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ORIGINAL ARTICLE

Application of phosphatidylethanol (PEth) in whole blood in comparison to ethyl glucuronide in hair (hEtG) in driving aptitude assessment (DAA)

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Abstract For driving aptitude assessment (DAA), the analysis of several alcohol biomarkers is essential for the detection of alcohol intake besides psycho-medical exploration. In Switzerland, EtG in hair (hEtG) is often the only direct marker for abstinence monitoring in DAA. Therefore, the suitability of phosphatidylethanol (PEth) was investigated as additional biomarker. PEth 16:0/18:1 and 16:0/18:2 were determined by online-SPE-LC-MS/MS in 136 blood samples of persons undergoing DAA and compared to hEtG, determined in hair segments taken at the same time. With a PEth 16:0/18:1 threshold of 210 ng/mL for excessive alcohol consumption, all ($n = 30$) but one tested person also had hEtG values ≥30 pg/mg. In 54 cases, results are not in contradiction to an abstinence as neither PEth (<20 ng/mL) nor hEtG (<7 pg/mg) was detected. In eight cases, both markers showed moderate consumption. Altogether, PEth and hEtG were in accordance in 68 % of the samples, although covering different time periods of alcohol consumption. With receiver operating characteristic analysis, PEth was evaluated to differentiate abstinence, moderate, and excessive alcohol consumption in accordance with hEtG limits. A PEth 16:0/18:1 threshold of 150 ng/mL resulted in the best sensitivity (70.6 %) and specificity (98.8 %) for excessive consumption. Values between 20 and 150 ng/mL passed for moderate consumption, values <20 ng/mL passed for abstinence. As PEth mostly has a shorter detection window (2–4 weeks) than hEtG (up to 6 months depending on hair length), changes in drinking behavior can be detected earlier by PEth than by hEtG analysis alone. Therefore, PEth helps to improve the diagnostic information and is a valuable additional alcohol marker for DAA.

Keywords Phosphatidylethanol (PEth) in blood · Alcohol biomarker · Ethyl glucuronide in hair (hEtG) · Online-SPE-LC-MS/MS

Introduction

In Switzerland, administrative regulations require a driving aptitude assessment (DAA) for convicted driving under the influence (DUI) of alcohol offenders having a blood alcohol concentration (BAC) of 1.6 ‰ and higher, as prolonged excessive alcohol misuse is suspected above this limit [1, 2]. In consequence of a DUI of alcohol offense, the driver's license is revoked. The driver has to pass a DAA, in which the driver's alcohol consumption habits are evaluated. Generally, the affected persons have to prove abstinence over a time period of 6 months (after traffic relevant alcohol misuse) up to 12 months (alcohol dependency) prior to re-granting the driver's license, and after re-granting the driver's license, minimal 12 months of a controlled alcohol abstinence are stipulated [3].

For DAA, direct (ethyl glucuronide (EtG), ethyl sulfate (EtS), fatty acid ethyl esters (FAEE)) and indirect markers (carbohydrate-deficient transferrin (CDT), γ-glutamyl transferase (GGT), mean corpuscular volume (MCV), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT)) are used [4]. However, EtG in hair (hEtG) often is the only direct marker for abstinence monitoring during DAA in Switzerland.

Hair samples mainly consist of head hair from the posterior region (0–5 cm) of the scalp, sometimes also from the arms, legs, and chest.

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EtG is a non-volatile, water-soluble metabolite of ethanol (Fig. 1) [5]. It is formed by a non-oxidative phase II metabolism, catalyzed enzymatically by UDP-glucuronosyltransferases, which conjugate ethanol with activated glucuronic acid [6]. This reaction constitutes only a very small fraction (<0.1 %) of the ingested alcohol dose [7, 8]. Depending on the amount of consumed alcohol EtG is detectable in blood, urine, tissues, sweat, and hair long time after termination of ethanol elimination [5, 9].

Compared to the smaller detection windows of EtG in body fluids, EtG is detectable for several months in hair depending on the hair length. Therefore, hEtG is of advantage for long-term abstinence monitoring in DAA. To differentiate prolonged excessive from moderate alcohol consumption, the Society of Hair Testing (SOHT) proposes a threshold of 30 pg/mg hEtG. A threshold of 7 pg/mg is "non-contradicting to self-reported abstinence." This expression is used, as occasional alcohol consumption cannot always be excluded, and if samples less than 3 cm or greater than 6 cm are analyzed for EtG, the results should be interpreted with caution [8, 10–12].

A direct marker, which has also a potential in the field of abstinence monitoring, is phosphatidylethanol (PEth), as it is formed directly after alcohol intake [13, 14].

