Set-up of a population-based model to verify alcohol abstinence via monitoring of the direct alcohol marker phosphatidylethanol 16:0/18:1

Katleen Van Uytfanghe[1], Liesl Heughebaert[1], Emmanuel Abatih[2], Christophe P. Stove[1$]

1. Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium

2. Fostering Innovative Research Based on Evidence (FIRE), Ghent University, Krijgslaan 281, 9000 Ghent, Belgium

$ corresponding author: tel: +3292648135; christophe.stove@ugent.be

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List of abbreviations, in order cited

PEth, Phosphatidylethanol; VAMS, Volumetric absorptive microsampling; LC-MS/MS, Liquid chromatography – tandem mass spectrometry; LLOQ, Lower limit of quantification; QC, Quality control; CV, Coefficient of variation; PI, Prediction interval; ROC, Receiver operating characteristic; EtG, Ethylglucuronide; CDT, Carbohydrate deficient transferrin

Abstract (max 250 words – current 249)

BACKGROUND AND AIMS

Phosphatidylethanol 16:0/18:1 (PEth) is a biomarker for alcohol intake. It has a half-life of 7/8 days. Chronic alcohol consumption causes high PEth values. It can take weeks before PEth values fall below the decision limit for “alcohol abstinence”. Our aim was to validate whether alcohol abstinence can be determined based on two consecutive PEth results above the decision limit.

DESIGN

Observational study.

SETTING

Belgium, February 2019. The study was linked to a social initiative in Belgium, “Tournée Minérale”.

PARTICIPANTS

Adults (>18 years, n=796) with varying drinking habits who self-reportedly refrained from alcohol consumption during the study.

MEASUREMENTS

A validated liquid chromatography – tandem mass spectrometry method was used to quantify PEth in participants’ dried blood samples, collected at 3 time points via remote fingerprick-based self-sampling.

FINDINGS

A population-based algorithm to evaluate abstinence based on 95% prediction limits was developed by fitting a linear mixed effect model to discern patterns in PEth elimination over time. It took intra- and interindividual variability into consideration. The algorithm was included in a 2-step decision tree, assessing whether (i) PEth values fell within the prediction interval, and (ii) the slope between 2 PEth values was consistent

with no alcohol consumption. Data for 74 participants reporting no alcohol intake during the study were used for validation. With a detection limit of “4 units spread over 14 days”, the sensitivity and specificity of the decision tree was 89%.

CONCLUSIONS

Claims of alcohol abstinence can be verified using a 2-step decision tree for phosphatidylethanol 16:0/18:1 (PEth) values, even when those values are above the limit for “alcohol abstinence”.

Keywords (6-10): Phosphatidylethanol, half-life, abstinence testing, elimination rate, prediction model, alcohol biomarker

INTRODUCTION

Phosphatidylethanol is an important direct biomarker for alcohol intake (1-4). Phosphatidylethanol refers to a range of different phosphatidylethanol analogues, with the most abundant analogue phosphatidylethanol 16:0/18:1 (in what follows 'PEth') being typically monitored (5-9). Alone or in combination with other biomarkers, monitoring of PEth increases the sensitivity of detecting alcohol consumption (1, 5, 10, 11). The meaning of PEth values and changes in these values in relation to alcohol consumption is still not fully understood. Information available from existing studies is limited because the findings are based on (i) a limited number of persons with excessive alcohol intake being abstinent for only a few weeks or on (ii) very specific patient groups (5, 6, 11-14). However, the main application is testing individuals from a more general population for (a change in) their drinking behavior (e.g. in the context of driver's license regranting or workplace testing). This is the focus of only two smaller studies (15, 16). Non-detectable PEth values clearly indicate no alcohol consumption ('abstinence'). There is also growing consensus to refer to PEth values below 20 ng/mL (0.028 µM) as 'being compatible with abstinence or minimal alcohol consumption during the weeks prior to sampling' (17-19). This threshold takes other alcohol exposures into consideration such as use of alcohol-containing mouthwash solutions (20). Given the half-life of this biomarker, it can take weeks or months for PEth values to fall below 20 ng/mL (0.028 µM) after cessation of alcohol consumption. This can make the interpretation of PEth values difficult, especially when monitoring individuals with alcohol use disorders.

The goal of this study was to obtain information on PEth values at the start of the study and the changes in PEth values in volunteers (with varying drinking habits) who self-reportedly refrained from alcohol consumption during the study. Two more objectives

were further investigated: (i) the half-life, in comparison to existing data (5, 12, 21-27); and (ii) the possibility to identify abstinence based on two PEth results above the decision limit for “alcohol abstinence”, performed several weeks apart.

MATERIALS AND METHODS

Study population

Participants

Adults (>18 years) who usually drink alcohol but who self-reportedly refrained from alcohol consumption for one month were included. Participants were recruited via the ‘Tournée Minérale’ initiative (see **Supplemental S1** and reference (28)). The Ghent University Hospital Ethics Committee approved the study (reference 2018/1514). Consenting participants registered via a dedicated website. The only incentives to participate were that participants would get insight in (i) their own drinking behavior compared to their ‘peers’ (i.e. the other participants, anonymized) and (ii) how their PEth levels would decrease while abstaining from alcohol consumption.

Sampling by the participants

Participants received a sampling kit at the end of January, 2019. (see **Supplemental S1**). They collected three fingerprick dried blood microsamples (early, mid and end of February 2019) via self-sampling at home using 10 µL volumetric absorptive microsampling (VAMS) devices (Mitra®, Neoteryx). To maximize correct self-sampling, participants were instructed remotely via a leaflet and a video (see **Supplemental S2**), as well as via e-mail. Following the instructions, after sampling, the blood in the VAMS devices was dried for at least two hours before being put in a sachet with desiccant.

The devices were stored at room temperature, protected from direct sunlight. Samples were sent via mail to the laboratory.

