosis of chronic alcohol consumption (Peterson, 2004). Nevertheless, it has been shown that CDT has a high specificity, but a limited sensitivity (Andresen-Streichert et al., 2018). Acceptable sensitivity and specificity are achieved only when combined with the results obtained from other markers (Morini et al., 2011; Sillanaukee and Olsson, 2001). Conversely, hEtG is the ideal marker for the verification of alcohol consumption and it is highly recommended to when checking to see if a subject is suitable or not to drive (Cinquetti et al., 2023; Marques et al., 2014). The reasons are linked to the characteristics of high sensitivity and specificity and to the coverage in chronological terms that the analysis of EtG offers. According to Italian legislation, driving licenses must not be issued or confirmed to

**Table 1**
Distribution of interest variables according to ETG classification.

| Variables | | ETG | | P | OR (CI95%)*** |
|---|---|---|---|---|---|
| | | Negative | Positive | | |
| **SEX** | Female | 28 (10.0%) | 2 (3.6%) | 0.194* | 1.00 |
| | Male | 253 (90.0%) | 53 (96.4%) | | 2.93 (0.67 – 12.68) |
| **Age** | | 41.06 (±12.08) | 44.65 (±10.92) | **0.041**** | **1.02 (1.00 – 1.05)** |
| **CDT** | Negative | 278 (98.9%) | 53 (96.4%) | 0.190* | 1.00 |
| | Positive | 3 (1.1%) | 2 (3.6%) | | 3.49 (0.57 – 21.43) |

* Fisher's Exact test
** Student's t test.
*** Logistic regression. OR = Odds ratio. CI95% = Confidence Interval 95%. Significance level of 5%.

drivers who are addicted to alcohol or who cannot dissociate driving from drinking. Surely a wider time window allows a better detection of an excessive alcohol consumption.

As can be seen from the data obtained from this study, EtG is certainly appropriate to use, instead of the traditional markers of alcohol assumption, in the determination of new direct indicators of excessive alcohol consumption, certainly more reliable and with greater sensitivity. Among the subjects who participated in this study, in fact, 53 showed a negativity to CDT and a positivity to EtG. The use of CDT alone to monitor chronic alcohol consumption would lead, in these cases, to a wrongful confirmation, return or release of the driving licence for most subjects. On the other hand, EtG, being a direct marker of alcohol intake, is present on hair matrix only after an alcohol consumption and therefore it is more reliable, sensitive and specific. CDT analysis can be applied in case of subjects without keratin matrix or with a keratin matrix altered following treatments. In these cases, it is advisable to carry out several samplings spaced out by at least 15 days: this is because CDT, with its half-life of 7–10 days, gradually normalizes and then falls below the thresholds values after two weeks of abstinence. In this way, a sufficiently large time window is reached to assess fitness to drive. CMLs can therefore use CDT in these cases as an alternative test for the driving licence regranting. On the contrary, for hEtG it is sufficient to take a single 3 centimeters head-proximal hair segment that covers a time span of about three months, large enough to verify a real excessive consumption not suitable with the requirements for fitness to drive.

Results similar to those obtained in this research project have also emerged in a study by Pirro et al., in which the research of EtG on hair was compared with the evaluation of CDT in serum and it was found that CDT and EtG have a perfectly overlapping specificity, but the EtG has a sensitivity significantly higher with respect to CDT (Pirro et al., 2011). In addition, a study by Kharbouche et al. (2012) shows that EtG analyses on hair matrix have a diagnostic capability to detect excessive and chronic consumption of alcohol. Also, according to the Guidelines on the Assessment of Fitness for Driving in Subjects with use/misuse of containing alcohol beverages, the prescription of EtG on hair and CDT on blood is recommended, as the two associated markers increase the possibility of a correct diagnosis of alcohol consumption, by investigating two different metabolites on two different matrices (COMLAS, 2022). If possible, it would be preferable to analyse two different markers with the same diagnostic window, such as EtG and Ethyl palmitate (EtPa) as suggested by Pragst et al. (2010).