The term PEth represents a group of phospholipid homologues, which are ethyl esters of phosphatidic acid (Fig. 1). These homologues have two fatty acid chains bound to the glycerol backbone in sn-1 and sn-2 position and phosphoethanol in sn-3 position. The fatty acid chains typically contain 14–22 carbon atoms with 0–6 double bonds [15, 16]. PEth homologues are present in cell membranes [17] and are biosynthesized from phosphatidylcholine (PC) catalyzed by the enzyme phospholipase D (PLD) [18], as long as ethanol is present in the organism. Due to an elimination half-life of about 4 days [17], accumulation of PEth is observed after repeated drinking. Therefore, PEth has been discussed as marker for detection of prolonged excessive alcohol consumption in several studies [16, 19–23].

Furthermore, drinking studies [14, 24] with a repeated alcohol consumption leading to a BAC of 1 g/kg on each of five or ten subsequent days showed a correlation between degree of alcohol consumption and detected PEth concentrations. The potential of PEth in abstinence monitoring was shown in another drinking study: After a single drinking event leading to an estimated BAC of 1 g/kg and followed by an abstinence period of 2 weeks, PEth was detectable for 3 up to 12 days [25].

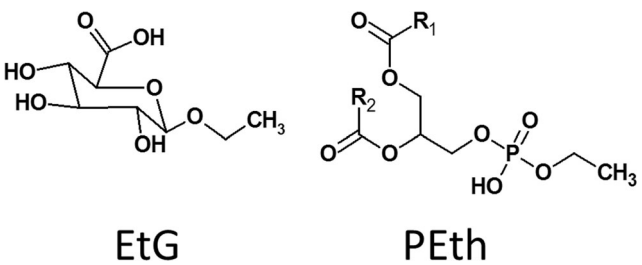

Fig. 1 Structural formulas of ethyl glucuronide (EtG) and phosphatidylethanol (PEth); R_1 and R_2, fatty-acyl-rests

In this study, we investigated the suitability of PEth in blood as an additional diagnostic marker for the assessment of drinking behavior by comparing the PEth concentrations in whole blood with EtG in proximal hair segments of up to 5 cm length, which is used in routine analysis for DAA in Switzerland and in Germany.

Material and methods

Study design

Blood and corresponding hair samples (hair segments of 0–5 cm) were obtained from 136 persons undergoing DAA at the Department of Traffic Sciences of the Institute of Forensic Medicine at the University of Bern, Switzerland. Samples were collected between December 2014 and December 2015. Blood was taken on the same day as hair samples and was analyzed for PEth homologues 16:0/18:1 and 16:0/18:2 by a validated online-SPE-LC-MS/MS method [25]. Hair was analyzed for EtG by a modified LC-MS/MS method according to Kummer et al. [26]. Hair samples were stored at room temperature. Blood samples were stored at 4 °C prior to analysis and were analyzed one week after sampling at the latest.

Chemicals and materials

PEth 16:0/18:1 and PEth 16:0/18:2 were provided by Avanti Polar Lipids (Alabaster, USA). EtG and deuterated EtG were purchased from Lipomed (Arlesheim, Switzerland). Ammonium acetate, acetone and dichloromethane were obtained from Merck (Darmstadt, Germany).

2-Propanol was obtained from Fisher Scientific (Loughborough, UK), methanol (MeOH) was from Biosolve BV (Valkenswaard, Netherlands) and acetonitrile (MeCN) was supplied by Agros Organics (New Jersey, USA). Formic acid (98 %) was provided by Fluka (Switzerland). HPLC solvents were of gradient grade; all other solvents were of analytical grade.

H_2O dest. was produced in-house with a Milli-Q water system from Millipore (Billerica, USA).

Deuterated standards were synthesized in our laboratory from phosphatidylcholine PC 16:0/18:1 and phosphatidylcholine PC 16:0/18:2, respectively, and D_6-ethanol catalyzed by phospholipase D [1].

EDTA monovettes (volume 3.4 mL) were obtained from Sarstedt (Nümbrecht, Germany). Lithium-heparinized whole blood, which was used as blank blood, was donated by volunteers who were abstinent from alcohol for at least 4 weeks. Blank hair samples were donated from teetotalers.

Determination of PEth in whole blood

PEth was analyzed in 200 μL of whole blood by online-SPE-LC-MS/MS with a QTrap 3200 mass spectrometer (AB Sciex, Toronto, Canada). Analytical separation was performed by a Luna RP-C5 column, 50 mm × 2 mm, 5 μm particle size (Phenomenex, Brechbühler, Schlieren, Switzerland), by gradient elution using ammonium acetate (10 mM)/MeCN (30:70, *v*/*v*) and 2-propanol. The precise details of the validated method can be found in [25].