Questionnaire – participant information

A questionnaire was provided along with the sampling kit to collect information about the participants' (i) demographics; (ii) general alcohol drinking behavior (using the AUDIT-C screening questionnaire) (29); (iii) index of mean weekly consumption in January (consumed units/week) and (iv), an alcohol consumption diary for February (in units/day, for those that failed to remain abstinent). To help estimate how many units of alcohol the participants consumed (one unit being defined as ~10 g ethanol), we provided examples of different standard drinks. We limited the personal information to height, weight, sex, age, ethnicity and relevant medical background such as gastric bypass, sleeve gastrectomy or any stomach/intestinal problems (**Supplemental S3**).

Phosphatidylethanol measurement

We quantified PEth 16:0/18:1 using a validated and ISO17025-accredited liquid chromatography – tandem mass spectrometry (LC-MS/MS) method with a limit of detection of 1.7 ng/mL (0.002 µM) (18). The lower limit of quantitation (LLOQ) in this study, defined as level with a CV of 20%, was 4 ng/mL (>0.006 µM), **Supplemental S4**. In brief, we extracted PEth from VAMS samplers by adding 250 µL of extraction solvent (2 mM ammonium acetate 0.01% formic acid in a 2/8/0.2 water/isopropanol/formic acid mixture) and 60 µL methanol containing 25 ng/mL (0.034 µM) PEth-D5 as internal standard. Following liquid-liquid extraction using 1 mL n-hexane, we collected the n-hexane fraction, dried and reconstituted it in 50 µL of injection solvent, and injected 5 µL onto an XBridge BEH Phenyl XP column. We used

a Shimadzu Prominence LC coupled to a QTRAP 5500 instrument (SCIEX) for LC-MS/MS analysis.

We analyzed all three samples obtained from one participant within one analytical run. Each analytical run included a calibration curve (blank and 8 calibrators, measured at the start and end of the run), the three samples from 30 participants, and duplicates of two different internal QC samples.

Data analysis

We designed the study initially as a descriptive study. Analysis plans were not pre-registered and were exploratory. Power calculations to estimate the minimal sample size were not performed.

We used data from all participants for demographics and descriptive statistics.

For data analysis on dependent variables and covariates, we used data from participants not reporting to have consumed alcoholic beverages during the study, and only using PEth values >4 ng/mL (>0.006 µM).

All statistical tests were performed at the 95% confidence level.

Demographics and descriptive statistics: phosphatidylethanol concentrations during the study

We used Microsoft Excel for Office 365 for basic data analysis and MedCalc, version 19.5.1 for statistics and regression analysis. Due to violations of parametric assumptions, we used mainly non-parametric tests (Mann-Whitney test, Kruskal-Wallis test, spearman's coefficient or rank correlation (rho) and Chi2 test).

Dependent variables: half-life

We calculated the half-life ($t_{1/2}$) using the following equation: $t_{1/2} = t * ln(2)/ln(N_0/N_t)$ (t: time-interval between N_0 and N_t, N_0: PEth concentration at the start, N_t: PEth concentration at the end), over three time intervals: early – mid, mid – end and early - end February. Outliers were identified using the method of Tukey, after logarithmic transformation (30). Within-subject and between-subject variation in half-life was calculated using ANOVA. Weighted linear regression (1/x) was used to estimate the relation between the PEth half-life and the initial PEth concentration.

Covariates: prediction of the decrease of PEth values in time – evaluation of abstinence

We established a prediction model by fitting a linear regression based mixed effect model to discern patterns in PEth elimination over time. We used normalized PEth scores (i.e. the log(result +1)) of time point 1, 2 or 3 which were divided by the log(result +1) of time point 1) as response variable and time as the fixed independent variable. Log transformations were used to ensure the linearity assumption was met. We used random effects to account for intra- and interindividual variability in PEth scores. Modeling was done using R 4.0.2. Details can be found in **Supplemental S5.**

We excluded from the data analysis results of participants (n=21) for whom the PEth concentration at the second or third sampling exceeded that of the first and/or second sampling, or those which were within 1 ng/mL (0.0015 µM) of each other (which is indicative of not being abstinent). As such, 1031 data points were available to set up the model, with an accompanying prediction interval (95% PI).

We incorporated the prediction model in a decision tree to objectively support or reject a claim of abstinence in individual cases. If, when applying the prediction model, the result was outside the PI, this was considered compatible with not being abstinent. For results within the PI, we further considered the slope of the regression line between

two consecutive samplings. When the difference of an individual slope versus the slope of the regression function of the prediction model exceeded the 99-centile of the differences for the slopes of all individual data pairs in the dataset used to establish the prediction model, this was also considered as compatible with not being abstinent. We evaluated the validity of the decision tree with two subgroups: (i) participants (n=44) that reported drinking 1 or more units in February 2019; (ii) participants reporting to have drunk alcohol free (i.e. ≤0.5% alcohol content) beverages, or having eaten liquor-containing sweets (n=30) - these were considered “negatives”. We calculated the sensitivity, specificity, positive and negative predictive values (31). We established a receiver operating characteristic (ROC) curve by varying the reported alcohol intake in the first subgroup in steps of “2 units consumed spread over 14 days” as the definition of “positives”.

Application in a forensic context

We tested the applicability of the decision tree in cases from our routine toxicology work (n=12).

RESULTS

Study population

Within 5 days after the launch, subscription to the study was closed as sufficient participants (796) had been recruited. 687 (86%) participants completed the entire study and sent back the sampling kit. **Figure 1** illustrates the loss in participants based on the feedback received. Most received packages (664, 97%) were complete (both the questionnaire and the three samples were present). Of the sample sets, 570 (85%) contained samples of good quality in three subsequent samplings. **Supplemental S6** provides an overview of the participants' demographics. The majority of the participants was female (426, 63%) and European (668, 99%), with an even spread in the age range 18 to 60 years. The index of mean weekly consumption in January ranged from none to over 50 units (see **Supplemental S7**), with 268 (40%) of the participants reporting to drink 1-5 units per week. From the AUDIT-C questionnaire we derived that the largest cohort of our participants (268, 40%) drinks two or three times a week, with a frequency of one or two drinks per day (297, 44%). With respect to binge drinking (more than 6 units in succession), 249 (37%) participants reported to do this less than monthly. **Supplemental S7** provides the details on the answers to the AUDIT-C questionnaire, and the final AUDIT-C score. Using the Mann-Whitney test, statistically significant differences between the AUDIT-C scores for men (median 6; n=242) and women (median 4; n=422) were seen ($p < 0.0001$). The findings were inconclusive as to whether or not differences in AUDIT-C scores were observed for the different age categories, with a median of 4 or 5 in all categories (18-25; n=76, 26-30; n=105, 31-35; n=70, 36-40; n=92; 41-45; n=83, 46-50; n=77, 51-55; n=64, 56-60; n=49, 61-80; n=47).