## 5. Conclusions

The issue of excessive alcohol intake is receiving increasing attention in the scientific, social and regulatory fields. With regards to the diagnosis of alcohol dependence for the procedure of regranting licenses, the present study which compares CDT on blood and EtG on hair, reiterates the fundamental role of hEtG as a marker of excessive alcohol assumption. The traditional indicators, such as CDT, that is characterized by a low sensitivity, are not ideal to evaluate the driving ability. The introduction in the laboratory practice of new markers, like EtG, is a valuable tool for the diagnosis and detection of alcohol misuse conditions. EtG is proving to be very useful for the diagnosis of alcoholism, being a direct metabolite of ethanol and showing greater reliability than traditional markers, which can be influenced by other factors. Further studies to evaluate the accuracy of other markers, such as Phosphatidylethanol, in comparison to EtG are still needed.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Author contributions

All authors have approved the final article.

## CRediT authorship contribution statement

**Mietti Gianmario:** Writing – original draft. **Buratti Erika:** Data curation. **Cerioni Alice:** Formal analysis, Conceptualization. **Cingolani Mariano:** Methodology. **Tassoni Giovanna:** Data curation. **Cippitelli Marta:** Writing – review & editing, Resources. **Arrais Ribeiro Isabella Lima:** Formal analysis. **Scendoni Roberto:** Project administration. **Froldi Rino:** Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

## Acknowledgments

We would like to thank the Medical Committee for the Licenses of Ascoli Piceno and in particular Dr. Pietro Alessandrini and Dr. Francesco Brandimarti for providing us with patient serum CDT data.

## References

Andresen-Streichert, H., Müller, A., Glahn, A., Skopp, G., Sterneck, M., 2018. Alcohol biomarkers in clinical and forensic contexts. Dtsch. Arzteblatt Int. 115 (18), 309–315. https://doi.org/10.3238/arztebl.2018.0309.

Arndt, T., 2001. Carbohydrate-deficient transferrin as a marker of chronic alcohol abuse: a critical review of preanalysis, analysis, and interpretation. Clin. Chem. 47 (1), 13–27.

Automobile Club d'Italia (ACI), Istituto Nazionale di statistica (ISTAT), 2022. Report Incidenti Stradali 2021.

Bianchi, V., Premaschi, S., Raspagni, A., Secco, S., Vidali, M., 2015. A comparison between serum carbohydrate-deficient transferrin and hair ethyl glucuronide in detecting chronic alcohol consumption in routine. Alcohol Alcohol. 50 (3), 266–270. https://doi.org/10.1093/alcalc/agv005.

Bortolotti, F., De Paoli, G., Tagliaro, F., 2006. Carbohydrate-deficient transferrin (CDT) as a marker of alcohol abuse: a critical review of the literature 2001-2005. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 841 (1-2), 96–109. https://doi.org/10.1016/j.jchromb.2006.05.005.

Chung, T., Creswell, K.G., Bachrach, R., Clark, D.B., Martin, C.S., 2018. Adolescent binge drinking. Alcohol Res. Curr. Rev. 39 (1), 5–15.

Cinquetti, A., Terranova, C., Aprile, A., Favretto, D., 2023. Driving license regranting: hair EtG, serum CDT, and the role of sociodemographic and medicolegal variables. Drug Test. Anal. *15* (9), 953–961. https://doi.org/10.1002/dta.3426COMLAS. Linee di Indirizzo sulla Valutazione dell'Idoneità alla Guida in Soggetti con uso/abuso di bevande alcolcihe. 2022.

Crunelle, C.L., Cappelle, D., Covaci, A., van Nuijs, A.L., Maudens, K.E., Sabbe, B., Dom, G., Michielsen, P., Yegles, M., Neels, H., 2014. Hair ethyl glucuronide as a biomarker of alcohol consumption in alcohol-dependent patients: role of gender differences. Drug Alcohol Depend. 141, 163–166. https://doi.org/10.1016/j.drugalcdep.2014.05.014.

Crunelle, C.L., Yegles, M., De Doncker, M., Dom, G., Cappelle, D., Maudens, K.E., van Nuijs, A.L., Covaci, A., Neels, H., 2015. Influence of repeated permanent coloring and bleaching on ethyl glucuronide concentrations in hair from alcohol-dependent patients. Forensic Sci. Int. 247, 18–22. https://doi.org/10.1016/j.forsciint.2014.11.023.