Determination of EtG in hair

EtG was determined in hair with a limit of quantification (LOQ) of 3 pg/mg by a validated modified LC-MS/MS method according to [26] with a Ultimate 3000 LC-system (Dionex, Thermo Fisher Scientific, Reinach, Switzerland) coupled to a QTrap 5500 mass spectrometer (AB Sciex, Toronto, Canada). This method is used for routine analysis in the accredited laboratory (ISO 17025) of the Institute of Forensic Medicine, Bern, for samples from DAA according to the guidelines of the "Swiss Society of Legal Medicine" (Schweizerische Gesellschaft für Rechtsmedizin (SGRM)) [3].

Briefly, approximately 30 mg of hair was washed with three different solvents (4 mL each of H_20, acetone, and dichloromethane) by subsequent shaking for 4 min and removal of the solvent between washing steps. After 1 h of drying, the hair was grinded for 5 min (30 1/s) with a ball-mill (MM400, Retsch, Haan, Germany). The hair powder was extracted for 2 h by ultra-sonication with 1 mL D5-EtG containing deionized H_2O. Further purification of the extract was achieved by SPE with Oasis Max cartridges (3 cc/60 mg; Waters, Baden-Dättwil, Switzerland). The samples were analyzed in MRM mode with online preconcentration using a Hypercarb column, 3.0 × 2.1 mm, 5 μm (Thermo Fisher Scientific, Reinach, Switzerland), and after switching a two-way valve, back-flow elution to the analytical column Zorbax Eclipse, 50 mm × 4.6 mm, 1.8 μm (Agilent, MSP Kofel, Zollikofen, Switzerland) and gradient elution. The following transitions were used: EtG: m/z 221/75 as quantifier and m/z 221/85 as qualifier. For the deuterated internal standard D5-EtG, the transitions m/z 226/75 (quantifier) and m/z 226/85 (qualifier) were used.

Statistic evaluation

Receiver operating characteristic analysis (ROC) was performed with GraphPad Prism 6 by classifying the PEth results into three groups depending on hEtG thresholds, suggested by the SOHT (hEtG ≥ 30 pg/mg, excessive alcohol consumption; 7 pg/mg ≤ hEtG < 30 pg/mg, moderate alcohol consumption; hEtG < 7 pg/mg, "non-contradicting to self-reported abstinence") [10, 11].

Results

One hundred thirty-six samples from persons undergoing DAA were analyzed. The controlled persons were between 19 and 78 years old (mean age 42 ± 15 years), 88.2 % were male (120 male, 16 female).

The PEth and hEtG results were distributed into three groups: Excessive alcohol consumption, moderate alcohol consumption, and sobriety (abstinence from alcohol) according to the thresholds suggested by the SOHT [10, 11] for hEtG. For PEth 16:0/18:1, the proposed thresholds (210 and 20 ng/mL) were obtained from a Swedish study [27] and from results of drinking studies [14, 24, 25], meaning that PEth 16:0/18:1 values below 20 ng/mL (LOQ of the method) were rated as consistent with abstinence, and PEth 16:0/18:1 values above 210 ng/mL were classified to excessive alcohol consumption. For PEth 16:0/18:2, the following thresholds were used: PEth 16:0/18:2 ≥ 100 ng/mL, excessive alcohol consumption; 20 ng/mL ≤ PEth 16:0/18:2 < 100 ng/mL, moderate alcohol consumption; PEth 16:0/18:2 < 20 ng/mL, consistent with abstinence.

With hEtG analysis, excessive alcohol consumption was detected in 37.5 % of the samples; and 44.1 % of the samples were consistent with abstinence. Moderate alcohol consumption was detected in 18.4 % of the samples. With PEth 16:0/18:1, elevated alcohol consumption was detected only in 22.8 % of the samples, but 58.1 % of the samples were already consistent with abstinence. Moderate alcohol consumption was detected in 19.1 % of the samples.

Twenty-nine of 30 persons with PEth 16:0/18:1 ≥ 210 ng/mL had hEtG ≥ 30 pg/mg. Only one person was classified into the group of excessive alcohol consumers by PEth analysis whereas hEtG indicated moderate consumption (PEth 450 ng/mL, hEtG 29 pg/mg).

In 54 cases, neither PEth nor hEtG was detected. In eight cases, PEth was 20 ≤ PEth < 210 ng/mL, and hEtG was between 7 and 30 pg/mg, both markers showing moderate alcohol consumption.

Nine tested persons showed excessive alcohol consumption after analyzing hEtG, but PEth in blood was negative. There were no tested persons with negative hEtG values and PEth values above the threshold for excessive alcohol consumption of 210 ng/mL.

There was a little overlapping of different PEth and hEtG results between excessive and moderate alcohol consumption and between moderate alcohol consumption and abstinence. For example hEtG showed excessive alcohol consumption, but the corresponding PEth result showed moderate alcohol consumption (8.8 %), or hEtG showed moderate alcohol consumption, but PEth was not detectable (11.8 %). There were also tested persons with negative hEtG results, whereas PEth was detectable in blood, demonstrating moderate alcohol consumption (4.4 %). Thus, very recent alcohol consumption

might have taken place, which could not be detected in hair, because of the gap of approximately 14 days that hair needs to grow out of the scalp before it can be cut. All results are summarized in detail in Table 1.