Phosphatidylethanol concentrations during the study

As no minimum consumption of alcohol was specified, also participants that had only consumed one or a few units of alcohol in January were present in our study, yielding PEth values that were very low or not quantifiable at the onset of the study. Nevertheless, from all samples taken early February, 572 (84%) had quantifiable PEth levels (>4 ng/mL (>0.006 µM)). Almost four weeks later, this had decreased to 273 (41%). **Figure 2** shows the PEth levels and the summary statistics according to the sampling moment. For both the AUDIT-C score and the index of mean weekly consumption, a correlation was found with the first PEth level (see **Supplemental S8**), with a Rho of 0.71 and 0.70, respectively ($p < 0.0001$). Rho between the two measures of alcohol intake itself (index of mean weekly consumption and AUDIT-C score) was 0.77 ($p < 0.0001$).

Half-life

The box-and Whisker plots of the calculated half-lives, excluding outliers and samples from individuals that claimed to have been drinking, are shown in **Supplemental S9**. The median and range of the different half-lives are shown in **Table 1**, the overall median half-life being 7.9 days. Using the Kruskal-Wallis test, statistically significant differences were seen between the three calculated half-lives ($p<0.001$). More specifically, a statistically significant difference was seen between the half-life of PEth early - mid February and the other two half-lives. Within-subject and between-subject variations in half-life were 1.7 days and 1.0 day, respectively. The findings were inconclusive as to whether or not there is an association between sex or AUDIT-C score and the PEth half-lives ($p>0.05$; Mann-Whitney; see **Table 1**). The same applied to the relation of the PEth half-life and the initial PEth concentration ($r<0.1$ and $p>0.05$;

Figure 3). There is no evidence that high PEth levels eliminate faster or slower than low PEth levels and *vice versa*.

To exclude that the median half-life reported would be skewed due to unreported drinking by a subset of the participants, we re-assessed it, arbitrarily excluding the 10% highest PEth half-lives. This only lowered the median half-lives by ≤0.2 days.

Prediction of the decrease of PEth-values in time – evaluation of abstinence

Supplemental S5 describes the R-script (also available online via https://peth.be/app), along with the details of the prediction model, as well as its validation. This prediction model was included in a decision tree to assess abstinence of persons for whom two or three quantifiable PEth values are available, at time points separated one to several weeks in time, see **Figure 4.** It should allow to make an objective statement on whether an individual has remained abstinent or not since the prior sampling. **Figure 4** also shows examples of the data used to assess sensitivity/specificity. **Table 2** and the ROC curve in **Figure 5** show the change in false positive versus true positive rate when the cut-off for defining a sample as positive was changed in steps of “2 units drunk over a period of 14 days”. If “4 units over 14 days” would still be allowed, sensitivity and specificity are both 89%. The latter means that at least the first of those 4 units was drunk 14 days before the sampling.

Application in a forensic context

We implemented the decision tree successfully in our routine toxicology lab (see **Supplemental S10**), either as part of a driving license regranting procedure, or in cases related to organ transplantations.

DISCUSSION

Summary

This study is the largest to date to study the decline in PEth values in participants with a broad range of alcohol use who refrained from alcohol consumption during the study. The recruitment of such a large cohort of participants was only possible via the social initiative “Tournée Minérale”. The study had a relatively low drop-out rate, which could be attributed to a simplified specimen collection procedure that can be performed by the participants at home. The collected samples were sent to the lab via regular mail. The data collected in this study allowed to gain unique insights in the variability of the PEth elimination rate. This enabled the development of a population-based algorithm to estimate cessation of alcohol consumption with 95% probability in individuals in whom PEth-values were not yet below the decision limit for “alcohol abstinence”. We finally developed a decision tree which yields a sensitivity/specificity of 89% to score compatibility with abstinence by looking at the relation between 2 consecutive PEth values and with a detection limit of “a consumption of 4 units of alcohol spread over 14 days”.

Strengths

The strengths of the study are 3-fold. First, we were able to collect samples from participants with a broad range of alcohol use, from almost abstainers to persons with excessive alcohol intake (reflected by the initial PEth levels: <4 to 1142 ng/mL). Second, although non-supervised sampling inherently implies a risk, sampling can be considered reliable. This is discussed in detail elsewhere and can be concluded based on (i) the limited requests for extra sampling material, (ii) visual control of the samples (only 9% being scored as under- or overfilled), (iii) assessment of samples taken at the

same sampling moment, and (iv) a questionnaire (32). Third, by implementing VAMS as a sampling strategy, self-sampling at home allowed the entire study to be conducted remotely. This, in combination with the high response rate, the good quality of the samples and the successful implementation in our routine forensic toxicology work demonstrates the applicability of the testing strategy we put forward in this manuscript. Major application fields we envisage are driving license regranting programs, monitoring of transplant patients or randomized clinical trials.

Limitations

The limitations of the study are threefold. First, it is possible that the self-reported alcohol consumption of January was underestimated (33, 34). The questionnaire was only sent out in late January, asking the participants to recall their alcohol use. This is not a real issue as these data were only used to get a rough idea on how to classify the participants. It is noteworthy though that we did find consistency between the AUDIT-C scores and the self-reported alcohol consumption, as confirmed by their good correlation (0.77, $p < 0.0001$) and the fact that also PEth levels correlated well with these two measures of alcohol intake. These two observations, which also align with findings from other studies, indicate that at least the majority of the participants completed the questionnaire rather truthfully (35-38).