Fagan, K.J., Irvine, K.M., McWhinney, B.C., Fletcher, L.M., Horsfall, L.U., Johnson, L., O'Rourke, P., Martin, J., Scott, I., Pretorius, C.J., Ungerer, J.P., Powell, E.E., 2014. Diagnostic sensitivity of carbohydrate deficient transferrin in heavy drinkers. BMC Gastroenterol. 14, 97. https://doi.org/10.1186/1471-230X-14-97.

Fleming, M.F., Anton, R.F., Spies, C.D., 2004. A review of genetic, biological, pharmacological, and clinical factors that affect carbohydrate-deficient transferrin levels. Alcohol. Clin. Exp. Res. 28 (9), 1347–1355. https://doi.org/10.1097/01.alc.0000139815.89794.be.

Foti, R.S., Fisher, M.B., 2005. Assessment of UDP-glucuronosyltransferase catalyzed formation of ethyl glucuronide in human liver microsomes and recombinant UGTs.

Forensic Sci. Int. 153 (2-3), 109–116. https://doi.org/10.1016/j.forsciint.2004.12.003.
Gareri, J., Rao, C., Koren, G., 2014. Examination of sex differences in fatty acid ethyl ester and ethyl glucuronide hair analysis. Drug Test. Anal. 6 (Suppl 1), 30–36. https://doi.org/10.1002/dta.1653.
General Assembly of Society of Hair Testing (SoHT), 2019. Consensus for the Use of Alcohol Markers in Hair for Supporting the Assessment of Abstinence and Chronic Alcohol Consumption.
Golka, K., Wiese, A., 2004. Carbohydrate-deficient transferrin (CDT)–a biomarker for long-term alcohol consumption. J. Toxicol. Environ. Health Part B Crit. Rev. 7 (4), 319–337. https://doi.org/10.1080/10937400490432400.
Gruppo di lavoro SIBioC, Bianchi, V., Pacifici, R., Palmi, I., Pichini, S., Vernocchi, A., Merlini, G., Ceriotti, F., Plebani, M., Gruppo di lavoro GFTI-SIMLA, Tagliaro, F., Bernini, M., Bortolotti, F., Caligara, M., Cassandro, P., De Giovanni, N., Snenghi, R., Bertol, E., 2010. Transferrina carboidrato-carente (CDT): strategie analitiche ed interpretative - Documento di consenso delle Società Scientifiche SIBioC e Gruppo Tossicologi Forensi Italiani afferente alla Società Italiana di Medicina Legale e delle Assicurazioni (GTFI-SIMLA), 34, pp. 128–138..
Hastedt, M., Büchner, M., Rothe, M., Gapert, R., Herre, S., Krumbiegel, F., Tsokos, M., Kienast, T., Heinz, A., Hartwig, S., 2013. Detecting alcohol abuse: traditional blood alcohol markers compared to ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) measurement in hair. Forensic Sci. Med. Pathol. 9 (4), 471–477. https://doi.org/10.1007/s12024-013-9416-8.
Helander, A., 2003. Biological markers in alcoholism. J. Neural Transm. (66), 15–32. https://doi.org/10.1007/978-3-7091-0541-2_2 (Supplementum.
Høiseth, G., Morini, L., Polettini, A., Christophersen, A., Mørland, J., 2009. Ethyl glucuronide in hair compared with traditional alcohol biomarkers–a pilot study of heavy drinkers referred to an alcohol detoxification unit. Alcohol. Clin. Exp. Res. 33 (5), 812–816. https://doi.org/10.1111/j.1530-0277.2009.00900.x.
Kharbouche, H., Faouzi, M., Sanchez, N., Daeppen, J.B., Augsburger, M., Mangin, P., Staub, C., Sporkert, F., 2012. Diagnostic performance of ethyl glucuronide in hair for the investigation of alcohol drinking behavior: a comparison with traditional biomarkers. Int. J. Leg. Med. 126 (2), 243–250. https://doi.org/10.1007/s00414-011-0619-9.
Lendoiro, E., de Castro, A., Jiménez-Morigosa, C., Gomez-Fraguela, X.A., López-Rivadulla, M., Cruz, A., 2018. Usefulness of hair analysis and psychological tests for identification of alcohol and drugs of abuse consumption in driving license regranting. Forensic Sci. Int. 286, 239–244. https://doi.org/10.1016/j.forsciint.2018.03.023.