As also the PEth homologue 16:0/18:2 was included in the column-switching LC-MS/MS method, this homologue was also compared to hEtG. Here, a value of PEth 16:0/18:2 ≥ 100 ng/mL was used as threshold to differentiate excessive from moderate alcohol consumption. PEth 16:0/18:2 values below 20 ng/mL (LOQ of the method) were regarded as consistent with abstinence. With these thresholds, the distributions of results obtained by PEth 16:0/18:1 were close to the results obtained by hEtG. With PEth 16:0/18:2 elevated alcohol consumption was detected in 22.8 % (hEtG: 37.5 %), abstinence was detected in 61.0 % (hEtG: 44.1 %) and moderate alcohol consumption was detected in 16.2 % (hEtG: 18.4 %) of the samples.

For PEth 16:0/18:1 and 16:0/18:2, the used thresholds to differentiate abstinence, moderate, and excessive alcohol consumption were evaluated by ROC analysis. Using the software GraphPad Prism 6, the PEth results were classified into three groups depending on hEtG (Fig. 2). All tested persons with hEtG ≥ 30 pg/mg were assumed to have prolonged excessive drinking habits, persons with hEtG between 7 and 30 pg/mg were classified to moderate alcohol consumption and persons with hEtG < 7 pg/mg were assumed to be abstainers or at least there was no contradiction to abstinence.

Using the suggested "Swedish threshold" of PEth 16:0/18:1 ≥ 210 ng/mL for the differentiation of excessive from moderate alcohol consumption, there is a specificity of 98.8 % and a sensitivity of 58.8 %. With a specificity of 100 % at a threshold of 470 ng/mL, there is only a sensitivity of 45.1 %. For the differentiation of excessive from moderate alcohol consumption, the PEth 16:0/18:1 threshold of 150 ng/mL resulted in the best sensitivity (70.6 %) and specificity (98.8 %) (Fig. 3a).

For the differentiation of drinking habits ("non-contradicting self-reported abstinence" from "moderate alcohol consumption"), the PEth 16:0/18:1 threshold of 20 ng/mL (LOQ) resulted in the best compromise for sensitivity (67.1 %) and specificity (90.0 %). With a specificity of 100 % at a threshold of 87 ng/mL, there is only a sensitivity of 53.9 %. Using the LOD of our method of 10 ng/mL, there is a specificity of 86.7 % and a sensitivity of 71.1 % (Fig. 3c). PEth 16:0/18:1 values between the suggested thresholds of 20 and 150 ng/mL would pass for moderate alcohol consumption habits.

Using the suggested "threshold" of PEth 16:0/18:2 ≥ 100 ng/mL, there is a specificity of 98.8 % and a sensitivity of 58.8 %. With a specificity of 100 % at a threshold of 332 ng/mL, there would only be a sensitivity of 33.3 %. For the differentiation of excessive from moderate alcohol consumption, the PEth 16:0/18:2 threshold of 96 ng/mL resulted in the best sensitivity (62.8 %) and specificity (98.8 %) (Fig. 3b).

For the differentiation of abstinence from moderate alcohol consumption, the PEth 16:0/18:2 threshold of 20 ng/mL (LOQ) resulted in the best compromise for sensitivity (63.2 %) and specificity (90.0 %). With a specificity of 100 % at a threshold of 93 ng/mL, there would only be a sensitivity of 44.7 %. Using the LOD of our method as threshold, which is 10 ng/mL, there is a specificity of 88.3 % and a sensitivity of 67.1 % (Fig. 3d). PEth 16:0/18:2 values between the suggested thresholds of 20 and 96 ng/mL would pass for moderate alcohol consumption habits.

Discussion

Although PEth and hEtG cover different time periods of alcohol consumption, PEth 16:0/18:1 (thresholds 210 and 20 ng/mL)

Table 1 Classification of alcohol consumption habits using hEtG and PEth 16:0/18:1 from 136 tested persons undergoing DAA

Drinking habits		PEth ≥ 210 ng/mL; PEth < 20 ng/mL	PEth ≥ 150 ng/mL; PEth < 20 ng/mL
hEtG	PEth	In % (number of samples)	In % (number of samples)
Excessive	Excessive	*22.1 (30)*	*26.5 (36)*
Excessive	Moderate	*8.8 (12)*	*4.4 (6)*
Moderate	Excessive	0.7 (1)	0.7 (1)
Moderate	Moderate	5.9 (8)	5.9 (8)
Abstinence*	Moderate	4.4 (6)	4.4 (6)
Moderate	Abstinence*	11.8 (16)	11.8 (16)
Abstinence*	Abstinence*	39.7 (54)	39.7 (54)
Excessive	Abstinence*	6.6 (9)	6.6 (9)
Abstinence*	Excessive	0 (0)	0 (0)

