FACULTY OF PHARMACEUTICAL SCIENCES

THE DRIVER'S LICENSE REGRANTING PROCESS-GETTING INSIGHT BASED ON 2 YEARS OF EXPERIENCE

Emma Cardon

A Master dissertation for the study programme Master in Drug Development

Academic year: 2021 - 2022

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SUMMARY

If a person violates a drug and/or alcohol law in traffic, one of the possible punishments is a forfeiture of the right to drive. To get the driver's license back, one must undergo a so-called reintegration exam in which a person's fitness to drive is evaluated. In order to do so, alcohol and drug biomarkers are used to objectively assess someone's substance abuse.

Phosphatidylethanol (PEth) is one of the newest direct alcohol biomarkers. It is a group of phospholipids exclusively formed in red blood cell membranes in the presence of ethanol. Because of the strong correlation between PEth levels in blood and alcohol consumption, it is a useful alcohol biomarker to assess abstinence, moderate, significant and heavy alcohol use.

Still under debate is the value for the PEth cut-off or decision limit to conclude whether someone has been abstinent the past few weeks (or only consumed a minor amount of alcohol). Although there is a consensus among the United States laboratories to use a decision limit of 20 ng/mL, it is still an arbitrary threshold. It was the aim of this thesis to underpin this arbitrary limit with scientific data.

It could be determined that using 20 ng/mL as a decision limit to score abstinence or minor alcohol intake has a high specificity. This was derived from patient data so all possible sources of variation are included, also the measurement uncertainty. As the conclusion on whether or not someone has been abstinent can be life-changing, this finding is significant and could be the first step towards harmonization.

Based on case files of reintegration exams, we managed to get more insight into the reintegration exam and its participants. Most of the candidates were male (87%) with an average age of 39 years. The main part of the participants (89%) was declared unfit to drive. Subjects are likely to underestimate their alcohol use during the reintegration exam. Subjects with a high blood alcohol concentration on the occasion of the offense do not automatically have high PEth and hEtG levels at the reintegration exam. Overall, subjects with a higher hEtG level tend to have a higher PEth level although this is not always the case. An immunoassay rapid drug test can be useful to quickly detect drug use but results should be considered presumptive until confirmed, nevertheless it is still recommended to perform one.

SAMENVATTING

Bij het overtreden van een alcohol- of drugswet in het verkeer is een van de mogelijke straffen het verval van het recht tot sturen. Om het rijbewijs terug te krijgen dient men te slagen voor een zogenaamd ‘herstelonderzoek’, waarin geëvalueerd wordt of een persoon rijgeschikt is. Om dit te doen worden alcohol- en/of drugsmerkers gebruikt om op objectieve wijze middelenmisbruik te beoordelen.

Phosphatidylethanol (PEth) is een van de nieuwste alcoholmerkers. Het is een groep van fosfolipiden exclusief gevormd in aanwezigheid van ethanol. Door de sterke correlatie tussen de PEth concentratie in het bloed en de mate van alcoholconsumptie is het mogelijk onderscheid te maken tussen geheelonthouding, gematigd, significant en hevig alcoholgebruik. Nog steeds onder discussie is de waarde voor de PEth beslissingslimiet om te besluiten dat iemand de laatste weken geen (of een kleine hoeveelheid) alcohol heeft geconsumeerd. Ondanks de overeenkomst tussen laboratoria in de Verenigde Staten om een beslissingslimiet van 20 ng/mL te gebruiken, blijft dit een arbitraire waarde. Het doel van deze thesis was om deze arbitraire limiet te onderbouwen met wetenschappelijke data.

Er kon worden bepaald dat het gebruik van een beslissingslimiet van 20 ng/mL een hoge specificiteit heeft. Doordat dit werd bepaald via data van echte patiënten, omvat dit alle mogelijke vormen van variatie, ook de meetonzekerheid. Omdat de conclusie van of iemand wel of niet abstinent bleef van levensbelang kan zijn, is deze bevinding belangrijk en kan het een eerste stap betekenen naar harmonisatie toe.

Met behulp van de dossiers van herstelonderzoeken konden we meer inzicht krijgen over het herstelonderzoek en de deelnemers ervan. Het grootste deel van de kandidaten was mannelijk (87%), had een gemiddelde leeftijd van 39 jaar en werd als niet rijgeschikt verklaard (89%). Het blijkt dat deelnemers de neiging hebben hun alcoholgebruik te onderschatten. Het blijkt niet zo te zijn dat mensen met een hoge alcoholconcentratie in het bloed automatisch ook een hoge PEth concentratie hebben, gemeten bij het herstelonderzoek. Over het algemeen blijken deelnemers met een hoge hEtG concentratie ook een hoge PEth concentratie te hebben, al is dit niet altijd zo. De resultaten drugs sneltesten moeten altijd worden bevestigd in de context van rijvaardigheidsonderzoeken, maar gebruik ervan wordt wel aangeraden.

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LIST OF USED ABBREVATIONS

ALD: Alcoholic liver disease

ALT: Alanine transaminase

AST: Aspartate transaminase

AUD: Alcohol Use Disorder

AUDIT: Alcohol Use Disorders Identification Test

AUDIT-C: Alcohol Use Disorders Identification Test-Consumption

BAC: Blood Alcohol Concentration

BIVV: Belgische Instituut voor de Verkeersveiligheid

CDT: Carbohydrate-deficient transferrin

CI: Confidence interval

DAST-10: Drug Abuse Screening Test

DBS: Dried Blood Spots

EMCDDA: European Monitoring Centre for Drugs Addiction

ESI: Electrospray ionization

ETG: Ethyl glucuronide

ETS: Ethyl sulfate

FAEEs: fatty acid ethyl esters

GC-MS: Gas Chromatography-Mass Spectrometry

GGT: γ-glutamyl transpeptidase

HCT: Hematocrit

HETG: Ethyl glucuronide in hair

HPLC: High-performance liquid chromatography

LC-MS: Liquid chromatography mass spectrometry

LC-MS/MS: Liquid chromatography tandem mass spectrometry

LC-QTOF-MS: Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry

LOD: Limit of Detection

LOQ: Limit of Quantification

MAST: Michigan Alcoholism Screening Test

MCV: Mean corpuscular volume

MDE: 3,4-Methylenedioxy-N-ethylamphetamine

MDA: Methyleendioxyamfetamine

MDMA: 3,4-Methylenedioxymethamphetamine

NPV: Negative predictive value

PETH: Phosphatidylethanol

PPV: Positive predictive value

THC: Tetrahydrocannabinol

USDTL: United States Drug Test Lab

VAMS: Volumetric absorptive microsampling

WHO: World Health Organization

1. INTRODUCTION

1.1. ALCOHOL AND DRUGS IN TRAFFIC

In the Western society, ethanol is the most consumed drug. (1) The use of alcohol as a drug does not necessarily need to be problematic, a distinction can be made between low-risk drinking, risky or problem drinking and alcohol use disorder (AUD). (2) AUD mainly affects men and is world-wide one of the most prevalent mental disorder. People with this disorder have less control over their alcohol use resulting in chronic and heavy alcohol consumption. (3) Unhealthy alcohol use leads to an increased risk for cardiovascular disease, liver disease, cancer and other complications. According to the World Health Organization (WHO) in 2015, alcohol abuse is the cause of 6% of worldwide deaths. (1–6)

Alcohol plays a significant role in driving impairment and car accidents. The use of alcohol in traffic can lead to a reduced response time, poor concentration, lack of motor coordination, reduced recognition of own physical and psychological state, decreased visual perceptual skills and sleepiness. (7)

In Belgium, driving with a Blood Alcohol Concentration (BAC) of 0,5 g/L or higher is considered a criminal offense. The affected person can get a fine and their driving license will be revoked for a minimum of 3 hours. For professional drivers the BAC limit is 0,2 g/L, in practice, this means a zero tolerance for drinking alcohol in traffic. From 0,8 g/L, the fine can be a lot larger and the driving license will be taken away for at least 6 hours. (8)

According to the European Monitoring Centre for Drugs Addiction (EMCDDA) in the European Drug report of 2021, cannabis is the most used drug in Europe. This is followed by respectively cocaine, MDMA, amphetamines and opioids. Driving under the influence of drugs increases the risk of car crashes because of diminished concentration, risky behavior and altered information processing. (9,10) Typically, somebody who intensively uses drugs is less capable to recognize their level of intoxication and is more likely to drive under influence than someone who uses drugs moderately. (10)

For cannabis, the relative risk of getting seriously injured or killed is slightly increased. (10) Although the mean speed of driving would reduce, cannabis can lead to sleepiness, decreased concentration and decisiveness and increased reaction time.

(11) Furthermore, a high concentration of tetrahydrocannabinol (THC) is associated with hallucination and panic attacks. (11–13) A medium increased risk occurs after cocaine use, especially in sleep-deprived persons. Benzodiazepines can lead to sedation and light-headedness resulting in a medium increased risk. Driving under the influence of opioids gives a similar risk. (10) When taking amphetamines, a reduction of sleepiness and a feeling of euphoria might result in a lack of coordination, over-confidence in driving ability and aggressiveness. (10,14) After some time (hours to days) there is a crash phase, with extreme exhaustion and depression. This altogether makes amphetamines very dangerous to use in traffic with a highly increased risk of getting injured or killed. The combination of drugs and alcohol gives the most extreme increased risk. (10)

It is forbidden to drive under the influence of illegal drugs. Since 2010, police can do a saliva test that detects THC, amphetamine, MDMA, morphine or 6-acetylmorphine and cocaine or benzoylecgonine. The saliva test can be applied when the person shows 3 or more characteristics of recent drug use. If the test is positive, the person gets a driving ban for 12 hours and a lab will do a saliva or blood analysis. Whether or not a person gets a punishment depends on the result of this lab test. The driving license will be given back if a new saliva test is negative 12 hours after the first one was taken. According to annex 6 of the Royal Decree of 23 March 1998 on driving licenses, a person addicted to psychoactive substances that affect driving abilities or anyone that cannot stay abstinent from them is declared unfit to drive. For their driving license regranting they need a proven abstinence over 6 months. (15)

When a person violates one of the alcohol and/or drug laws, he or she can be punished by a magistrate. Examples of this punishment could be a forfeiture of the right to drive, a fine or a prison sentence. The duration of this forfeiture of the right to drive can range from a few days to months to a lifetime. To get the driver's license back, one must undergo a so-called reintegration exam. (8)

