Quantification of **hair EtG**, **urine EtG/EtS** and **C-DBS PEths** to assess the alcohol consumption in driver's licence regranting cases

Kummer Natalie[1], Wille Sarah[1], Poll Anneleen[2], Lambert Willy[3], Samyn Nele[1], Stove Christophe[3]

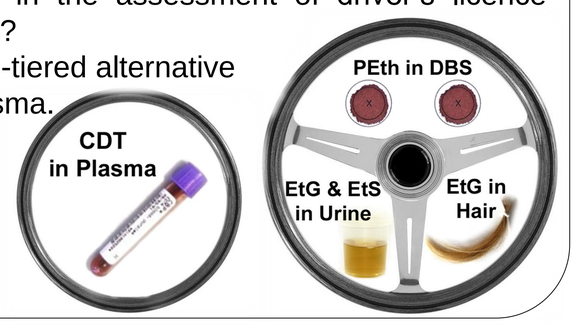
[1]National Institute of Criminalistics and Criminology, Brussels, Belgium, [2]Belgian Institute of Road Safety, Brussels, Belgium [3]Ghent University, Faculty of Pharmaceutical Sciences, Ghent, Belgium

Introduction

In Belgium, the analysis of indirect biomarkers (i.e. carbohydrate deficient transferrin (CDT%), GGT, AST, ALT and MCV)), is currently used to monitor the alcohol consumption in cases of fitness to drive assessment. The use of ethyl glucuronide (EtG) and ethyl sulfate (EtS) in **urine**, EtG in **hair**, and phosphatidylethanol species (PEths) in capillary dried blood spots (**C-DBSs**) to **detect excessive and chronic alcohol consumption and to monitor alcohol abstinence periods** was tested analysing samples from 50 volunteers for whom fitness to drive had to be assessed and for whom a blood analysis for indirect biomarkers was requested [1]. A **flowchart** to integrate the results of hair EtG, C-DBS PEths and urine EtG/EtS into the fitness to drive decision process was proposed (Figure 3).

Aim

Changing gears in the assessment of driver's licence regranting cases?
Evaluation of a 3-tiered alternative for CDT% in plasma.



Methods

EtG in pulverised hair (50 mg) was quantified by UPLC-ESI-MS/MS after a solid-phase extraction (BondElut SAX) [2]. UPLC-ESI-MS/MS was also used to quantify EtG and EtS in urine after protein precipitation with methanol [3]. PEths were quantified from 3 punches (3 mm) excised from C-DBS. A mixture of acetonitrile/buffer was used to extract the blood from the paper and n-hexane was used to extract PEths [4].

Detection of excessive and chronic alcohol consumption

17/50 volunteers had serum CDT% (<2.4%), hair EtG (<30 pg/mg) and C-DBS PEth 16:0/18:1 (<221 ng/mL) concentrations that **do not suggest excessive or chronic alcohol consumption**.

33/50 volunteers had at least one result (serum CDT%, hair EtG and/or C-DBS PEth 16:0/18:1) that **suggests excessive and chronic alcohol consumption** (Figure 1).

Hair EtG (24/50) and C-DBS PEths (29/50) are more sensitive to detect alcohol dependence than the currently used indirect biomarkers (13/50 for CDT%) and allow to disprove an abstinence period.