For PEth the following thresholds were used: PEth < 20 ng/mL: abstinence*; PEth ≥ 210 ng/mL, and after ROC analysis PEth ≥ 150 ng/mL: excessive alcohol consumption

*Non-contradicting self-reported abstinence according to SOHT [11]

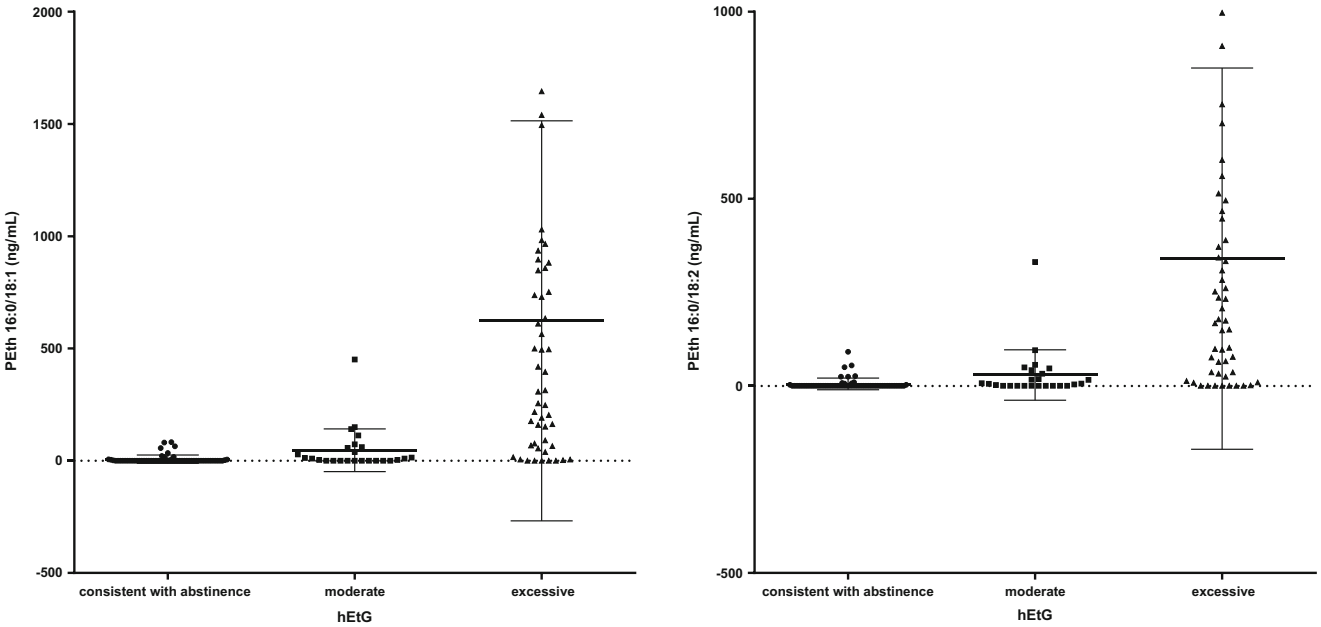

Fig. 2 Classification of PEth 16:0/18:1 and 16:0/18:2 results in comparison to hEtG (consistent with abstinence, hEtG < 7 pg/mg; moderate, 7 ≤ hEtG < 30 pg/mg; excessive, hEtG ≥ 30 pg/mg). Three data points (PEth 16:0/18:1 and PEth 16:0/18.2, respectively) are outside the axis limit in the column of excessive hEtG (PEth 16:0/18:1: 2155 ng/mL, PEth 16:0/18:2: 1495 ng/mL; PEth 16:0/18:1: 3545 ng/mL, PEth 16:0/18:2: 1945 ng/mL; PEth 16:0/18:1: 4870 ng/mL, PEth 16:0/18:2: 2685 ng/mL)

and hEtG results are in agreement in 68 % of tested persons undergoing DAA. By comparing PEth 16:0/18:2 (thresholds 100 and 20 ng/mL) to hEtG, 66 % of the samples are in agreement.

Using the thresholds resulting from ROC analysis, results are in agreement in 72 % of the samples for PEth 16:0/18:1 (thresholds 150 and 20 ng/mL) (see Table 1: numbers in line 2 and 3 in italics); for PEth 16:0/18:2, 68 % of the samples are in agreement in comparison to hEtG (PEth 16:0/18:2 thresholds 96 and 20 ng/mL).