In this exam, the person’s fitness to drive is evaluated and consists of at least 2 parts: a psychological and medical examination. For the psychological part, an interview should lead to a clear picture of someone’s behavioral problems, personality disorders and psychiatric problems that would impede driving under sober circumstances. Next to that, the amount of and the reasoning behind drug or alcohol

use will be evaluated. Any indication of recidivism, including previous convictions, is taken into consideration.(8)

For the medical part of the examination, a doctor will make a new estimation of the substance (ab)use, possibly supplemented with blood, urine and/or hair analyses. In addition, a medical examination is carried out together with thorough medical history research. The doctor can declare the person unfit to drive and if so, he or she must undergo a new reintegration exam to regain their driver's license. (8)

To evaluate someone's alcohol and illegal drug use, a non-invasive way to do so is via questionnaires. The Alcohol Use Disorders Identification Test (AUDIT) is a screening tool with 10 questions to evaluate someone's alcohol use based on self-report. The first three questions are now put in an abbreviated version called the AUDIT-consumption (AUDIT-c). (16,17) Similar self-report questionnaires are available e.g. the CAGE questionnaire and the Michigan Alcoholism Screening Test (MAST). (18)To detect drug use, the Drug Abuse Screening Test (DAST-10) is a tool with 10 questions. The substance use is rated through a scoring system whereas a score of three points or higher is evocative of substance use disorder. (19) These tests are widely used but may lead to an underestimate due to a reporting and/or recall bias. For this reason, there is a need for the use of biomarkers. (16–19)

1.2. ALCOHOL BIOMARKERS

Alcohol biomarkers supply objective measures for alcohol (ab)use which can be essential in clinical or forensic contexts. (20) Examples of where this can play a role include the screening for (relapsed) alcohol disorders, liver transplant eligibility, driver's license regranting process and post-mortem examination. (4) Alcohol biomarkers can be measured in body fluids or keratinous tissue. The analysis of body fluids e.g. blood and urine provides information about recent or current exposure, while keratinous tissue e.g. hair gives insight into long-term alcohol (ab)use. (4,21–25)

Indirect alcohol biomarkers allow to make an estimate about the alcohol consumption as they measure changes in the body due to alcohol. These include carbohydrate-deficient transferrin (CDT), alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT) and mean corpuscular volume (MCV). Direct biomarkers on the opposite measure ethanol itself or its metabolites. (26)Measuring ethanol in blood or breath is a highly specific tool to determine acute

alcohol intoxication. Disadvantageous, the window of detection is short and unpredictable, resulting in poor sensitivity. (4,23,26)

1.2.1 Indirect alcohol biomarkers

Carbohydrate-deficient transferrin (CDT%)

Transferrin is a glycoprotein responsible for iron transport. The most abundant isoform of transferrin is tetrasialotransferrin, which contains four terminal sialic acids. Carbohydrate deficient transferrin (CDT%) is a collective name for isoforms of transferrin with a deficiency in one or more terminal sialic acids. Although the mechanism is still unclear, heavy alcohol use (40-60 g/day for weeks to months) leads to an increase in CDT. Levels of CDT can also increase due to liver disease and pregnancy. To identify excessive drinking, CDT% has the highest sensitivity and specificity of all indirect biomarkers. (6)

Liver enzymes (GGT, AST, ALT)

As the metabolism of alcohol predominantly takes place in the liver, it is the first tissue to get affected by the toxic effects of ethanol. Moderate to heavy drinking (70 g/week for men, 60 g/week for women) leads to an increase in liver enzymes. (6)

Gamma-glutamyl transferase (GGT) is a microsomal enzyme, as the name suggests it assists the transfer of glutamyl to amino acids and peptides. Although heavy alcohol use may increase GGT concentrations, many other conditions do the same. Sensitivity and specificity for GGT as a biomarker for excessive alcohol use are strongly influenced by age, gender and comorbidities. This altogether makes GGT an ineffective biomarker to screen for prolonged excessive alcohol use. (6)

Aspartate transaminase (AST) and alanine aminotransferase (ALT) are enzymes indispensable in the Krebs cycle for the synthesis of certain amino acids. Liver damage, alcohol-induced or non-alcohol induced, leads to a serum increase of these enzymes. Taking the ratio of AST/ALT results in higher specificity and sensitivity than measuring the enzymes independently. Although a ratio >2 is a strong indicator of advanced alcoholic liver disease, it is not useful for detecting heavy alcohol use in absence of liver disease. Overall AST and ALT have poor sensitivity and specificity as an alcohol biomarker and should not be used on their own. (6)

Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) indicates the average volume of a person's red blood cells. Heavy, prolonged drinking (60 g/day for weeks to months) increases the MCV, people that drink heavy but irregular (60 g, 15-20 days/month) have normal MCV. Due to the slow turnover of red blood cells, it can take up months before a change in alcohol consumption habits is reflected in MCV. This indirect biomarker has poor sensitivity and specificity to determine alcohol use. (6)

Indirect biomarkers can be useful to screen for problematic alcohol use, but for abstinence monitoring they lack specificity and sensitivity. They are influenced by variate factors such as age, gender, diseases and other consumed substances. The use of direct biomarkers, preferably in combination, is more appropriate for abstinence monitoring as they have a higher sensitivity and specificity. (16,21–23)

1.2.2 direct alcohol biomarkers

After alcohol consumption, alcohol dehydrogenases rapidly metabolize about 90-98% of the ethanol in the liver. 2-8% will be excreted unchanged through the kidneys, lungs and sweat glands. Lastly, less than 1,5% undergoes non-oxidative metabolism to ethyl glucuronide (EtG), ethyl sulfate (EtS), phosphatidylethanol (PEth) and fatty acid ethyl esters (FAEEs). (4,6)

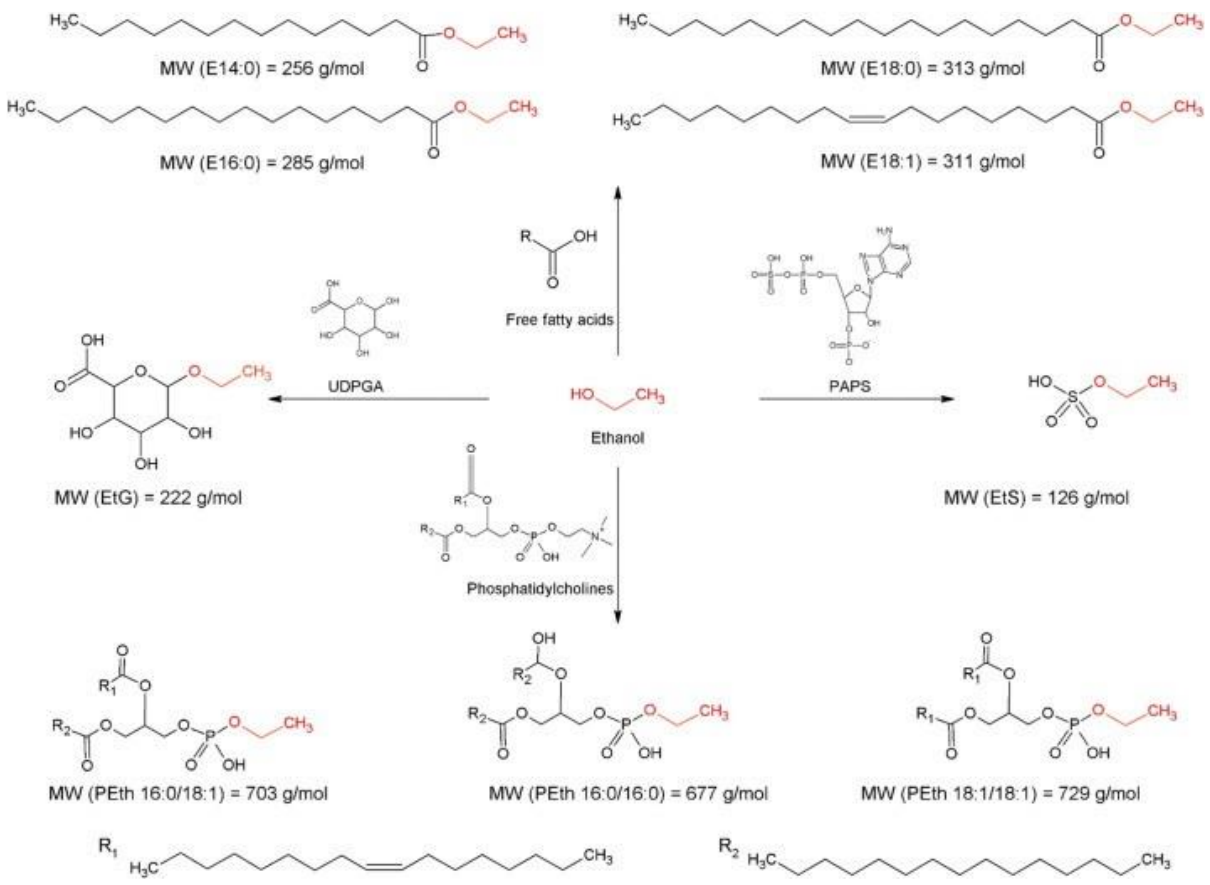

Figure 1.1: non-oxidative metabolism of ethanol (Kummer et al.,2016).(27)

Ethyl glucuronide (EtG)

Ethyl glucuronide is formed in the liver through enzymatic glucuronidation of ethanol. (4) EtG has a high sensitivity for ethanol and thus can differentiate between abstinence, social drinking and excessive drinking. (28) In urine, EtG can be detected 1 hour after consumption and stays detectable up to 48 hours, although for heavy alcohol consumption this could be up to 72 hours. (6)

The possibility of false positives should be taken into consideration when interpreting a result for EtG. A study done by Reisfield et al. measured EtG concentrations in urine up to four times the commonly used threshold of 500 ng/mL due to intensive use of ethanol-based hand sanitizer. (6,29) Microbes such as yeast in urine samples could form ethanol, which can lead to the post-sampling synthesis of EtG when further metabolized by bacteria. (6)