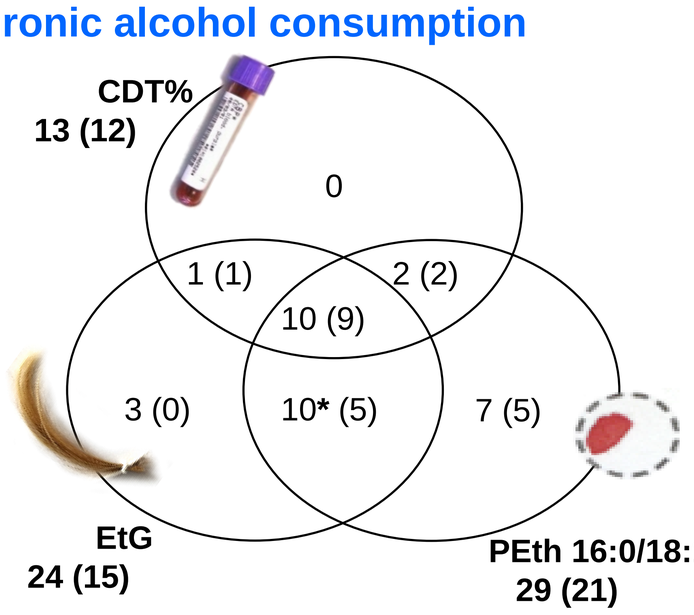


Figure 1 Numbers out of 50 volunteers with results above the cut-off values for CDT% (≥2.4%), EtG (≥30 pg/mg) and/or PEth 16:0/18:1 (≥221 ng/mL), suggesting alcohol misuse. The number of samples with EtG_{100} or EtS_{100} concentrations in urine above or equal to the LLOQ (≥100 ng/mL) is indicated between brackets.
* 9 CDT% values below the cut-off and one with a missing result.

Monitoring of alcohol abstinence period

Out of 13 cases which had been submitted to an abstinence period, **recent alcohol consumption** was suggested in 4 (EtG_{100}≥100 ng/mL) and 3 (EtS_{100}≥100 ng/mL) cases using urine results, while **strict abstinence was disproved** by C-DBS (PEth 16:0/18:1≥10 ng/mL) analysis in 7 cases and by hair (EtG≥7 pg/mg) analysis in 5 cases (3 volunteers did not provide a hair sample).

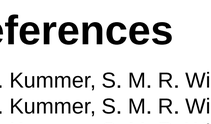
Hair EtG and C-DBSs PEths are more efficient to disprove an alcohol abstinence period than urinary EtG/EtS.

Inference processes

Different information can be obtained from the three methods and allows to infer about the evolution of the drinking pattern (as illustrated in Figure 2). The flowchart presented in Figure 3 allows to interpret the results of hair C-DBS and urine together.

In hair

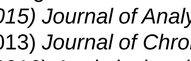
EtG = 9 pg/mg
Cut-off = 30 pg/mg



last 6 months
no alcohol misuse

In C-DBS

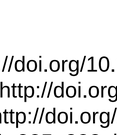
PEth 16:0/18:1 = 1056 ng/mL
Cut-off = 221 ng/mL
PEth 18:1/18:1 in C-DBS = 33 ng/mL
LLOQ = 10 ng/mL
PEth 16:0/16:0 in C- DBS= 86 ng/mL
LLOQ = 19 ng/mL



last month
alcohol misuse

In urine

EtG_{100} > 170678 ng/mL
EtS_{100} > 170678 ng/mL
LLOQ = 100 ng/mL



last days
recent alcohol intake

Increase of the alcohol consumption

Figure 2 Hypothetical drinking pattern inferred from the results of hair, C-DBS and urine samples analysis.

Figure 3 Flowchart showing the influence of the results of EtG in hair, PEth 16:0/18:1 in C-DBS and EtG_{100} and EtS_{100} in urine on the fitness to drive decision.

Conclusion

The non- or minimally invasive sampling strategies tested in this study (hair, urine and C-DBS) allow sampling to be performed directly during the fitness to drive assessment by regular staff members. Each of the strategies applied here provides a different level of information and can be used separately or combined. In conjunction with the physical/psychological assessments, the approach proposed here allows to obtain a more detailed view on (the evolution of) the alcohol consumption of a subject. This results in a better judgment about the fitness to drive and hence has the potential to improve the driver's licence regranting process.

References

[1] N. Kummer, S. M. R. Wille, A. Poll, *et al.* (2016) *Drug and Alcohol Dependence*, *165*, 191–197. http://doi.org/10.1016/j.drugalcdep.2016.06.012
[2] N. Kummer, S. M. R. Wille, V. Di Fazio, *et al. (2015) Journal of Analytical Toxicology*, *39*(1), 17–23. http://doi.org/10.1093/jat/bku108
[3] N. Kummer, S. M. R. Wille, V. Di Fazio, *et al.* (2013) *Journal of Chromatography B*, *929*, 149–154. http://doi.org/10.1016/j.jchromb.2013.04.011
[4] N. Kummer, A. -S. Ingels, S. M. R. Wille, *et al.* (2016) *Analytical and Bioanalytical Chemistry*, *408*(3), 825–838. http://doi.org/10.1007/s00216-015-9169-1

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