Of the tested persons, who were classified into the group with moderate alcohol consumption habits (7 pg/mg ≤ hEtG < 30 pg/mg), only one person had a PEth 16:0/18:1 value above the threshold of 150 ng/mL (sensitivity 70.6 %, specificity 98.8 %) resulting from ROC analysis. However, the hEtG result is very close to be classified as excessive alcohol consumption (hEtG 29 pg/mg and PEth 450 ng/mL). These results show that all PEth values above this threshold are representing excessive alcohol consumption habits with a specificity of 98.8 %, and that the PEth 16:0/18:1 threshold for differentiation of excessive and moderate alcohol consumption can be lowered to 150 ng/mL for persons undergoing DAA.

There is a relatively high number of persons undergoing DAA, who showed excessive alcohol consumption habits (37.5 % detected by hEtG analysis, 22.8 % detected by PEth analysis). The reason for these findings could be that alcohol consumption habits were assessed soon after withdrawal of the driver's license, and drinking habits might not have been changed in the meantime.

The reasons for minor discrepancies between PEth and hEtG results are due to the different detection windows of the matrices and analytes utilized (blood: PEth, hair: EtG). As PEth has a shorter detection window (ca. 2–4 weeks) than hEtG (several months depending on the hair length) [8], changes in drinking behavior can be detected earlier by PEth than by hEtG analysis alone. Furthermore, PEth can be directly detected in blood a few hours after alcohol ingestion, whereas EtG first needs to be incorporated into the hair bulbus. As the scalp hair growth rate can vary from 0.6 to 1.5 cm/month [28] with an average rate of approximately 1 cm/month, and an additional time period for hair growth (of 10–14 days) has to take place until the incorporated EtG is found in the proximal segment of head hair, a segment of 5–6 cm length corresponds to a retrospective time window of approximately 5–6 months [3].

Therefore, PEth shows changes in drinking habits earlier than hEtG. Withdrawal or relapse to drinking can be shown directly after drinking or cessation of drinking by PEth analysis. Additional PEth analysis during DAA increases the sensitivity for uncovering alcohol consumption, although a single blood sampling cannot cover a 5-month period—which is the approximated period covered by EtG analysis with a 5-cm proximal hair segment.