Second, as the study was performed remotely, there was no witnessed sampling and participants could have falsified the samples. While it should be noted that there would be no reason to do so, as inclusion was voluntarily, unpaid, and in principle on the participant's request, we cannot exclude this theoretical possibility. Third, there was no objective assessment of abstinence using another biomarker. The latter was not possible due to the study design. Measuring urinary ethylglucuronide (EtG) regularly

or providing all 796 participants with a e.g transdermal sensor for alcohol monitoring was not feasible and would require an entirely different study set-up. However the trustworthiness of the responses as mentioned before, combined with the fact that there was no consequence whatsoever if the participants failed to remain abstinent, strengthens the belief that the vast majority of the participants did sample themselves or would have declared alcohol consumption. The fact that we might have included some individuals that may have been drinking limited amounts of alcohol during the study actually implies a reduced risk of false positives, or that the developed decision tree might underpredict. On the other hand, the robustness of our model is supported by the unprecedented large size of our data set and the robustness of the median half-life to the exclusion of the top 10% half-lives. We therefore believe it does include many possible variables and confounders that may also be present in a real-life setting. Given the potentially severe consequences of false positives (i.e. falsely scoring an individual as non-abstinent), this gives the benefit of the doubt to the individual.

Comparison to existing literature

PEth half-life

Earlier reports on the half-life of PEth stem from studies that were conducted under more controlled conditions i.e., with regards to participation, however those studies were conducted with small study populations (n up to 57) and/or over shorter times (around 14 days) (5, 12, 21-27). The design of this study, using a larger population and longer duration, determined a half-life of 7.9 days, which is consistent with findings by other recent studies. (5, 12). The finding that the half-life calculated for the first half of the study is shorter than that for the second part, or for the complete study, can be related to a steeper decline of PEth levels early on in the elimination phase (5, 39).

Being aware of this, we aimed at minimizing this effect by asking participants to start sampling only on the second day after the start of cessation of alcohol consumption (12, 21). The inter-individual variability (1 day, 13%) in PEth half-life is consistent with previous observations (5, 12, 22, 27), and could not be associated with sex, AUDIT-C score or initial PEth level. The intra-individual variability was of the same magnitude (1.7 days, 21%).

Evaluation of abstinence

It is easy to conclude abstinence when PEth levels are not detectable (21, 22). However, this is less evident when PEth values are above the decision limit (i.e. 20 ng/mL (0.028 µM)). Therefore, we developed a decision tree with the intention to verify cessation of alcohol intake in individuals before their PEth values fall below the decision limit. This is particularly relevant for people who had an excessive alcohol intake and for whom it can take weeks to have PEth values below the decision limit. In this study, final PEth values lay above the decision limit in 11% (n=58) of the participants reporting to not have drunk during the study.

It is generally accepted that not a single testing strategy is capable of identifying abstinence with 100% selectivity and specificity in routine settings. Approaches such as daily monitoring of alcohol in blood or urine, twice a week monitoring of EtG in urine, monitoring carbohydrate deficient transferrin (CDT) in plasma, monitoring of EtG in hair, or using a combination of these biomarkers have limitations. These include (i) the short detection window for alcohol itself or EtG in urine or blood (1), (ii) the specificity and sensitivity of CDT in individuals with occasional alcohol consumption (40), (iii) the detection of EtG in hair requires a sufficient alcohol intake and, moreover, may be masked by hair treatment (41, 42). Our testing strategy has a detection limit of “4 units spread over 14 days”. This is in line with other reports on the detectability of a single

dose intake of ethanol (21, 22, 43). Overall, our testing strategy, assessing the relation between 2 consecutive PEth values, yielded a sensitivity/specificity of 89% to score compatibility with abstinence. This approach offers an added-value compared to other well-established procedures assessing biomarkers for ethanol intake.

Conclusion

In conclusion, this study allowed us to (i) get insight into the (intra- and inter-individual variability in) PEth half-life and (ii) to establish a population-based prediction model to verify claims of abstinence, even when PEth values are still above the decision limit for compliance with abstinence. The described approach appears useful to monitor short- to mid-term cessation of alcohol consumption.

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REFERENCES

1. Andresen-Streichert H, Muller A, Glahn A, Skopp G, Sterneck M. Alcohol Biomarkers in Clinical and Forensic Contexts. Deutsches Arzteblatt international. 2018;115(18):309-15.
2. Nanau RM, Neuman MG. Biomolecules and Biomarkers Used in Diagnosis of Alcohol Drinking and in Monitoring Therapeutic Interventions. Biomolecules. 2015;5(3):1339-85.
3. Nguyen VL, Haber PS, Seth D. Applications and Challenges for the Use of Phosphatidylethanol Testing in Liver Disease Patients (Mini Review). Alcoholism, clinical and experimental research. 2018;42(2):238-43.
4. Ulwelling W, Smith K. The PEth Blood Test in the Security Environment: What it is; Why it is Important; and Interpretative Guidelines. Journal of forensic sciences. 2018;63(6):1634-40.
5. Helander A, Bottcher M, Dahmen N, Beck O. Elimination Characteristics of the Alcohol Biomarker Phosphatidylethanol (PEth) in Blood during Alcohol Detoxification. Alcohol and alcoholism (Oxford, Oxfordshire). 2019;54(3):251-7.
6. Gnann H, Thierauf A, Hagenbuch F, Rohr B, Weinmann W. Time dependence of elimination of different PEth homologues in alcoholics in comparison with social drinkers. Alcoholism, clinical and experimental research. 2014;38(2):322-6.
7. Aboutara N, Jungen H, Szewczyk A, Sterneck M, Muller A, Iwersen-Bergmann S. Analysis of six different homologues of phosphatidylethanol from dried blood spots using liquid chromatography-tandem mass spectrometry. Drug Test Anal. 2021;13(1):140-7.
8. Gnann H, Engelmann C, Skopp G, Winkler M, Auwarter V, Dresen S, et al. Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS. Analytical and bioanalytical chemistry. 2010;396(7):2415-23.
9. Nalesso A, Viel G, Cecchetto G, Mioni D, Pessa G, Favretto D, et al. Quantitative profiling of phosphatidylethanol molecular species in human blood by liquid chromatography high resolution mass spectrometry. Journal of chromatography A. 2011;1218(46):8423-31.
10. Simon TW. Providing context for phosphatidylethanol as a biomarker of alcohol consumption with a pharmacokinetic model. Regulatory toxicology and pharmacology : RTP. 2018;94:163-71.
11. Rohricht M, Paschke K, Sack PM, Weinmann W, Thomasius R, Wurst FM. Phosphatidylethanol Reliably and Objectively Quantifies Alcohol Consumption in Adolescents and Young Adults. Alcoholism, clinical and experimental research. 2020.
12. Hill-Kapturczak N, Dougherty DM, Roache JD, Karns-Wright TE, Javors MA. Differences in the Synthesis and Elimination of Phosphatidylethanol 16:0/18:1 and 16:0/18:2 After Acute Doses of Alcohol. Alcoholism, clinical and experimental research. 2018;42(5):851-60.
13. Moore KE, Santiago Rivera OJ, Anderson B, Johnson JE, Hahn JA, Kurth ME, et al. Phosphatidylethanol Levels Among Incarcerated Women: The Influence of Pre-incarceration Alcohol Consumption and Length of Abstinence. Alcoholism, clinical and experimental research. 2018;42(3):500-7.
14. Neumann J, Beck O, Helander A, Bottcher M. Performance of PEth Compared With Other Alcohol Biomarkers in Subjects Presenting For Occupational and Pre-Employment Medical Examination. Alcohol and alcoholism (Oxford, Oxfordshire). 2020;55(4):401-8.
15. Schrock A, Hernandez Redondo A, Martin Fabritius M, Konig S, Weinmann W. Phosphatidylethanol (PEth) in blood samples from "driving under the influence" cases as indicator for prolonged excessive alcohol consumption. International journal of legal medicine. 2016;130(2):393-400.
16. Bean P, Brown G, Hallinan P, Becerra S, Lewis D. Improved recovery of repeat intoxicated drivers using fingernails and blood spots to monitor alcohol and other substance abuse. Traffic Injury Prevention. 2017;18(1):9-18.
17. Reisfield GM, Teitelbaum SA, Opie SO, Jones J, Morrison DG, Lewis B. The roles of phosphatidylethanol, ethyl glucuronide, and ethyl sulfate in identifying alcohol consumption among participants in professionals health programs. Drug Test Anal. 2020;12(8):1102-8.

