
PEthNET
PEth in Mind
LUCERNE 2024
MAY 31 & JUNE 1

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Organization and Imprint

Venue
HSLU (Lucerne University of Applied Sciences and Arts)
[redacted]

Date
31st of May and 1st of June 2024

Conference Homepage
www.peth-net.org/2024-peth-in-mind-conference/

Conference Organization

Organizational Lead
Marc Luginbühl, President, PEth-NET
Frieder Wurst, Vice-President, PEth-NET
Frederike Stöth, Secretary General, PEth-NET
Marion Luginbühl, Treasurer, PEth-NET
Wolfgang Weinmann, Scientific Advisory Board, PEth-NET

Session Chairs

Session 1: Christophe Stove	Frederike Stöth
Session 2: Wolfgang Weinmann	Marc Luginbühl
Session 3: Katleen van Uytfanghe	Frieder Wurst

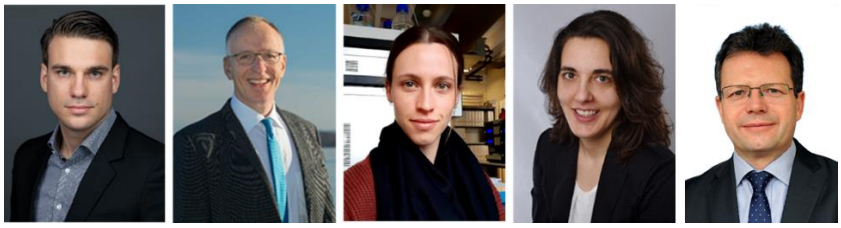
Welcome Reception
[redacted]

Dinner Restaurant
[redacted]

Inquiries, Help, and Support
contact@peth-net.org
[redacted]

Welcome Message

3rd Conference of the Society of PEth Research



Dear colleagues,
Dear PEth-NET members,
Dear conference visitors,

We are delighted to extend a warm welcome to you at the heart of Switzerland for the 2024 conference of the Society of PEth Research (PEth-NET).

We once again have selected a special location for our PEth-NET conference. The city of Lucerne, is renowned for its well-preserved medieval architecture, including the iconic Chapel Bridge and Water Tower, creating a charming and timeless atmosphere. We hope that the beautiful view onto the Swiss Alps will inspire us to delve into insightful discussions and groundbreaking research.

After dedicated efforts over the past months, we are pleased to present an engaging program for you. Embracing the theme "PEth in Mind," our conference will concentrate on the analysis and research of the direct alcohol biomarker phosphatidylethanol. Our sincere appreciation goes to our esteemed speakers for their numerous captivating contributions.

The first day of the conference will emphasize a deep dive into the technical dimensions of PEth analysis, covering methodologies, cutoff concentrations, sampling strategies, harmonization, and inter-laboratory comparisons. Two dedicated workshop sessions centered on the future of PEth research and harmonization will empower us to direct and align our upcoming projects. The day will culminate with a carefully selected conference dinner with the title “Beer and Wine Challenge” at the Museum of Transport, fostering continued exchange of knowledge and experiences.

On the second day of the conference, the spotlight will be on the practical application of PEth in both clinical and forensic contexts, specifically highlighting areas such as driving aptitude assessment and organ transplantation screening.

Enjoy the days here in Lucerne with us.
We are looking forward to your active participation, stimulating scientific discussions, and interesting personal talks.

With kind regards,

The conference organizers

Marc Luginbühl	Frieder Wurst	Frederike Stöth	Marion Luginbühl	Wolfgang Weinmann
President	Vice-President	Secretary General	Treasurer	Scientific Adv. Board