EtG can also be determined in hair which gives a long-term idea about alcohol consumption habits. The window of detection is depending on the length of hair given that hair grows with an average speed of 1,1 cm/month.(30) EtG in hair (HEtG) is stable even up to 12 cm hair strands with no or a minimal washout effect. (28) Analytical methods need to be very sensitive as only very small amounts of EtG (pg/mg) are incorporated in hair.(4) Over the years hEtG is routinely applied in many contexts like driving license regranting due to its higher specificity and sensitivity than some other alcohol markers. (28) False negatives appear due to bleaching or permanent hair dye while hair products with ethanol can lead to a false positive result. (26)

Ethyl sulfate (EtS)

Ethyl sulfate is formed through sulfate conjugation of ethanol. Just like EtG it is detectable in urine 1 hour after alcohol consumption and stays detectable for the same amount of time as EtG. Unintentional ethanol exposure could also lead to false-positive EtS results in urine. The sensitivity and specificity of EtS for recent alcohol use are comparable to EtG. (6) EtS in urine is sometimes measured to confirm EtG findings. (4)

Fatty acid ethyl esters (FAEE)

Fatty acid ethyl esters are the result of the esterification of fatty acids and ethanol. (31). HEtG and FAAEs in hair have a comparable window of detection but because of the low specificity of FAEEs in hair, it is currently only used in combination with hEtG. This could be profitable as FAEEs are less likely to cause false negatives after chemical hair treatment. (4)

Phosphatidylethanol (PEth)

One of the newest direct biomarkers for alcohol is blood phosphatidylethanol (PEth). A PEth molecule contains a glycerophospholipid central chain and two carboxylic acid side chains. The two side chains can vary which results in at least 48 PEth analogs. In more recent articles, PEth usually refers to the most abundant analog (36-46%) namely PEth 16:0/18:1. The percentage of the 16:0/18:1 analog depends on the levels of drinking, the ingested fat through food and other factors. (22,26) In what follows, “PEth” refers to PEth 16:0/18:1, unless otherwise specified.

PEth is exclusively formed in red blood cell membranes in the presence of ethanol and therefore a very specific marker for alcohol exposure. The enzyme phospholipase D catalyzes the reaction between ethanol and phosphatidylcholine with the formation of PEth as a result. (22,23) This process starts immediately when alcohol is consumed, even in low doses. (16) After the formation, it will degrade very slowly which results in a long half-life of approximately 7/8 days. (32,33) Consequently, PEth has a broad window of detection of several weeks. (23)

PEth can only be measured in whole blood as it is almost exclusively located at the surface of red blood cells. (33) One limitation of PEth measurement in whole blood is the possibility of in vitro formation post-sampling. If ethanol is present in the blood sample, this can result in falsely raised concentrations. One way to avoid this problem is by adding a phospholipase D inhibitor, e.g. $NaVO_3$, to the samples. (34)

The complexity and costs of the (pre-)analytical procedure for the determination of PEth were strongly reduced due to the possibility of using dried blood spots (DBS) after a fingerprick. (4,21,33,35) Advantages of DBS include an easy sampling process and the possibility to ship through the post as they are non-hazardous. In contrast to conventional venous blood samples where PEth is stable at a storage temperature of -80°C, DBS can be stored and shipped at room temperature. Sampling does not require an exact measurement as a fixed diameter sub-punch will be taken to analyze. (35,36)

A problem that occurs with the use of conventional DBS is a phenomenon called the hematocrit (Hct) bias. A drop of blood with a high hematocrit level also has a high viscosity, consequentially, it will spread less on the DBS paper. Because a fixed-size sub-punch is taken, the amount of blood and analyte will be higher in a sample with a high hematocrit level compared to a low hematocrit level. One way to overcome this problem is volumetric absorptive microsampling (VAMS). This technique allows sampling an accurate blood volume (~10 µL) with an absorptive tip. It has been proven that this blood volume is independent of Hct if the Hct level is between 0,20 and 0,70. The devices, e.g. the Mitra ® devices, are user-friendly as evaluated by users. (33,37)

A big strength of PEth is the strong correlation between PEth levels in blood and alcohol consumption. (25) One single alcohol consumption, leading to a blood alcohol

content of 1 g/L, was detectable for 12 days. (33) It has been proven useful for monitoring abstinence as well as moderate, significant and heavy alcohol use. (25) For chronic and excessive alcohol use, PEth has a sensitivity of 95% and specificity of 100%. Because of the long window of detection, PEth is still detectable in alcohol abusers weeks after withdrawal depending on their initial PEth value. (33)

Still under debate is the value for the PEth cut-off or decision limit to conclude whether or not someone has been abstinent the past few weeks. This conclusion can be life-changing, so high confidence in the decision limit is needed. One example is the role of assessing alcohol intake in organ transplantation. A common indication for liver transplantation is alcoholic liver disease (ALD), remaining abstinent after transplantation is important, especially in these patients. The survival rate is higher in ALD patients who achieve abstinence post-transplantation than in those continuing to consume alcohol. Through pre-transplant screening, PEth can be a useful tool to evaluate someone's drinking habits. Post-transplant screenings with a high sensitive marker that can detect low alcohol intake may help individuals in remaining sober. (39–41)

Accidental intake of ethanol through mouthwash or hand sanitizer for example also does not lead to a PEth value above 20 ng/mL, a commonly used decision limit to conclude abstinence. Reisfield et al. did a study where 15 participants gargled 4 times a day with an ethanol-containing mouthwash for 12 days; None of the blood samples exceeded the 20 ng/mL decision limit. (42) From the same research group, a study was set up where 15 participants used an alcohol-based hand sanitizer multiple times a day for 12-13 consecutive days. They concluded that reaching a level of 20 ng/mL was very unlikely. (43)

One of the difficulties surrounding the decision limit is the lack of big studies validating or underpinning the numerical values used. There is a consensus among the United States laboratories to use a value of 20 ng/mL, as suggested by the United States Drug Test Lab (USDTL). Similarly, Swedish laboratories agreed on using a 35 ng/mL threshold. (26,44,45) One of the issues is the measurement uncertainty of the method, which contains the accuracy and precision. When someone has a PEth value of 20 ng/mL, in 50% of the cases the PEth value will be higher, meaning that they will have a positive test result while they possibly remained abstinent. Some labs then agreed

to use a higher cut-off to exclude these false positives, like the Swedish laboratories. Consequently, sensitivity and specificity of the PEth measurement are affected by adding a margin for safety. In general, a higher cut-off results in a higher specificity and a lower sensitivity than a lower cut-off. (46)

To distinguish between moderate and excessive alcohol use, concentrations ranging from 150 to 221 ng/mL are often used. In the national harmonization of Sweden for PEth, they established a cut-off of 210 ng/mL. The Ghent University's Laboratory of Toxicology (UGent) uses a cut-off of 270 ng/mL to account for measurement uncertainty and the inconsistency between laboratories. (46)

1.3. DRUG BIOMARKERS

Substance use can be indicated through urine, blood, saliva and hair. For recent drug exposure, blood and saliva are useful as they appear immediately but shortly, with a small window of detection (1-3 days). Drugs appear in urine hours after consumption and are detectable for days to weeks. In clinical and forensic toxicology, urine is frequently used. After consumption, drugs are detectable in hair after one week and persist until the hair is cut which means that the window of detection is especially long (weeks, months or even years). (47)

Cannabis

There are more than 100 different, identified cannabinoids but delta-9-tetrahydrocannabinol (THC) is the main psychoactive compound in cannabis. (12) The main metabolites of THC are 11-hydroxy-THC (11-OH-THC) and its oxidated form 11-Carboxy-THC (11-COOH-THC). The glucuronide conjugate of 11-COOH-THC is also an abundant metabolite of THC. While THC and hydroxy-THC are quickly metabolized, carboxy-THC persists longer in biological matrices e.g. plasma. Although the exact mechanism of incorporation is still unknown, carboxy-THC is found in hair in very low concentrations (<10 pg/mg). The determination of this metabolite is crucial to differentiate between passive drug exposure and active consumption. (48–52)

Cocaine

Cocaine has a short half-life (+/- 40 minutes) as it is rapidly metabolized. Although there are many metabolites, there are 2 main pathways: spontaneous hydrolysis to benzoylecgonine and enzymatic hydrolysis to ecgonine methyl ester. Because only small amounts of unchanged cocaine appear in urine, most screening

tests for cocaine use detect benzoylecgonine. This metabolite is detectable in urine for at least 48 hours but up to two weeks for heavy cocaine users. (53) Although cocaine itself could be determined in hair, a positive result could be attributed to external contamination. For this reason, benzoylecgonine and ecgonine methyl ester are the main target compounds in conventional hair analysis. (54)

Opiates

Testing for opiate use in urine is usually done by targeting morphine. A positive sample can be hard to interpret because codeine (a prescription drug), heroine (an illicit drug) and other opiates all metabolize to morphine. More specific analytical methods can identify which opiates are present in a sample. The ratio of morphine/codeine could distinguish between morphine use and codeine use. The detection of heroin or its active metabolite 6-monoacetylmorphine is a criterium to conclude heroin use. (55)

Amphetamines

Amphetamines refer to a group of drugs that contains both prescribed and illegally produced amphetamine, methamphetamine and other amphetamine-like drugs. (14) 3,4-Methylenedioxymethamphetamine (MDMA), also known as “ecstasy”, is a widely used amphetamine-like drug, chemical similar drugs include 3,4-methyleendioxyamfetamine (MDA) and 3,4-Methylenedioxy-N-ethylamphetamine (MDAE or MDE). Amphetamine, methamphetamine, MDA and MDE are all completely synthetic substances and are widely used for recreational purposes. (56) All can be detected unchanged in urine and are incorporated in hair. (57)

Benzodiazepines

Benzodiazepines are used to treat several disorders, for example anxiety and epilepsy. Commonly used benzodiazepines include alprazolam, lorazepam, clonazepam and diazepam. They can be misused by combining with alcohol or illegally used for recreational purposes. (58) Finding an optimal screening method for benzodiazepines is hard because a large number of benzodiazepines are available Because of extensive metabolism, detection should be focused on the identification of excreted metabolites. (59) Many benzodiazepines have common metabolites, mostly nordiazepam and oxazepam which are prescribed drugs themselves. (60)

Immunoassays for drug screening

Immunoassay drug tests, for example, the Nal Von Minden test, detect drugs or their metabolites in blood, urine or saliva utilizing antibodies. It is a quick and inexpensive method, therefore they are often used as a “point-of-care” or “roadside” test. Unfortunately, they are also subjected to false positives and negatives. For this reason, results should never just be taken as true and in a judicial context will always have to be confirmed through additional tests. (61) This confirmation is even implemented, e.g. in the Belgian law on driving under the influence of drugs. (15)

2. OBJECTIVES

The objectives of this master thesis were 2-fold:

1. Underpin confidence in the PEth decision limit used to conclude compliance with abstinence
2. Compile evidence to get more insight into the drivers' license regranting processes, as applied at the institute of forensic medicine led by Dr. Evy De Boosere based on the case files archived since 2020

2.1. PETH DECISION LIMIT

In various studies, various decision limits or cut-offs are used to conclude whether a person has been abstinent, or only had a minor alcohol intake in the past couple of weeks. We aimed to document the origin of these various decision limits and verify whether they are scientifically underpinned. Despite the variety of limits used, there is a growing international consensus to use 20 ng/mL phosphatidylethanol 16:0/18:1 (PEth) as a decision limit. As this conclusion of abstinence or not can be life-changing, a high confidence in the decision limit is needed. It is the goal of this study to seek confidence, based on real data gathered in the Laboratory of Toxicology at Ghent University. To do so, a big dataset was used of 465 subjects who refrained from drinking alcohol for one month, PEth measurements were obtained at the start, after 2 weeks and after 4 weeks.