18. Van Uytfanghe K, Ramirez Fernandez MDM, De Vos A, Wille SM, Stove CP. Quantitation of phosphatidylethanol in dried blood after volumetric absorptive microsampling. Talanta. 2021;223(Pt 1):121694.
19. Helander A, Hansson T. [National harmonization of the alcohol biomarker PEth]. Lakartidningen. 2013;110(39-40):1747-8.
20. Reisfield GM, Teitelbaum SA, Jones JT, Mason D, Bleiweis M, Lewis B. Blood Phosphatidylethanol (PEth) Concentrations Following Regular Exposure to an Alcohol-Based Mouthwash. Journal of analytical toxicology. 2020.
21. Schrock A, Thierauf-Emberger A, Schurch S, Weinmann W. Phosphatidylethanol (PEth) detected in blood for 3 to 12 days after single consumption of alcohol-a drinking study with 16 volunteers. International journal of legal medicine. 2017;131(1):153-60.
22. Javors MA, Hill-Kapturczak N, Roache JD, Karns-Wright TE, Dougherty DM. Characterization of the Pharmacokinetics of Phosphatidylethanol 16:0/18:1 and 16:0/18:2 in Human Whole Blood After Alcohol Consumption in a Clinical Laboratory Study. Alcoholism, clinical and experimental research. 2016;40(6):1228-34.
23. Varga A, Hansson P, Johnson G, Alling C. Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics. Clinica chimica acta; international journal of clinical chemistry. 2000;299(1-2):141-50.
24. Wurst FM, Thon N, Aradottir S, Hartmann S, Wiesbeck GA, Lesch O, et al. Phosphatidylethanol: normalization during detoxification, gender aspects and correlation with other biomarkers and self-reports. Addiction biology. 2010;15(1):88-95.
25. Nguyen VL, Paull P, Haber PS, Chitty K, Seth D. Evaluation of a novel method for the analysis of alcohol biomarkers: Ethyl glucuronide, ethyl sulfate and phosphatidylethanol. Alcohol (Fayetteville, NY). 2018;67:7-13.
26. Gnann H, Weinmann W, Thierauf A. Formation of phosphatidylethanol and its subsequent elimination during an extensive drinking experiment over 5 days. Alcoholism, clinical and experimental research. 2012;36(9):1507-11.
27. Viel G, Boscolo-Berto R, Cecchetto G, Fais P, Nalesso A, Ferrara SD. Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. International journal of molecular sciences. 2012;13(11):14788-812.
28. Tournée Minérale 2021 [Available from: https://www.tourneeminerale.be/.
29. Bradley KA, DeBenedetti AF, Volk RJ, Williams EC, Frank D, Kivlahan DR. AUDIT-C as a brief screen for alcohol misuse in primary care. Alcohol Clin Exp Res. 2007;31(7):1208-17.
30. Tukey JW. Exploratory Data Analysis: Addison-Wesley, Reading, MA; 1977.
31. Simundic AM. Measures of Diagnostic Accuracy: Basic Definitions. EJIFCC. 2009;19(4):203-11.
32. Van Uytfanghe K, Heughebaert L, Stove CP. Self-sampling at home using volumetric absorptive microsampling: coupling analytical evaluation to volunteers' perception in the context of a large scale study. Clinical chemistry and laboratory medicine. 2020.
33. Poikolainen K. Underestimation of recalled alcohol intake in relation to actual consumption. British journal of addiction. 1985;80(2):215-6.
34. Livingston M, Callinan S. Underreporting in alcohol surveys: whose drinking is underestimated? Journal of studies on alcohol and drugs. 2015;76(1):158-64.
35. Cherrier MM, Shireman LM, Wicklander K, Yeung W, Kooner P, Saxon AJ, et al. Relationship of Phosphatidylethanol Biomarker to Self-Reported Alcohol Drinking Patterns in Older and Middle-Age Adults. Alcoholism, clinical and experimental research. 2020;44(12):2449-56.
36. Piano MR, Tiwari S, Nevoral L, Phillips SA. Phosphatidylethanol Levels Are Elevated and Correlate Strongly with AUDIT Scores in Young Adult Binge Drinkers. Alcohol and alcoholism (Oxford, Oxfordshire). 2015;50(5):519-25.
37. Afshar M, Burnham EL, Joyce C, Clark BJ, Yong M, Gaydos J, et al. Cut-Point Levels of Phosphatidylethanol to Identify Alcohol Misuse in a Mixed Cohort Including Critically Ill Patients. Alcoholism, clinical and experimental research. 2017;41(10):1745-53.