About PEth-NET

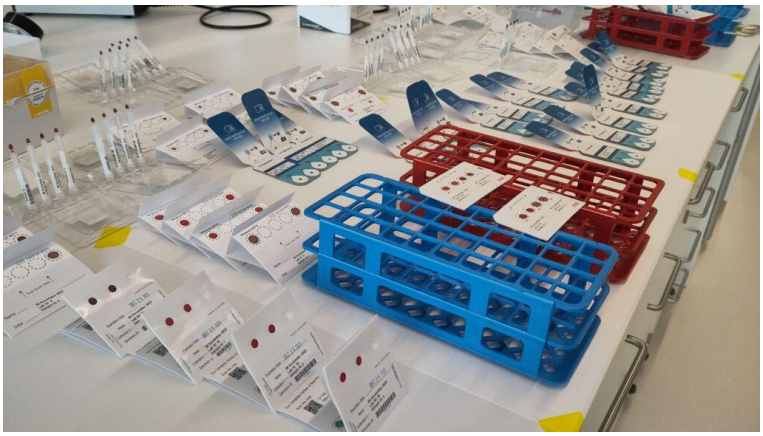
“The Society of PEth Research (PEth-NET)” is a non-profit organization. By bringing together representatives from academia, industry, research, regulatory and standard-setting bodies, the association aims to:

- Provide a platform to unify global experts for PEth research
- Provide information about PEth testing and novel publications
- Promote the analysis of PEth for the assessment of drinking behavior
- Promote research in forensic, clinical, and occupational sciences
- Organize meetings and workshops
- Encourage scientific cooperation and exchange among members and external partners

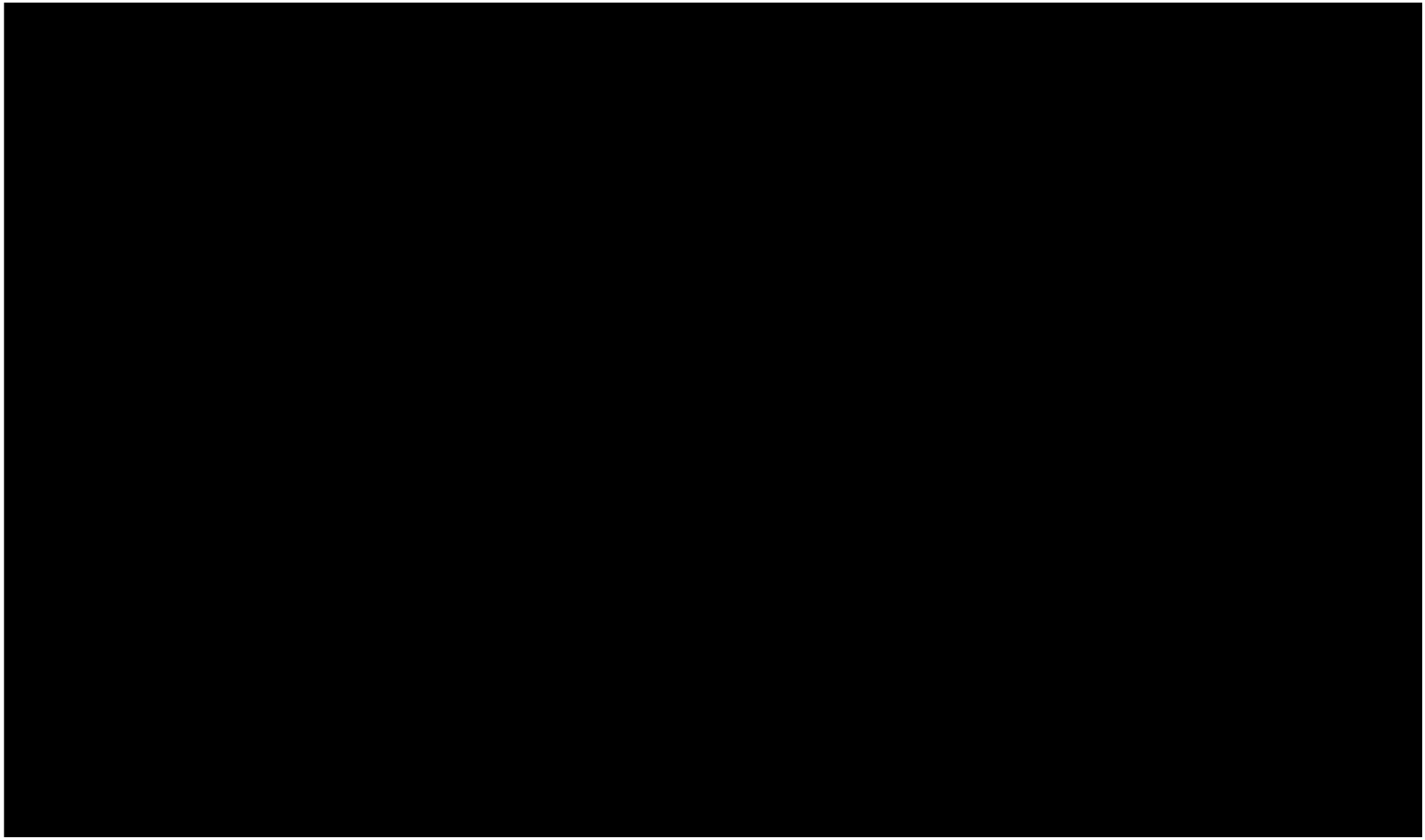
Individuals who feel addressed by the mission of The Society of PEth Research (PEth-NET) are highly encouraged to apply for membership via contact@peth-net.org.