Marques, P.R., Tippetts, A.S., Yegles, M., 2014. Ethylglucuronide in hair is a top predictor of impaired driving recidivism, alcohol dependence, and a key marker of the highest BAC interlock tests. Traffic Inj. Prev. 15 (4), 361–369. https://doi.org/10.1080/15389588.2013.824569.
Morini, L., Varango, C., Filippi, C., Rusca, C., Danesino, P., Cheli, F., Fusini, M., Iannello, G., Groppi, A., 2011. Chronic excessive alcohol consumption diagnosis: comparison between traditional biomarkers and ethyl glucuronide in hair, a study on a real population. Ther. Drug Monit. 33 (5), 654–657. https://doi.org/10.1097/FTD.0b013e318232950f.
Oppolzer, D., Barroso, M., Passarinha, L., Gallardo, E., 2017. Determination of ethyl glucuronide and fatty acid ethyl esters in hair samples, 10.1002/bmc.3858 Biomed. Chromatogr. 31 (4). https://doi.org/10.1002/bmc.3858.
Peterson, K., 2004. Biomarkers for alcohol use and abuse–a summary. Alcohol. Res. Health. J. Natl. Inst. Alcohol Abus. Alcohol. 28 (1), 30–37.
Pirro, V., Valente, V., Oliveri, P., De Bernardis, A., Salomone, A., Vincenti, M., 2011. Chemometric evaluation of nine alcohol biomarkers in a large population of clinically-classified subjects: pre-eminence of ethyl glucuronide concentration in hair for confirmatory classification. Anal. Bioanal. Chem. 401 (7), 2153–2164. https://doi.org/10.1007/s00216-011-5314-7.
Pragst, F., Rothe, M., Moench, B., Hastedt, M., Herre, S., Simmert, D., 2010. Combined use of fatty acid ethyl esters and ethyl glucuronide in hair for diagnosis of alcohol abuse: interpretation and advantages. Forensic Sci. Int. 196 (1-3), 101–110. https://doi.org/10.1016/j.forsciint.2009.12.028.
Sillanaukee, P., Olsson, U., 2001. Improved diagnostic classification of alcohol abusers by combining carbohydrate-deficient transferrin and gamma-glutamyltransferase. Clin. Chem. 47 (4), 681–685.
Snenghi, R., Forza, G., Favretto, D., Sartore, D., Rodinis, S., Terranova, C., Nalesso, A., Montisci, M., Ferrara, S.D., 2015. Underlying substance abuse problems in drunk drivers. Traffic Inj. Prev. 16 (5), 435–439. https://doi.org/10.1080/15389588.2014.968656.
Società scientifica COMLAS, Ministero della Salute, SIBIOC, SIML, PUBLIEDIT, 2022. Linee di indirizzo per la valutazione dell'idoneità alla guida in soggetti con uso/abuso di bevande alcoliche.
Torrente, M.P., Freeman, W.M., Vrana, K.E., 2012. Protein biomarkers of alcohol abuse. Expert Rev. Proteom. 9 (4), 425–436. https://doi.org/10.1586/epr.12.38.
Vezzoli, S., Bernini, M., De Ferrari, F., 2015. Ethyl glucuronide in vitreous humor and blood postmortem specimens: analysis by liquid chromatography-electrospray tandem mass spectrometry and interpreting results of neo-formation of ethanol. Ann. Dell'Ist. Super. Sanita 51 (1), 19–27. https://doi.org/10.4415/ANN_15_01_05.
Vidali, M., Bianchi, V., Bagnati, M., Atzeni, N., Bianchi, A.M., Bellomo, G., 2014. False negativity to carbohydrate-deficient transferrin and drugs: a clinical case. Biochem. Med. 24 (1), 175–179. https://doi.org/10.11613/BM.2014.020.
Whitfield, J.B., Dy, V., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W., 2008. Measuring carbohydrate-deficient transferrin by direct immunoassay: factors affecting diagnostic sensitivity for excessive alcohol intake. Clin. Chem. 54 (7), 1158–1165. https://doi.org/10.1373/clinchem.2007.101733.
World Health Organization (WHO), 2018. Global Status Report on Alcohol and Health.