38. Schrock A, Wurst FM, Thon N, Weinmann W. Assessing phosphatidylethanol (PEth) levels reflecting different drinking habits in comparison to the alcohol use disorders identification test - C (AUDIT-C). Drug and alcohol dependence. 2017;178:80-6.
39. Luginbuhl M, Weinmann W, Butzke I, Pfeifer P. Monitoring of direct alcohol markers in alcohol use disorder patients during withdrawal treatment and successive rehabilitation. Drug Test Anal. 2019;11(6):859-69.
40. Bortolotti F, Sorio D, Bertaso A, Tagliaro F. Analytical and diagnostic aspects of carbohydrate deficient transferrin (CDT): A critical review over years 2007-2017. J Pharm Biomed Anal. 2018;147:2-12.
41. Jones AW. Alcohol, its absorption, distribution, metabolism, and excretion in the body and pharmacokentic calculations. Wires Forensic Sciences. 2019;1:e1340.
42. Walsham NE, Sherwood RA. Ethyl glucuronide and ethyl sulfate. Advances in clinical chemistry. 2014;67:47-71.
43. Helander A, Hermansson U, Beck O. Dose-Response Characteristics of the Alcohol Biomarker Phosphatidylethanol (PEth)-A Study of Outpatients in Treatment for Reduced Drinking. Alcohol and alcoholism (Oxford, Oxfordshire). 2019;54(6):567-73.

TABLES

Table 1. Mean, median and SD of the three half-lives, for the complete dataset and according to sex or AUDIT-C score. Note, calculations are based on available data, i.e. for some individuals gender or AUDIT-C score were missing. For those comparisons, these subjected were not accounted for.

	Half-life of PEth		
	Early - mid February (days)	**Mid - end February (days)**	**Early - end February (days)**
All subjects			
N	358	189	204
Median	7.67	8.11	8.14
CI	7.47 – 7.88	7.80 – 8.60	7.94 – 8.36
5% - 95%	5.09 – 11.51	5.43 – 13.20	6.14 – 11.96
Male			
N	152	80	90
Median	7.51	8.25	8.15
CI	7.12 – 7.77	7.73 – 8.86	7.79 – 8.51
5% - 95%	4.95 – 11.20	5.59 – 13.65	5.89 – 12.20
Female			
N	203	107	112
Median	7.79	8.11	8.11
CI	7.49 – 8.15	7.55 – 8.65	7.79 – 8.38
5% - 95%	5.13 – 11.66	5.26 – 12.82	6.22 – 10.91
P(Mann-Whitney) male vs female	0.08	0.90	0.68
AUDIT C ≤ 4			
N	85	23	26
Median	7.85	8.09	8.36
CI	7.41 – 8.44	7.07 – 10.45	7.90 – 9.52
5% - 95%	4.58 – 11.67	5.74 – 14.19	6.50 – 12.19
AUDIT C > 4			
N	262	162	174
Median	7.62	8.14	8.10
CI	7.32 – 7.84	7.80 – 8.59	7.82 – 8.33
5% - 95%	5.29 – 11.37	5.37 – 12.81	6.16 – 11.94
P(Mann-Whitney) AUDIT C ≤ 4 vs AUDIT C > 4	0.39	0.55	0.25

Table 2. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) to score compatibility with abstinence by looking at the relation between 2 consecutive PEth values, when the cut-off for defining a sample as positive was changed in steps of “2 units drunk over a period of 14 days” (at least one unit was consumed 14 days before sampling).

	Cut-off in “units drunk over a period of 14 days”					
	0	**2**	**4**	**6**	**8**	**10**
False negatives	17	11	3	1	1	1
False positives	3	4	5	8	11	12
True negatives	27	33	41	43	43	43
True positives	27	26	25	22	19	18
Sensitivity (%)	61	70	89	96	95	95
Specificity (%)	90	89	89	84	80	78
positive predictive value (%)	90	87	83	73	63	60
negative predictive value (%)	61	75	93	98	98	98

FIGURE CAPTIONS

Figure 1 Overview of the initial and final number of participants based on their feedback and PEth values.