2.2. THE DRIVERS' LICENSE REGRANTING PROCESS

The case files of the reintegration exams deliver valuable information about the driver's license regranting process and the candidates that participate. To get more insight, we will investigate the following things: what are the demographical characteristics of the subjects in the case files? Do people who get caught driving with a high Blood Alcohol Content (BAC) typically have a high PEth and hEtG at the time of the reintegration exam? Similarly, is the same drug that a subject tested positive on while driving typically found back in urine at the reintegration exam? Is there any correlation between the concentration of PEth and hEtG? Furthermore, is there any reliability in the self-report of alcohol consumption? Lastly, we wanted to take a look at how many positive results in the rapid drug test were confirmed via Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) or Gas Chromatography-Mass Spectrometry (GC-MS).

3. MATERIALS AND METHODS

3.1. PETH DECISION LIMIT

To trace the origin of the PEth decision limits a literature study was done using the search term ‘PEth OR phosphatidylethanol” in Pubmed, with as filter ‘humans’. Only the articles for which the full text was available as open access or via the Ghent University Library were withheld. Articles published before 2009 could not be included as PEth was measured using a high-performance liquid chromatography (HPLC) method with an evaporative light scattering detection. This method measured the sum of all the PEth analogs. Since then, liquid chromatography mass spectrometry (LC-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods are used that allow quantifying single analogs of PEth. (38) Every article that matched the criteria was screened for a statement on the decision limit for PEth 16:0/18:1 to assess abstinence or minor alcohol intake. The argumentation to use a certain decision limit was studied in every article, if a reference was made to another article, the argumentation in this article was studied as well.

To validate the use of the 20 ng/mL as a decision limit, we used a dataset from a big study where 678 volunteers self-reportedly refrained from alcohol consumption for 4 weeks. Participants were adults who usually drink alcohol recruited via the ‘Tournée Minérale’ initiative. Per subject, 3 samples were obtained with a 2 weeks intervals (PEth 1 (day 1 of abstinence), Peth 2 (~day 14 of abstinence), PEth 3 (~day 28 of abstinence)) via self-sampling using 10µL volumetric absorptive microsampling (VAMS) devices. Peth was quantified using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Based on the data obtained in this study, a population-based algorithm capable of predicting abstinence with 95% probability was set up. (32)

The PEth values of every subject were linked with the days in the study on which they were obtained, and the median of the final sampling day was calculated. Based on the regression model, the starting value was derived that would lead to an upper limit of the 95% prediction interval of 20 ng/mL on the median of the final sampling day. Because the decrease of PEth is time-dependent, it was necessary to determine what the upper limit would be, for that starting value, for every final sampling day.

For data stratification, we excluded every subject that admitted to drinking. Subjects for whom the ratio PEth 2/PEth 1 or PEth 3/PEth 2 was higher than 0.7, were also excluded as this might indicate alcohol consumption. Subjects with a third sample taken on day 31 or later were left out because the regression model is only accurate up until day 30. We also excluded participants with incomplete data or when the initial PEth was lower than 4 ng/mL, which was the LOD.

The specificity, sensitivity, negative predictive value and positive predictive value were calculated using data from 465 participants. If the participant's initial PEth concentration was higher than the starting value, a concentration >20 ng/mL after four weeks of abstinence was considered correct (or as expected based on the regression model) ('true positive'), while a concentration <20 ng/mL was considered incorrect ('false negative'). For those individuals with an initial PEth concentration lower or equal to the starting value, a final concentration <20 ng/mL was considered correct ('true negative'; compatible with abstinence), whereas a concentration >20 ng/mL was considered incorrect ('false positive'). The same classification algorithm was applied for 10 different switching points (with a range of 80-270 ng/mL). For every subject with a last sampling day different from the median, the upper limit of the 95% prediction interval for that specific day was used.

Following formulas were used to calculate the specificity, sensitivity, positive predictive value and negative predictive value (62):

Table 3.1: formulas to calculate specificity, sensitivity, PPV and NPV.

Specificity	$TN^{a}/(TN+FP^{b})$
Sensitivity	$TP^{c}/(TP+FN^{d})$
Positive predictive value (PPV)	TP/(TP+FP)
Negative predictive value (NPV)	TN/(TN+FN)

[a]TN: True Negative

[b]FP: False Positive

[c]TP: True Positive

[d]FN: False Negative

Data-analysis and calculations were performed with Excel.

3.2. THE DRIVERS' LICENSE REGRANTING PROCESS

365 cases were available from reintegration exams performed by Dr. Evy De Boosere. These files contain information about the committed violation of the law, as well as previous convictions. Date of birth, gender, marital status and profession are all reported just like their medical history. The subjects were asked to estimate the number of alcohol units they consume in a week. The subjects were also asked if they take any illicit drugs. If he or she indicated any, the type and amount of illicit drug use were noted. Depending on the anamnesis, subjects could be asked to deliver a urine, hair and/or fingerprick blood sample. If they agreed on delivering a urine sample, the Nal Von Minden rapid test was performed so subjects immediately can be confronted with the results. After the examination, the samples are transferred to the laboratory of toxicology where they are analyzed. They perform the following analysis: PEth-measurement in the fingerprick blood sample via LC-MS/MS, untargeted screening using high-resolution mass spectrometry of the urine. Depending on the results of the latter and the anamnesis, targeted analysis of the urine samples is done to detect cocaine, cannabis, amphetamines and related substances using gas chromatography–mass spectrometry (GC/MS). In the final stage, depending on the outcome of these first analyses, hair analysis is performed as well, for the following class of substances, including their metabolites: cannabinoids, cocaine, benzodiazepines, ketamine, methadone, opiates, amphetamines and ethylglucuronide.

9 databases were used for the data analysis of this part of the study: the demographics of the subject (age, sex, …), PEth values, hEtG values, self-report of alcohol consumption, results from the Nal Von Minden test, results from the saliva drug test at the time of the incident, Blood Alcohol Content (BAC) at the time of the incident, the untargeted and targeted screening of urine.

To perform data analysis, numerical values were needed for all input data. For that reason the following adjustments were made:

Table 3.2: adjustments of PEth and hEtG values to perform data-analysis.

PEth < 10 ng/mL	to	5 ng/mL
hEtG < 3 pg/mg	to	0 pg/mg
hEtG < 5 pg/mg	to	4 pg /mg

hEtG < 7 pg/mg	to	4 pg/mg

Calculations of the BAC (g/L) were done by multiplying the breath alcohol concentration (mg/L) with a factor 2.3. (63)

Data-analysis was performed using Excel and MedCalc.

For each subject, an estimate was made based on his or her statements whether their alcohol consumption falls under "never", "10 or fewer units per week" or "more than 10 units per week". In some cases, individuals were specifically asked how many units of alcohol they consumed per week. To analyze the data, the mean value of the self-reportedly amount of units was taken.

For five predefined drug classes, including amphetamines, cocaine, cannabinoids, opiates and benzodiazepines, an immunoassays drug screening test was performed (Nal Von Minden). Cases with positive screen results were then reflexed to definitive testing via LC-QTOF-MS. An electrospray ionization (ESI) source was used in positive mode to perform a urinalysis. To collect mass spectra, a full scan mode was used, in both parent and product mode. For data analysis, a broad library with databases gathered from literature or other sources was used, together with a 360-compound list including retention time and structural formula specific to the settings of the used Q-TOF mass analyzer. A positive result in the untargeted screening was mostly confirmed and quantified via GC-MS, with the exception of benzodiazepines.

4. RESULTS AND DISCUSSION

4.1. PETH DECISION LIMIT

Figure 4.1 describes the outcome of the literature search. 632 articles matched our search criteria, 82 of those articles mentioned a decision limit for PEth 16:0/18:1 to assess abstinence or minor alcohol intake. Overall, 3 decision limits were commonly used: 20 ng/mL, 35 ng/mL and 8 ng/mL.