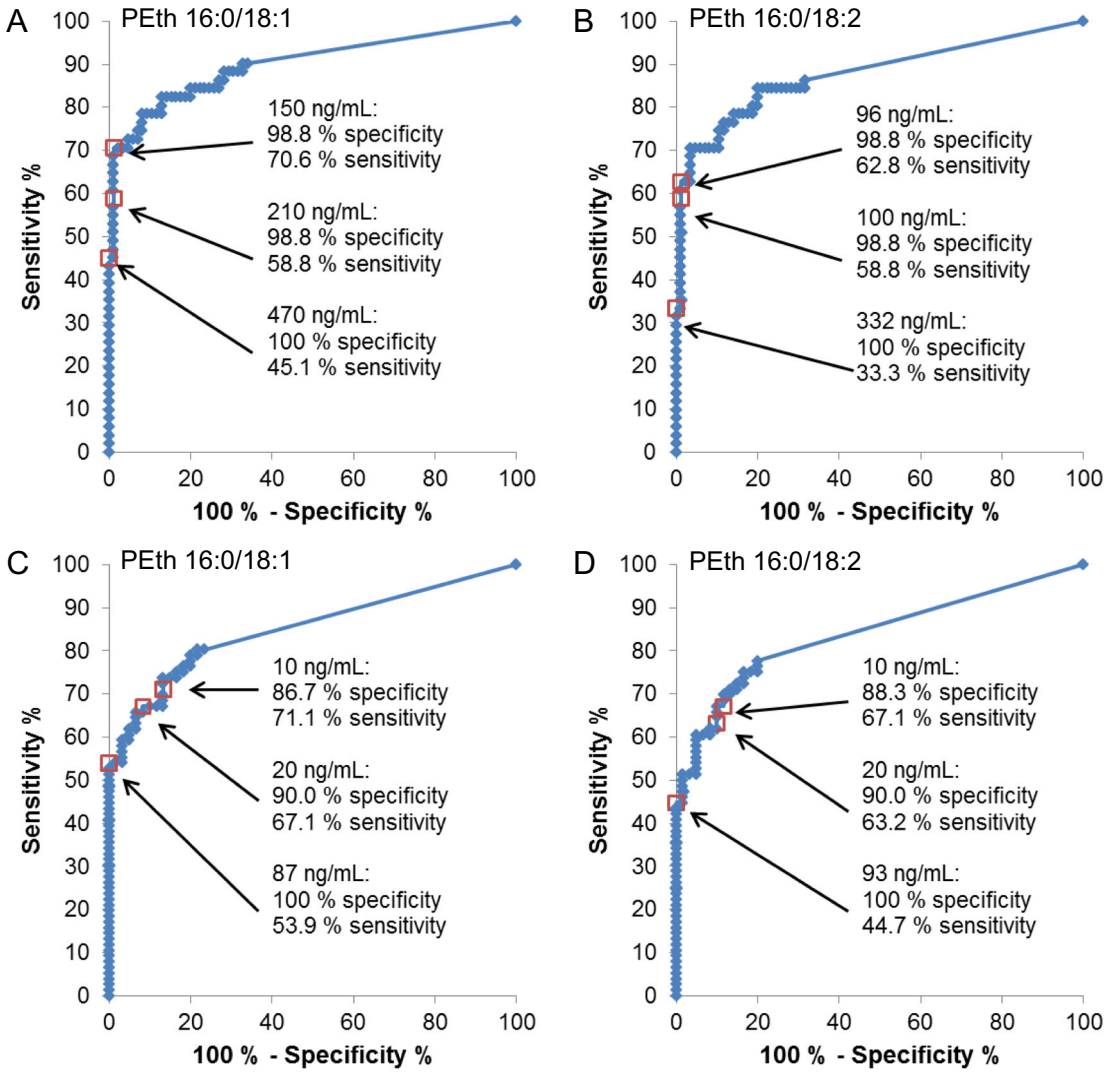

Fig. 3 ROC analysis for PEth vs. hEtG. **a** PEth 16:0/18:1 vs. hEtG to differentiate excessive from moderate alcohol consumption (AUROC 0.899, *P* value <0.0001). **b** PEth 16:0/18:2 vs. hEtG to differentiate excessive from moderate alcohol consumption (AUROC 0.876, *P* value <0.0001). **c** PEth 16:0/18:1 vs. hEtG to differentiate abstinence from moderate alcohol consumption (AUROC 0.85, *P* value <0.0001). **d** PEth 16:0/18:2 vs. hEtG to differentiate abstinence from moderate alcohol consumption (AUROC 0.84, *P* value <0.0001)

Conclusion

PEth is a valuable additional marker for DAA and helps to improve diagnostic information. As PEth has a shorter detection window (ca. 2–4 weeks) than hEtG (of a 5-cm proximal hair segment; approximately 5 months), changes in drinking behavior can be detected earlier by PEth in blood than by hEtG analysis alone. Another advantage of PEth analysis is that unlike hair, blood is always available in sufficient amounts.

Therefore, we strongly recommend including the two most abundant PEth homologues in human blood, PEth 16:0/18:1 and PEth 16:0/18:2, in routine analysis for DAA. PEth should be analyzed in 2–3 blood samples over 6 months, as elevated PEth concentrations would demonstrate a lack of abstinence which might cause, prior to re-granting the driver's license, a longer withdrawal, or, after re-granting the driver's license, a new withdrawal for a non-compliant person.

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Compliance with ethical standards