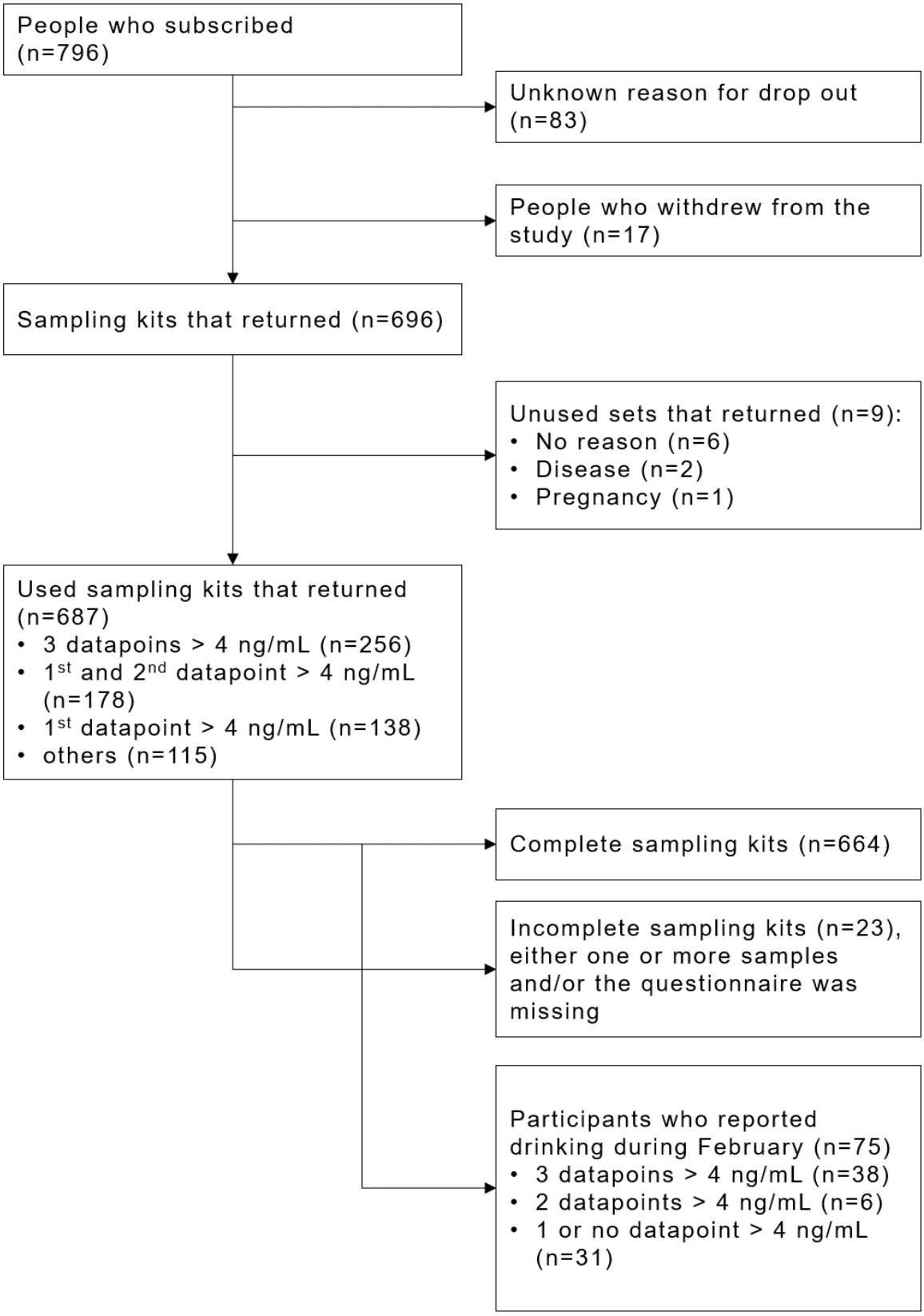
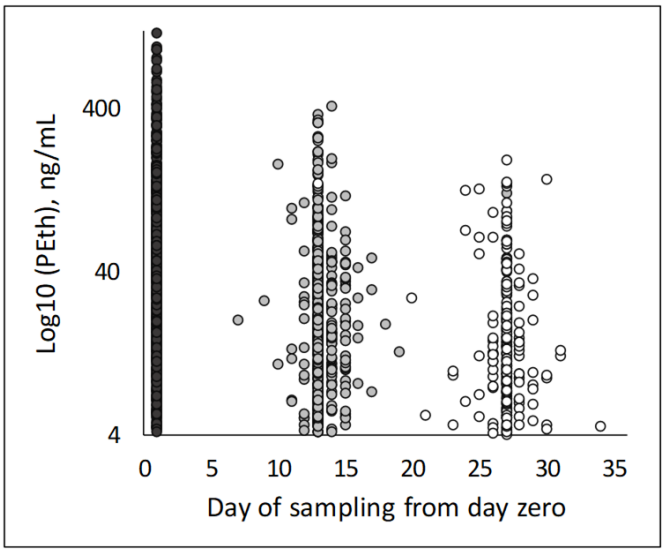
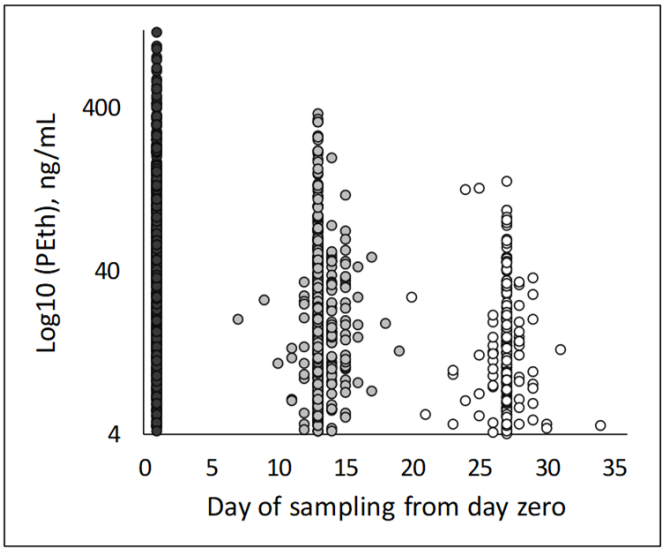


Figure 2

Observed PEth levels according to the sampling date. Dark grey: first sampling, light grey: second sampling, white: third sampling. The upper panel shows all results >4 ng/mL, and the accompanying descriptive statistics. In the lower panel the results for participants reporting to drink are excluded.



Early February	minimum	< 4ng/mL
	maximum	1142
	median	37
	> 4ng/mL	572 (84%)
Mid February	minimum	< 4ng/mL
	maximum	405
	median	15
	> 4ng/mL	442 (65%)
End February	minimum	< 4ng/mL
	maximum	190
	median	7
	> 4ng/mL	273 (41%)



Early February	minimum	< 4ng/mL
	maximum	1142
	median	37
	> 4ng/mL	509 (84%)
Mid February	minimum	< 4ng/mL
	maximum	363
	median	15
	> 4ng/mL	388 (64%)
End February	minimum	< 4ng/mL
	maximum	140
	median	6
	> 4ng/mL	229 (38%)

Figure 3

Weighted linear regression (1/x) analysis to estimate the relation between the PEth half-life (y-axis) and the initial PEth concentration (x-axis). Plot A shows the half-life calculated for the period early to mid February; B for mid to end February; and C for early to end February.