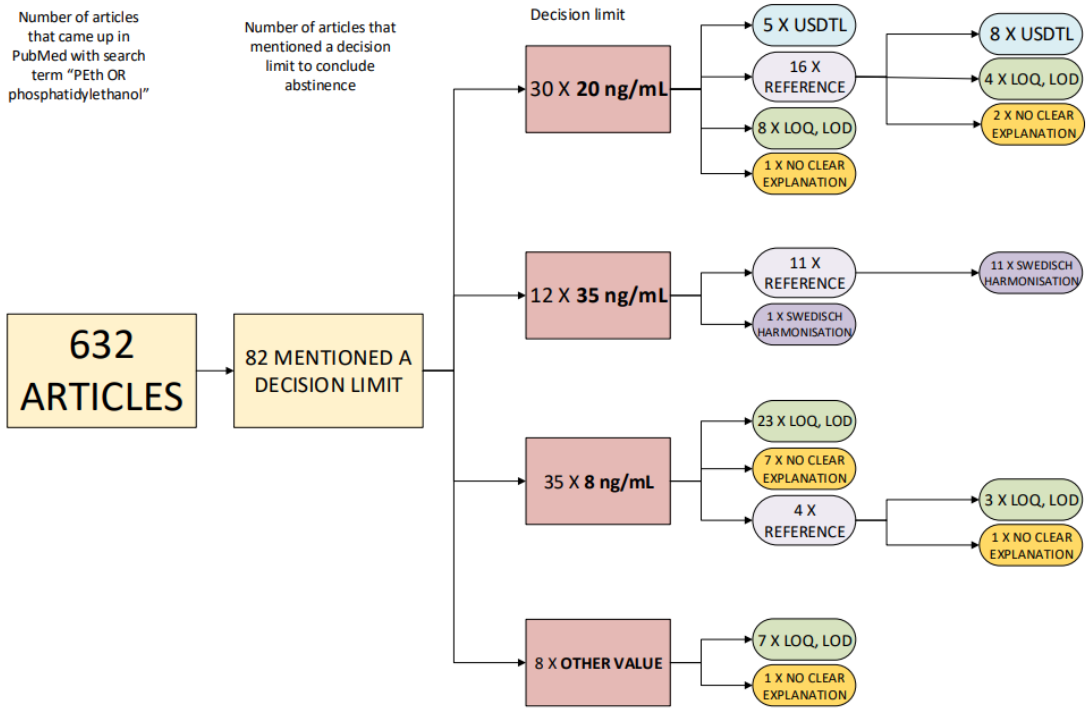

Figure 4.1: different decision limits in articles.

In 2009, the first article came up that used a decision limit of 20 ng/mL, this was based on the limit of quantification (LOQ). (20) Seven other articles used 20 ng/mL for the same reason. (25,40,64–68) The first article that referred to the USDTL agreement was published in 2013 (45) and was followed by four other articles. (21,26,44,69) fourteen articles referred to another study as to reason for the use of 20 ng/mL(33,38,42,70–80), eight of those studies, in turn, referred to the USDTL, four to the LOQ and two studies did not mention the motivation for the decision limit value of 20 ng/mL.

In 2013, Swedish laboratories decided to harmonize and use a 35 ng/mL decision limit. (81) Since then, eleven articles referred to this agreement. (38,82–91)

35 articles used the 8 ng/mL decision limit, most of them (23 articles) referred to the limit of detection (LOD) or LOQ.(21,39,92–112) Seven articles did not explain why 8 ng/mL was used (113–119), four articles referred to another article(120–123) (three of them mentioned the LOD or LOQ, one article did not mention 8 ng/mL).

Eight articles used another value (a range from 2,0 ng/mL to 10 ng/mL), seven of them referred to the LOQ or LOD of the method. (16,34,124–128) One article suggests a decision limit of 10 ng/mL without any further explanation. (23)

Altogether, this literature study clarifies the need to harmonize between laboratories and do more research to find a common decision limit value with a scientific background. The next step in our research was to look if we could underpin the use of 20 ng/mL as a decision limit because even though there is a consensus among the United States laboratories to use this value, it is still an arbitrary threshold.

In order to achieve this goal, a real patient database was used of 465 participants who refrained from alcohol for one month. 3 samples were taken on day 1, after 2 weeks and after 4 weeks. The third sampling day was at minimum on day 20, at maximum on day 34 with a median on day 27. Based on the regression model, we derived that the starting value that would lead to an upper limit of the 95% prediction interval of 20 ng/mL on day 27 of abstinence is 120 ng/mL.

To validate the use of 20 ng/mL as a decision limit for compliance with abstinence or minor alcohol intake, it is more important to consider the specificity compared to the sensitivity. The specificity represents the probability of a negative test result for an individual that should be negative, in other words, a high specificity is needed to not falsely accuse someone of drinking when they did not. The specificity for a switching point of 120 ng/mL was found to be 100%. This could be interpreted as if someone starts with a PEth concentration beneath 120 ng/mL, the probability of ending up with a concentration lower than 20 ng/mL after 4 weeks of abstinence is 100%. The negative predictive value (NPV) is the probability of not having drunk in an individual with a negative test result. For a concentration of 120 ng/mL, this NPV was 88%. Even for higher switching points, were based on the predicition model, one shouldn’t end up below 20 ng/mL, the specificity remains > 90%.

Table 4.1: specificity, sensitivity, PPV and NPV calculated for different switching points.

SP (ng/mL)	*270*	*245*	*220*	*200*	*180*	*160*	*140*	*120*	*100*	*80*
Specificity	94	95	96	97	97	99	100	100	100	100
Sensitivity	97	97	91	86	83	74	68	54	45	38
PPV	50	63	70	77	77	91	98	98	98	98
NPV	100	100	99	98	98	96	94	88	83	78

As previously mentioned, the decision limit to conclude abstinence or minor alcohol intake can be life-changing. This study was a first step toward scientifically underpinning the use of 20 ng/mL as a decision limit, based on real patient data. More research is needed to confirm these results. Harmonization between laboratories around the world should be pursued, whereby collaboration between different research groups in different countries will be necessary.

4.2. THE DRIVERS' LICENSE REGRANTING PROCESS

4.2.1. Population characteristics

The study population mainly consists of men (87%) with an average age of 39 years on the day of the reintegration exam. In 2015, the Belgian Institute for road safety (BIVV, "Belgische Instituut voor de Verkeersveiligheid") did similar research on reintegration exams in Belgium and the socio-demographic characteristics of participants. (8) The average age and percentage of males are in agreement with what was reported by BIVV (89% males and 41 years old). Figure 4.2 shows the absolute amount of subjects per age group.

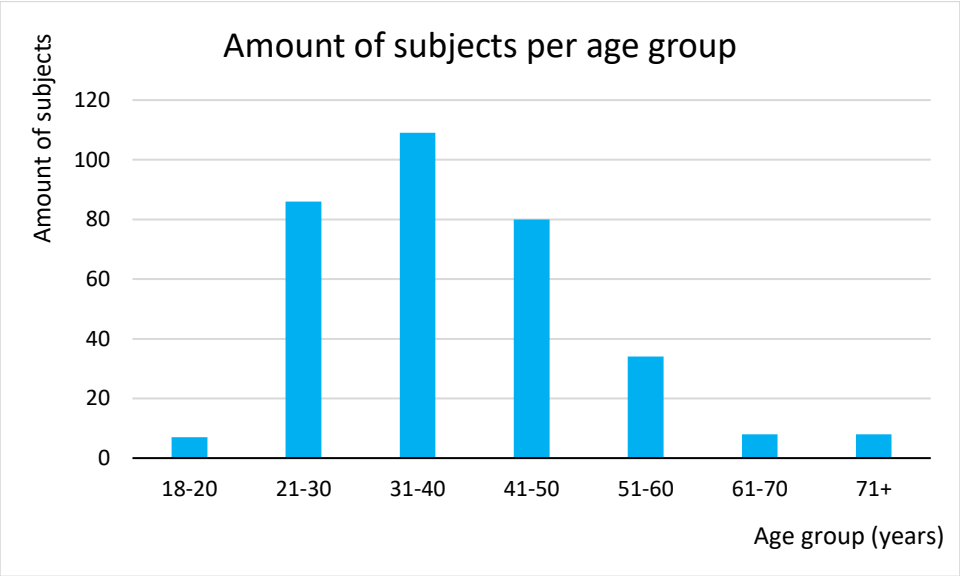

Figure 4.2: Amount of subjects per age group.

Most subjects (109 subjects) are between 31 and 40 years old, followed by the 41-50 and the 21-30 age group. Only a few subjects were younger than 20 years (5 people) or older than 70 years (7 people).

The average time between the day of the offense and the (first) visitation for the reintegration exam was 298 days (~9 months). The average time was remarkably shorter for the examinations held in 2022 (179 days) than those held in 2020 (297 days) and 2021 (315 days). For future perspectives, striving for a shorter time between offense and reintegration exam could reduce recidivism as candidates with an alcohol or drug problem can earlier be declared unfit to drive.

Considering the incident, 156 subjects had a BAC higher than 0,5 g/L,145 subjects had a positive saliva drug test and 18 had both. 45% of the subjects with a BAC higher than 0,5 upon the incident were found to have a PEth higher than 270 ng/mL at the time of the reintegration exam, moreover in 21% of them some type of illicit drug was detected. For the subjects with a positive saliva test upon the incident, 16% of them for whom PEth was determined had a concentration above 270 ng/mL at the reintegration exam.

89% of the study population was declared unfit to drive, mostly because of alcohol abuse (39%) and drug abuse (34%). In 14% of the cases, both drug and alcohol use were problematic. For the others, the doctor was unable to make a statement about

the fitness to drive as more research would be needed, for example, due to the lack of a hair sample. The large amount of subjects that were declared unfit to drive is in contrast with what was reported by BIVV, only 8,2 % of their subjects were declared unfit. This could be explained by the difference in the amount of additional analyses in blood, urine and hair. Considering the research done by BIVV, CDT in blood was determined for 43,9% of the subjects. Urinalysis was done in only 8,6% of the cases, which could be explained by the low amount of drug-related convictions. On the other hand, PEth was determined for 85% of the subjects in our study population and untargeted screening of urine is done routinely (93% of the subjects). Note that CDT, is only sensitive for uncovering chronic and excessive alcohol use, people with social, but rather high alcohol intake might still have normal CDT values. Hence, if the prerequisite is abstinence, CDT is not a suited marker. (129) The latter shows the importance of performing additional analyses on top of the consultation with the doctor and psychologist.

4.2.2 The correlation between the self-report of alcohol use and PEth and hEtG

This study aimed to look if there is any reliability in the self-report of alcohol use, using PEth and hEtG as the "gold standard". Concentrations of PEth <20 ng/mL and ≥270 ng/mL have been used to assess, respectively, abstinence and excessive alcohol consumption in the last 4 weeks, with moderate drinking lying between 20 and 270 ng/mL. Similarly, a concentration of hEtG <5 pg/mg is compatible with abstinence, 5-30 pg/mg with moderate alcohol consumption and finally, a concentration ≥30 pg/mg reveals excessive alcohol consumption in the last few months (depending on the hair length). Figure 4.3 shows the box-and-whisker plot for the PEth concentrations plotted against the self-report of alcohol, expressed in the estimated amount of units of alcohol per week. Figure 4.4 is equivalent but shows the hEtG concentrations versus the self-report.