The PEth-NET Inter-Laboratory Comparison

Since 2022, PEth-NET is organizing inter-laboratory comparisons for the analysis of PEth 16:0/18:1 using microsampling devices. Thereby, each round four different authentic whole blood samples are provided to each participating laboratory. To prepare the individual samples, each participating laboratory is asked to ship the sampling device to spot a selected choice of blood (e.g. 10-25 µL). The next opportunity for participation will be: **Round 2/2024** (registration closes on the 27th of September 2024). For the registration and more information please visit www.peth-net.org.



Program Overview



PEth-NET Awards

The scientific advisory board members will have the chance to honor two outstanding contributions presented at the PEth-NET meeting, whereby two recognition awards will be provided.

PEth-NET Best Presentation Award

for the presentation that most appealed to the audience

PEth-NET Best Innovation Award

for the most innovative scientific approach

Scientific advisory board members, who are present at the meeting and who do not compete for any of the awards, can choose the best contribution in each category.

Welcome Reception

[redacted]

Date
Thursday, the 30th of May 2024

[redacted]

[redacted]

[redacted]

Conference Dinner

Beer & Wine Battle*

Beer or wine, which drink suits the dish better?
Convince yourself, say goodbye to principles!

Motto
Principles accompany us every day! Are you willing to set them aside this time and follow your taste buds? Why not have wine with salad and why only white wine with fish? Is red wine truly the perfect companion to meat and cheese, or does beer also fit?

Many questions that we will explore with you at the Beer & Wine Battle and try something new. We will enjoy an evening full of palate surprises and convince ourselves of the exciting combinations.

Program
[redacted]

Date
Friday the 31st of May 2024

Transfer
[redacted]

[redacted]

*Alcohol-free options will be available

Keynotes

The window of detection of PEth after consumption of low alcohol amounts

Wolfgang Weinmann
Professor of Forensic Toxicology at the University of Bern

“This talk will discuss whether low drinking amounts can result in a positive PEth concentration above 20 ng/mL”

The role of direct ethanol metabolites in the measurement of alcohol intake – what is new and what do the German evidence and consensus-based guidelines recommend in terms of application

Frieder Wurst
Professor for psychiatry and psychotherapy at the University of Basel

“A rich body of evidence strengthens the positions of direct ethanol metabolites in the measurement of alcohol use. Besides the clear recommendations, now for a widespread use of the guidelines the implementation through various organisations is crucial. Furthermore questions in the context of clinical use and establishing cut-offs will be discussed.”

Research and clinical uses of PEth, and future directions for broader application

Judith Hahn
Professor of Medicine in the HIV, ID, and Global Medicine Division, Department of Medicine, University of California, San Francisco

“In her plenary, Dr. Judy Hahn will review the uses of PEth in behavioral and HIV research, addiction research, and liver care, and discuss what steps and considerations are needed for a broader application of PEth.”