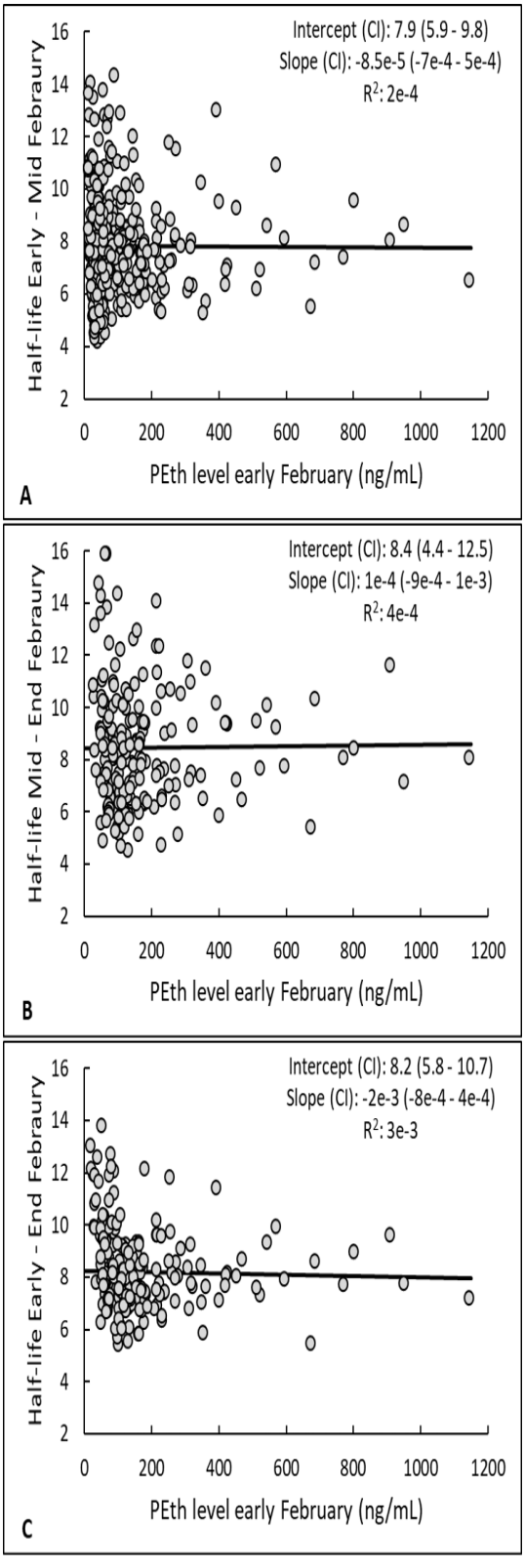


Figure 4

Graphical representation of the decision tree, with examples.A and B are two individuals who admitted drinking in February, C is a sample from a routine case where we used PEth measurements to follow-up abstinence from the onset on. The blue line represents the predicted regression line. The green lines represent the prediction interval around the regression line. Black dots represent the individual data used to calculate the regression model. The red line and red dots represent the individual case.

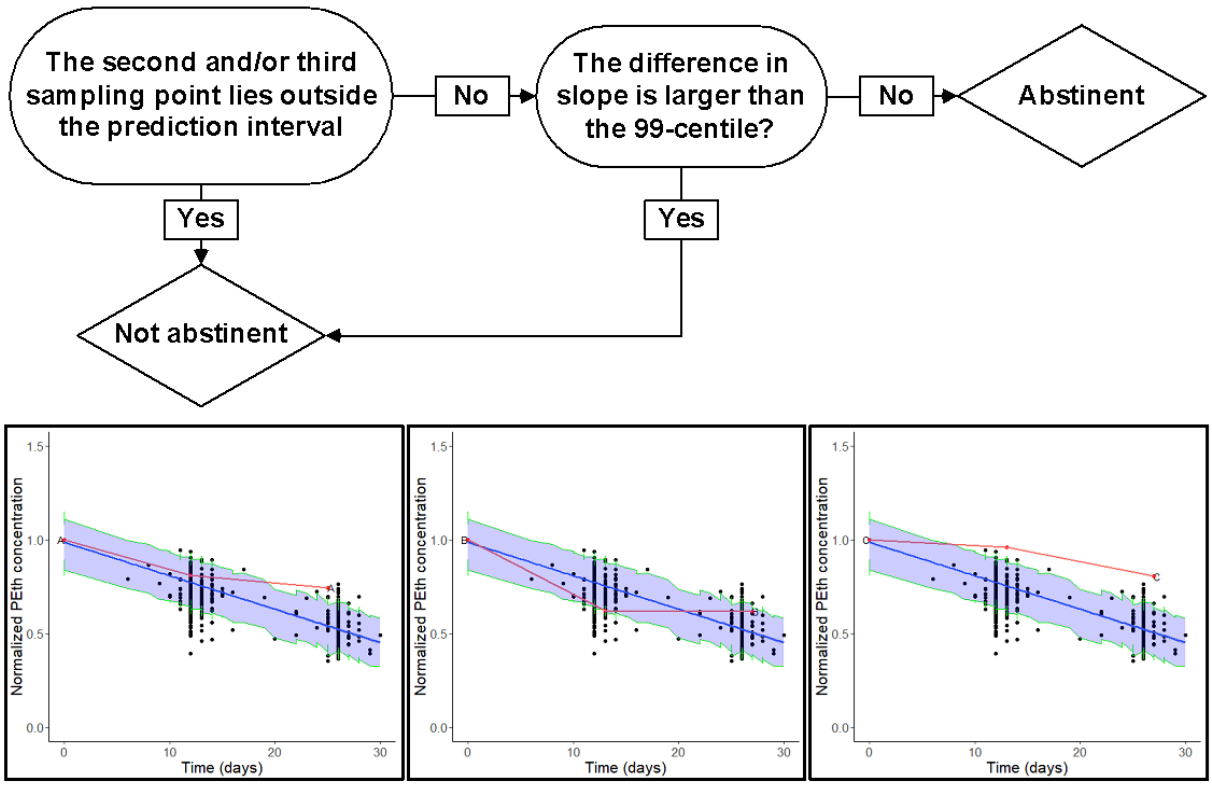


Figure 5

Receiver Operating Characteristic curve showing the change in false positive versus true positive rate when the cut-off for defining a sample as positive was changed in steps of “2 units drunk over a period of 14 days” (at least one unit was consumed 14 days before sampling). Numbers accompanying the data points indicate the respective cut-off.