The first group consists of 69 people, all claiming to never drink alcohol. Their PEth values ranged from 0 to 2000 ng/mL, with a median of 11,9 ng/mL. The second group (162 people) estimated their alcohol use at 10 units per week or less, their PEth concentration had a median of 135,5 ng/mL (range: 5 to 2090 ng/mL). For the third group, this median was 488 ng/mL with a minimum of 15 ng/mL and a maximum of 3510 ng/mL. The third group consists of 66 people and they all claim to drink more

than 10 units per week. This plot and the medians of the PEth values per group show that people tend to report a higher alcohol use if they consume more alcohol.

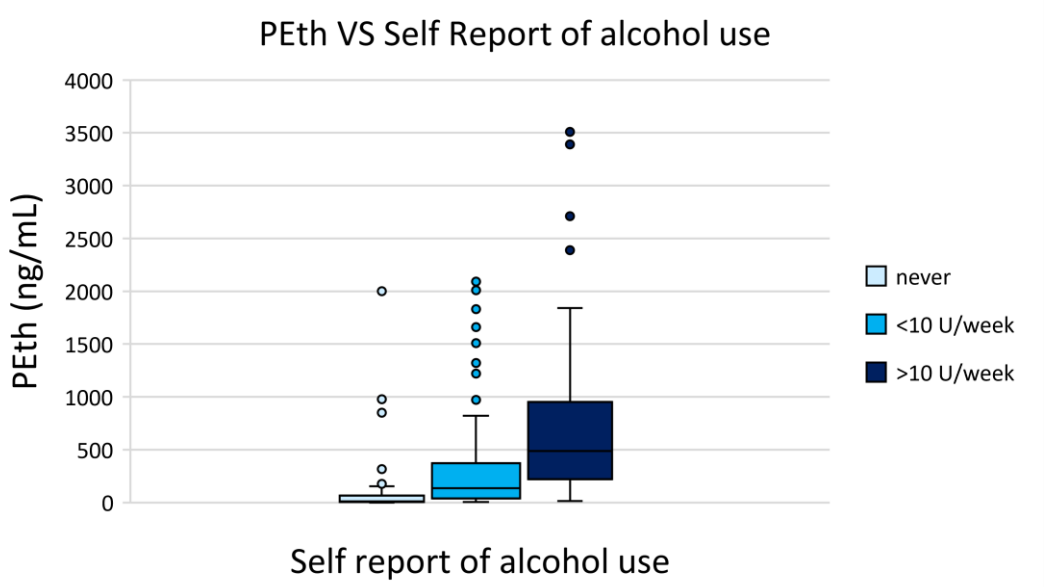

Figure 4.3: Box-and-Whisker plot for PEth levels in subjects that claim to drink: light-blue: never, blue: 10 or less units per week, dark-blue: more than 10 units per week. Dots represent individual values.

However, during the Tournée Minérale study in 2019, subjects were also asked to estimate the number of alcohol units consumed in a week. The median of the PEth value was 19,3 ng/mL for the group that estimated their alcohol consumption at 10 units per week or less. For the group describing their alcohol consumption as being more than 10 units, this median was 115,0 ng/ml. These lower medians for the Tournée Minérale database suggest that the self-report in our database could be an underestimate of the real alcohol consumption.

Figure 4.4 shows the same trend: the self-report of alcohol use tends to be higher in subjects with a higher hEtG value. The group that claims to never drink alcohol consists of 25 subjects, with hEtG values ranging from 0 to 189,4 pg/mg and a median of 10,2 pg/mg. The second group (55 people) estimates their alcohol use at 10 units per week or less, their hEtG concentrations have a median of 24,9 pg/mg (range: 0 to 39,7 pg/mg). For the group that claims to drink more than 10 units per week (15 people), the median is 39,6 pg/mg (with a range of 4 pg/mg to 1000 pg/mg).

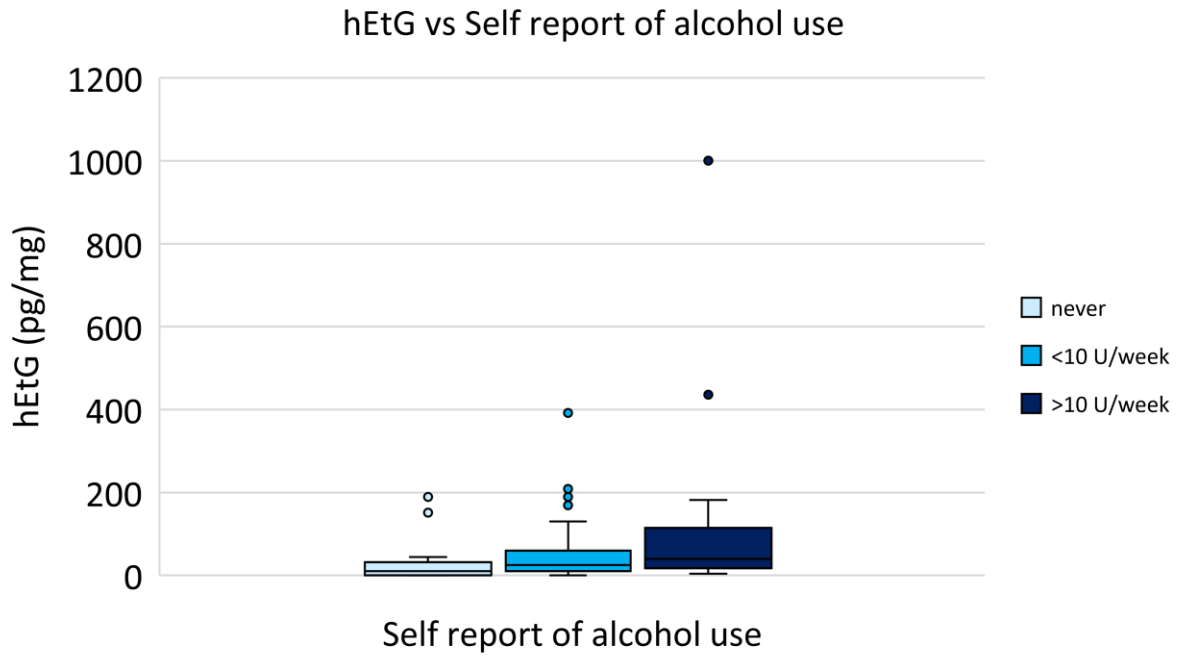

Figure 4.4: Box-and-Whisker plot for hEtG levels in subjects that claimto drink: light-blue: never, blue: 10 or less units per week, dark-blue: more than 10 units per week. Dots represent individual values.

The same trend is visible in these scatter plots (Figure 4.5 and 4.6) where PEth and hEtG concentrations are plotted against the mean amount of alcohol units a subject reported drinking per week. The correlation coefficients were, respectively, r=0,36 (p<0,0001) and r=0,42 (p=0,0044).

There would be two logical reasons for PEth and hEtG values to be higher than expected compared to their self-reports. First, it is likely in the context of reintegration exams that people will underestimate their alcohol use. Secondly, some people may tend to lower or stop their alcohol consumption just before the appointment resulting in a low self-report but a PEth and hEtG value that still represents the old drinking habits. This is especially true for hEtG as it takes 7-10 days to be incorporated into hair. (67)

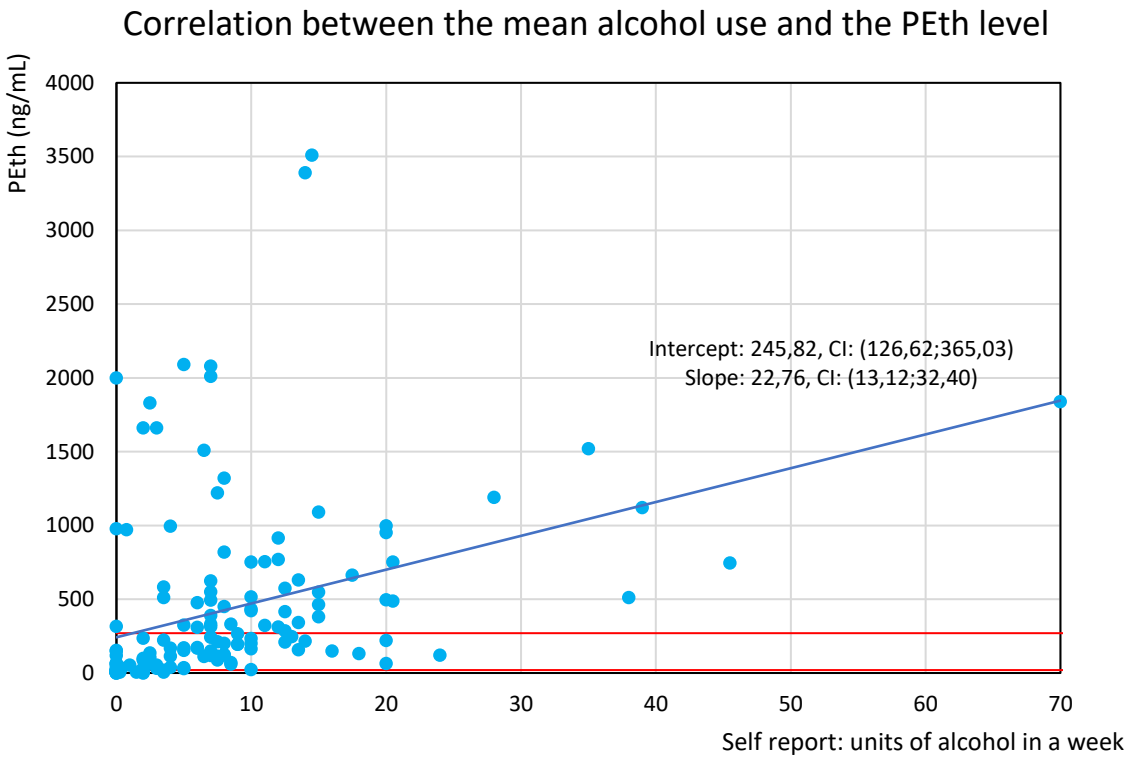

Figure 4.5: Correlation between the mean alcohol use per week and the PEth level. Red lines horizontally represent the decision limits of 20 ng/mL and 270 ng/mL to assess for abstinence and excessive alcohol use.