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References

1. Schröck A, Hernandez Redondo A, Martin Fabritius M, König S, Weinmann W (2016) Phosphatidylethanol (PEth) in blood samples from "driving under the influence" cases as indicator for prolonged excessive alcohol consumption. Int J Legal Med 130(2):393–400
2. The Federal Authorities of the Swiss Confederation (19. Dezember 1958 (effective: 20. May 2015)) Road Traffic Act Art. 15d. https://www.admin.ch/opc/de/classified-compilation/19580266/index.html. Accessed 22 Feb 2016
3. Schweizerische Gesellschaft für Rechtsmedizin (SGRM) (2014) Bestimmung von Ethylglucuronid (EtG) in Haarproben. http://www.sgrm.ch/uploads/media/EtG_FINAL_2014.pdf. Accessed 19 Jan 2016
4. Wurst FM, Yegles M, Alling C, Aradottir S, Dierkes J, Wiesbeck GA, Halter CC, Pragst F, Auwaerter V (2008) Measurement of direct ethanol metabolites in a case of a former driving under the influence (DUI) of alcohol offender, now claiming abstinence. Int J Legal Med 122(3):235–239
5. Seidl S, Wurst FM, Alt A (2001) Ethyl glucuronide-a biological marker for recent alcohol consumption. Addict Biol 6(3):205–212
6. Schwab N, Skopp G (2014) Identification and preliminary characterization of UDP-glucuronosyltransferases catalyzing formation of ethyl glucuronide. Anal Bioanal Chem 406(9–10):2325–2332
7. Bendroth P, Kronstrand R, Helander A, Greby J, Stephanson N, Krantz P (2008) Comparison of ethyl glucuronide in hair with phosphatidylethanol in whole blood as post-mortem markers of alcohol abuse. Forensic Sci Int 176(1):76–81
8. Schröck A, Thierauf A, Wurst FM, Thon N, Weinmann W (2014) Progress in monitoring alcohol consumption and alcohol abuse by phosphatidylethanol. Bioanalysis 6(17):2285–2294
9. Wurst FM, Thon N, Yegles M, Schröck A, Preuss UW, Weinmann W (2015) Ethanol metabolites: their role in the assessment of alcohol intake. Alcohol Clin Exp Res 39(11):2060–2072
10. Society of Hair Testing (SOHT) (2011) Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption 2011. http://www.soht.org/images/pdf/Revised%20Alcohol%20marker%20Consensus.pdf. Accessed 15 Apr 2014
11. Society of Hair Testing (SOHT) (2012) Use of Alcohol Markers in Hair for Abstinence Assessment 2012. http://www.soht.org/images/pdf/Use%20of%20Alcohol%20Markers%20in%20Hair%20for%20Abstinence%20Assessment%202012.pdf. Accessed 15 Apr 2014
12. Society of Hair Testing (SOHT) (2014) 2014 Consensus for the Use of Alcohol Markers in Hair for Assessment of both Abstinence and Chronic Excessive Alcohol Consumption. http://www.soht.org/images/pdf/2014%20Alcohol%20markers%20revision%2013JUN14%20FINAL.pdf. Accessed 05 Apr 2016
13. Gnann H, Weinmann W, Engelmann C, Wurst FM, Skopp G, Winkler M, Thierauf A, Auwarter V, Dresen S, Ferreiros Bouzas N (2009) Selective detection of phosphatidylethanol homologues in blood as biomarkers for alcohol consumption by LC-ESI-MS/MS. J Mass Spectrom 44(9):1293–1299
14. Gnann H, Weinmann W, Thierauf A (2012) Formation of phosphatidylethanol and its subsequent elimination during an extensive drinking experiment over 5 days. Alcohol Clin Exp Res 36(9):1507–1511
15. Gnann H, Engelmann C, Skopp G, Winkler M, Auwarter V, Dresen S, Ferreiros N, Wurst FM, Weinmann W (2010) Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS. Anal Bioanal Chem 396(7):2415–2423
16. Isaksson A, Walther L, Hansson T, Andersson A, Alling C (2011) Phosphatidylethanol in blood (B-PEth): a marker for alcohol use and abuse. Drug Test Anal 3(4):195–200
17. Varga A, Hansson P, Johnson G, Alling C (2000) Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics. Clin Chim Acta 299(1–2):141–150
18. Kobayashi M, Kanfer JN (1987) Phosphatidylethanol formation via transphosphatidylation by rat brain synaptosomal phospholipase D. J Neurochem 48(5):1597–1603
19. Viel G, Boscolo-Berto R, Cecchetto G, Fais P, Nalesso A, Ferrara SD (2012) Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. Int J Mol Sci 13(11):14788–14812
20. Winkler M, Skopp G, Alt A, Miltner E, Jochum T, Daenhardt C, Sporkert F, Gnann H, Weinmann W, Thierauf A (2013) Comparison of direct and indirect alcohol markers with PEth in blood and urine in alcohol dependent inpatients during detoxication. Int J Legal Med 127(4):761–768
21. Aradottir S, Asanovska G, Gjerss S, Hansson P, Alling C (2006) Phosphatidylethanol (PEth) concentrations in blood are correlated to reported alcohol intake in alcohol-dependent patients. Alcohol Alcohol 41(4):431–437
22. Nanau RM, Neuman MG (2015) Biomolecules and biomarkers used in diagnosis of alcohol drinking and in monitoring therapeutic interventions. Biomolecules 5(3):1339–1385
23. Mann K, Hoch E, Batra A, on behalf of the guideline working group (2016) S3-Leitlinie "Screening, Diagnose und Behandlung alkoholbezogener Störungen" AWMF-Register Nr. 076-001. http://www.awmf.org/uploads/tx_szleitlinien/076-001l_S3-Leitlinie_Alkohol_2016-02.pdf. Accessed 08 Mar 2016
24. Gnann H (2011) Phosphatidylethanol - Ein Alkoholkonsummarker auf dem Weg in die klinisch-forensische Routinediagnostik. Dissertation, University of Freiburg, Germany. http://www.freidok.uni-freiburg.de/volltexte/8428/pdf/Diss_Heike_Gnann.pdf. Accessed 15 Apr 2014: p. 101
25. Schröck A, Thierauf-Emberger A, Schürch S, Weinmann W (2016) Phosphatidylethanol (PEth) detected in blood for 3 to 12 days after single consumption of alcohol – a drinking study with 16 volunteers. Int J Legal Med. submitted in February 2016
26. Kummer N, Wille SM, Di Fazio V, Ramirez Fernandez Mdel M, Yegles M, Lambert WE, Samyn N (2015) Impact of the grinding process on the quantification of ethyl glucuronide in hair using a validated UPLC-ESI-MS-MS method. J Anal Toxicol 39(1):17–23
27. Helander A, Hansson T (2013) National harmonization of the alcohol biomarker PEth. Lakartidningen 110(39–40):1747–1748
28. Pianta A, Liniger B, Baumgartner MR (2013) Ethyl glucuronide in scalp and non-head hair: an intra-individual comparison. Alcohol Alcohol 48(3):295–302