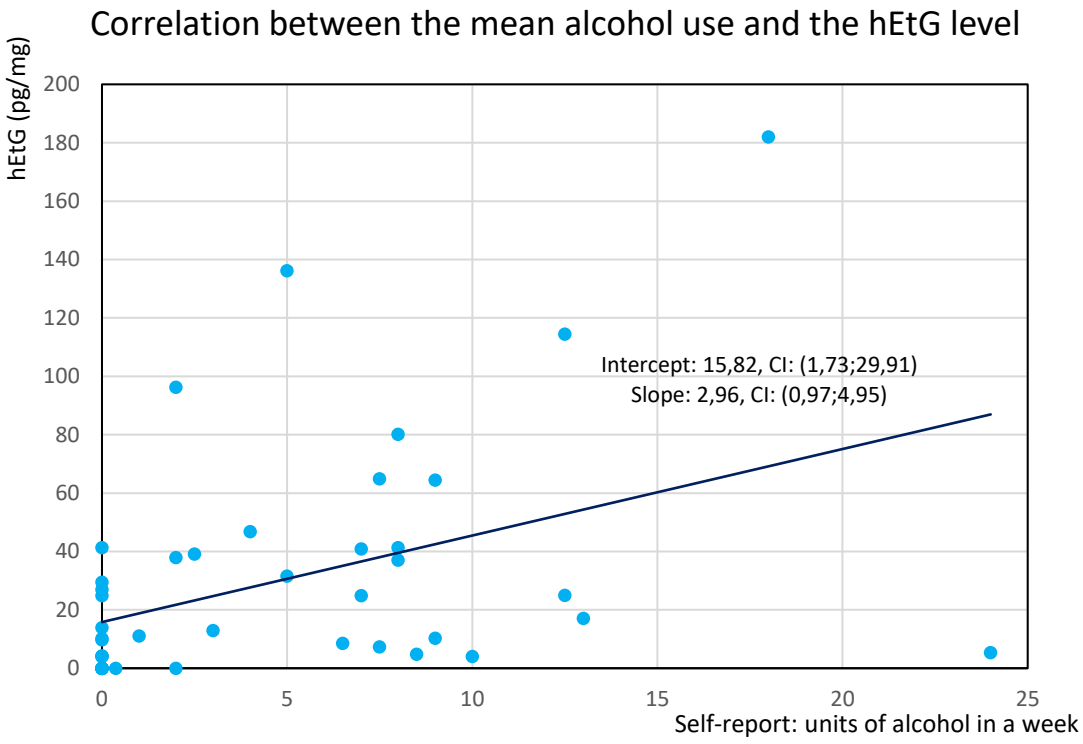

Figure 4.6: Correlation between the mean alcohol use per week and the hEtG level.

. To get a better understanding of the possible underreport by participants of the reintegration exam, Figure 4.7 shows the scatter plot for PEth values plotted against the self-report of alcohol use for our database and the Tournée Minérale database.

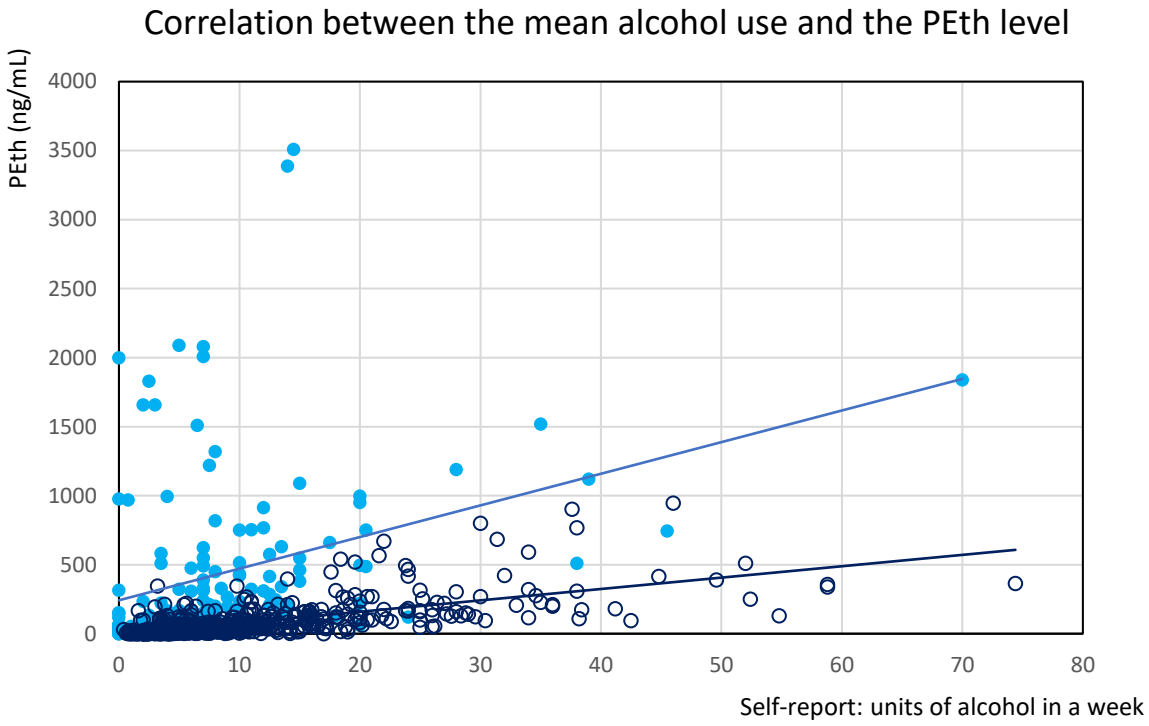

Figure 4.7: Correlation between the mean alcohol use per week and the PEth level. Blue: our database, dark-blue: Tournée Minérale database.

Compared to the data of this study, it seems that PEth values tend to be lower for the same units of alcohol per week in the Tournée Minérale dataset. A possible explanation for this is the context of the study, for Tournée Minérale there were no consequences for the subject considering their alcohol use and so little motivation to inaccurately report alcohol use. However, the driver's license regranting process forms part of a legal decision, subjects likely underreport their alcohol consumption on the reintegration exam as it decides on their fitness to drive. The self-report of their alcohol use should be taken with a grain of salt and has limited reliability. This demonstrates the importance of the use of objective biomarkers.

4.2.2. The correlation of PEth and hEtG with the blood alcohol content

The scatter plots in Figures 4.8 and 4.9 show PEth or hEtG plotted against the Blood Alcohol Concentration (BAC) measured on the occasion of the offense.

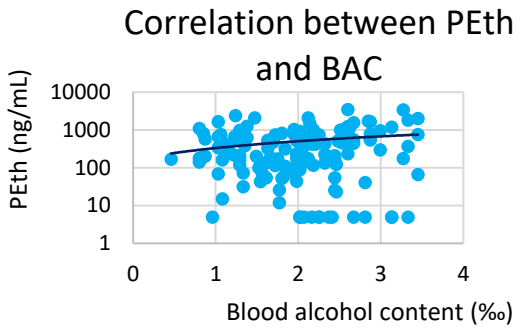

Figure 4.8: Correlation between PEth and BAC.

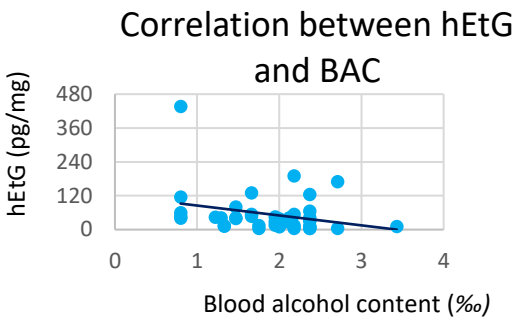

Figure 4.9: Correlation between hEtG and BAC.

Both graphs show that someone who was caught driving with a high alcohol percentage does not necessarily end up with a high PEth or hEtG on the day of the reintegration exam. One possible explanation could be the time between the offense and the reintegration exam in which the subject does not has his driver's license. In some cases, this could push a person into drinking more as they are not allowed to drive. In other cases, they would reduce the amount of drinking as they would want to have their driver's license back as soon as possible. Because of the time between the measurements (~9 months), these graphs say something about the ability to lower or stop the consumption of alcohol to get their driver's license back rather than if the people who drive with a high BAC usually are heavy drinkers. Remarkably, lots of subjects were not able to adjust their alcohol use regardless of the loss of their driver's license.

4.2.3. The correlation between PEth and hEtG

For 101 subjects, both PEth and hEtG were determined. Figure 4.10 shows the scatterplot where hEtG (pg/mg) is plotted against PEth (ng/mL). A positive correlation is found between PEth and hEtG, r=0,224, n=96, p=0,0284. In Figure 4.11 the results were distributed into groups according to the decision limits used by the Laboratory of Toxicology at Ghent University. PEth results were classified as followed: compatible with abstinence (<20 ng/mL), social drinker (20-150 ng/mL), social drinker with more

important alcohol use (150-270 ng/mL), suggestive for excessive alcohol use (270-500 ng/mL) and excessive alcohol use (>500 ng/mL). Similarly, the hEtG result for every subject was classified as compatible with abstinence (<5 pg/mg), moderate alcohol consumption (5-30 pg/mg) and excessive alcohol consumption (≥30 pg/mg). Both figures show that people with a higher PEth level tend to have a higher hEtG level.

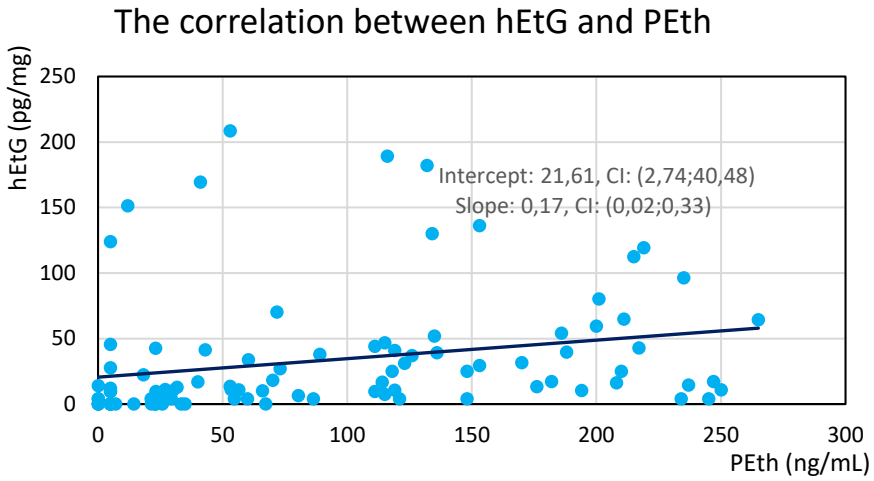

Figure 4.10: The correlation between hEtG and PEth.

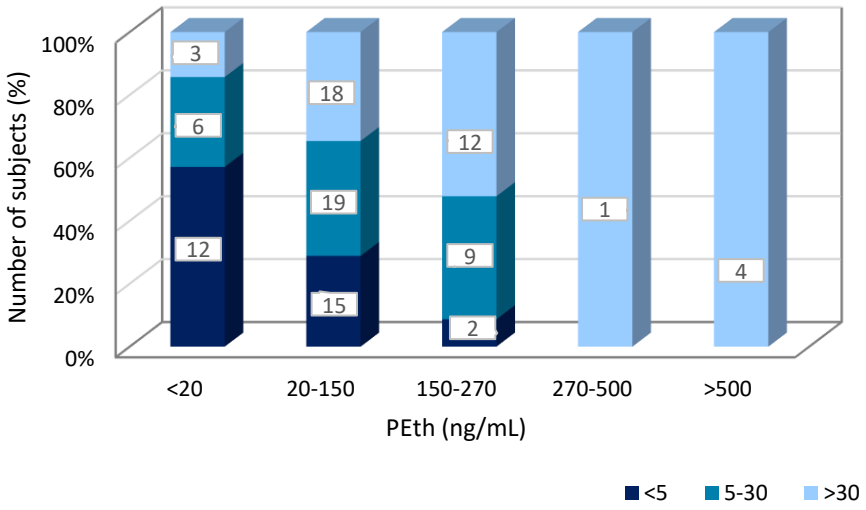

Figure 4.11: relative abundance of hEtG concentration groups in different PEth concentration groups. Numbers represent the absolute amount of subjects in every group.

Excessive alcohol consumption was detected in 38% of the samples in the hEtG analysis, while 29% of the sample were consistent with abstinence. For the PEth analysis, 21% of the samples were beneath 20 ng/mL, compatible with abstinence.

Only 5% of the samples showed a PEth value above 270 ng/mL, which could simply be explained by the procedure of the reintegration exam: PEth values above 270 ng/mL are already indicating for excessive alcohol use, therefore it is not necessary to measure hEtG and only a few subjects with PEth > 270 ng/mL could be included. 3 subjects with a PEth value <20 ng/mL had hEtG ≥ 30 pg/mg, which immediately shows the advantage of using hEtG additional to PEth. For example, one particular subject had a PEth <10 ng/mL but hEtG was 46 pg/mg, based on the value of PEth he may have been considered fit to drive, but the hEtG level revealed an excessive alcohol use and thus this subject did not pass the exam. 17 subjects had a PEth concentration higher than 20 ng/mL, but a hEtG level lower than 5 pg/mg. Those subjects probably only recently consumed alcohol, leaving EtG still undetectable in hair. In 45 % of the cases, the PEth and hEtG values were in agreement (PEth <20 ng/mL and hEtg <5 pg/mg, PEth 20-270 and hEtG 5-30 or PEth >270 ng/mL and hEtG >30 pg/mg). 56% of the subjects did not have corresponding PEth and hEtG concentrations, which could be due to the differences in the window of detection. PEth has a shorter window of detection (several weeks) than hEtG (several months, depending on the length of the hair), if a subject recently changed their drinking behavior, this would only be reflected in the PEth level. Additionally, EtG needs to be incorporated into hair before it can be detected, whereas PEth is detectable in blood almost immediately after alcohol consumption. (67)

4.2.4. Immunoassay rapid drug test

A total of 163 positive screens were observed in the immunoassays urine screening of 329 subjects. Every positive specimen was subjected to confirmatory testing using untargeted (QTOF) and targeted (GC-MS) screening. Table 4.1 shows the number of positive immunoassay screenings that were confirmed (left) and not confirmed (right) for every drug class.

Table 4.1: The amount of positive immunoassay drug screenings that were confirmed (left) or not confirmed (right) in urinalysis.

	Confirmed	Not confirmed
Carboxy-THC	74	8
Benzoylecgonine	29	0
Amphetamine	20	0
Benzodiazepine	14	11
Opioids	7	1

4.2: The amount of negative immunoassay drug screenings for cocaine that were confirmed (left) or resulted in a positive urinalysis (right).

	Expected negatives	Unexpected positives
Benzoylecgonine	270	22

Concerning carboxy-THC, a GC-MS analysis could not confirm the positivity in the Nal Von Minden test for 8 cases. An untargeted screening of the same samples came back negative for most of the samples. In 2 cases, the anti-epileptic drug lamotrigine was detected which has been shown to cause false positives in immunoassay screenings for phenylcyclohexylpiperidine. (130) However, there is no literature on how lamotrigine would cause a problem for carboxy-THC.

Because amphetamines are simple molecules, it is quite difficult to develop specific antibodies for these drugs. In literature, many compounds are described that cause false positives for amphetamine urine immunoassays, e.g. dimethylamine, ranitidine, and bupropion. (131) However, twenty positive urine immunoassays were all confirmed with the untargeted urine screening and additionally a GC-MS analysis.

One positive opiate immunoassays could not be confirmed by untargeted screening. Targeted screening of this urine sample showed positivity for benzoylecgonine and carboxy-THC, but negativity for opiates. Untargeted screening did not reveal any components that could clarify the positive result.

Out of the twenty-five positive benzodiazepine immunoassays, 11 of them could not be confirmed through untargeted screening. In urine benzodiazepine screens, a drug that has been identified to cause false positives is the anti-depressant sertraline (130), however, this was not found in any of the samples via untargeted urine screening. Compounds may be missed in the untargeted screening because of a general setting of e.g. the collision energy that could not be ideal to identify certain compounds. Using a different detection technique, for example, a high-performance liquid chromatography diode-array detector (HPLC-DAD), could bring more answers to this issue. (132)

All positive cocaine immunoassays were confirmed. However, untargeted screening of urine revealed cocaine use in twenty-two cases while negative in the immunoassay (see Table 4.2) This only affirms that the result of an immunoassay test should be considered presumptive until confirmed. (59)

However, it is still recommended to perform a rapid drug test. It is an inexpensive and quick method to get the first idea of someone's drug use. If a person thinks they can cheat the system and conceal their drug use, a confrontation with a positive result could lead to a more honest statement. Furthermore, a positive result in a rapid drug test can be a reason to do a targeted analysis regardless of the result of the untargeted analysis. Sometimes drug use can be detected this way if the untargeted analysis would have missed it.

4.2.5. Saliva road test

The purpose of this part of the study was to look at the result of the saliva road test and compare it to the urine screening at the time of the reintegration exam. This would give an idea of whether or not these people would change their drug intake. Table 4.2 gives an overview of the number of subjects that tested positive for drugs in the road test, and the percentage of these that still tested positive in the reintegration exam. Moreover, it also shows that sometimes drugs of abuse were detected upon the reintegration exam that were not detected in the road test, and vice versa.

Table 4.3: the amount of subjects that tested positive for a certain drug on the saliva road test (left), the amount of subjects that tested positive for the saliva road test, and tested positive for the same drug on the urinalysis at the reintegration exam (middle), the amount of subjects that tested positive on the urinalysis at the reintegration exam but did did not test positive for that drug at the saliva road test.

	Positives saliva road test (abs amount)	Positives urinalysis (abs amount)	Positive urinalysis (abs amount)
Cannabis	45	21	5
Cocaine	38	5	10
Opiates	4	0	1
Amphetamines	42	6	2

Forty-five subjects had a positive saliva test for carboxy-THC, twenty-one of these subjects had a positive urine test for carboxy-THC. For seven subjects, carboxy-THC was found in the urine while they did not test positive for cannabis use on the road test. Cocaine use was detected in thirty-eight subjects on the road test, for five of them the urinalysis was positive for cocaine. Ten subjects did not have a positive saliva road test for cocaine, although benzoylecgonine was detected in urine. For people tested positive for opiates on the road saliva test, none of them tested positive in the urine screening. Fifty-five subjects had a positive saliva test for amphetamines, only in eight of them, it was found at the urine screening.

To interpret the results, it is necessary to keep in mind the window of detection of urine (a few days up to a week). A negative urine screening only says something about very recent drug use and does not mean a subject stopped using drugs. Still, it is surprising that so many people use drugs shortly before the reintegration exam.

5. CONCLUSION

Because of a strong correlation between alcohol and phosphatididylethanol, it has been proven to be a useful biomarker to assess abstinence or minor alcohol intake. However, there is still a variety in decision limits used to do so and there is a need to harmonize between laboratories and scientifically underpin a decision limit. In this study, we tried and succeed to seek confidence in using a decision limit of 20 ng/mL, based on real patient data. This decision limit, to score abstinence or minor alcohol intake, includes all possible sources of variation, also the measurement uncertainty. The accompanying high specificity demonstrates that this cut-off can be used with high confidence to score ‘compatibility with abstinence or minor alcohol intake’.

Based on 365 case files of reintegration exams, we managed to get more insight into the reintegration exam and its participants. The majority of the candidates were male (87%) with an average age of 39 years. The main part of the participants (89%) was declared unfit to drive. Subjects are likely to underestimate their alcohol use during the reintegration exam which demonstrates the importance of the use of objective biomarkers. Subjects with a high blood alcohol concentration on the occasion of the offense do not automatically have high PEth and hEtG levels at the reintegration exam, the average time between both measurements was 9 months. Overall, subjects with a higher hEtG level tend to have a higher PEth level although this is not always the case. The latter could be explained by the difference in window of detection and the time for EtG to be incorporated into hair, whereas PEth is detectable in blood almost immediately after alcohol consumption. An immunoassay rapid drug test can be useful to quickly detect drug use but false negatives and positives occur so results should be considered presumptive until confirmed. This study reveals how hard it is for lots of people to stop using illicit drugs and to stop or lower alcohol consumption in order to get their driver’s license back. It shows the importance of reintegration exams and the importance of drug and alcohol biomarkers to prevent recidivism and possibly save human lives.

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