The applicability of phosphatidylethanol (PEth) in the liver transplant setting

Susana G. Rodrigues
MD, PhD, Consultant Hepatologist, Inselspital, Bern University Hospital, University of Bern

‘‘Data on phosphatidylethanol’s (PEth) applicability, use and reliability in the setting of liver diseases has been limited. This lecture aims to provide an evidence-based overview on the applications, challenges and remaining open questions concerning the incorporation of PEth testing in identifying alcohol intake in liver disease patients. The focus will be on its use in the liver transplant setting.”

The application of PEth in Belgium

Christophe Stove
PharmD, PhD, associate-professor at Ghent University, heading the Laboratory of Toxicology at the Faculty of Pharmaceutical Sciences

“Belgium has recently introduced the use of PEth. This lecture aims to provide an overview about the transformation process (from CDT), sampling procedures, regulatory aspects, and the legal framework for monitoring alcohol consumption behaviour with alcohol biomarkers.”

Experiences and insights into a population-based algorithm for abstinence prediction

Katleen van Uytfanghe
PhD, Quality manager and technical supervisor of the accredited reference laboratory (Ref4U) and routine laboratory at the Laboratory of Toxicology of the University of Ghent, Belgium

“This talk will focus on the use of a population-based algorithm capable of predicting abstinence while accounting for intra- and interindividual variability in PEth scores”

Oral Presentations

Lyso-Phosphatidylethanol: Another marker for alcohol consumption monitoring?

Bantle Matthias[1], Van Tieghem Lanya[2], Weinmann Wolfgang[1]

[1] Institute for Forensic Medicine, University of Bern, Switzerland
[2] Faculty of Pharmaceutical Sciences, University of Ghent, Belgium

Background and aim
The alcohol biomarker phosphatidylethanol (PEth) has been increasingly used in the last years for abstinence monitoring and drinking behaviour monitoring. However, only a snapshot of the current concentration can be obtained from a single measurement of PEth. To obtain a clear picture on the patient's alcohol consumption history, the absolute value needs to be put into temporal context of multiple analyses, or additional markers have to be analyzed. We present the first results on a series of structurally related markers, lyso-phosphatidylethanols (LysoPEth), which, in contrast to PEth, are missing one fatty acid chain. This may have an influence on its distribution in the body as well as on formation and elimination kinetics.

Methods
Reference compounds were synthesized using enzymatic pathways and characterized by LC-MS/MS (MRM 437.3→255.2, 437.3→181.1; using commercial LysoPEth 18:1 (Echelon Bioscience) for comparison). An in-vitro metabolism study was carried out by spiking blank blood with different amounts of ethanol and measuring the changes in concentration for different PEth and LysoPEth analogues over time from dried blood spots. Additionally, PEth-positive blood was incubated at 37 °C for 79 h to follow up on analyte stability. Finally, routine forensic and clinical samples as well as lyophilized synthetic and authentic quality controls were analysed for LysoPEth.

Results
LysoPEth 16:0 and its deuterated analogue were successfully synthesized starting from phosphatidylcholine 16:0/18:1 via phosphatidylethanol 16:0/18:1. Analysis using LC/MS-MS showed full conversion with minor residual amounts of LysoPEth 18:1. Two in-vitro metabolism studies showed an alcohol concentration-dependent formation of PEth and LysoPEth 16:0 over 56 and 104 h with PEth and LysoPEth reaching a stable target concentration within 72h. Incubation of PEth-positive blood showed a decline in PEth 16:0/18:1 concentration with an increase in LysoPEth 16:0 concentration. LysoPEth 16:0 and PEth 16:0/18:1 were found to be in a linear correlation for fresh capillary and venous blood, but not for lyophilized quality control blood samples.

Conclusion
LysoPEth 16:0 can be used as additional alcohol biomarker complementary to PEth. Further investigations are planned on formation and elimination kinetics and pathways, distribution within different matrices, and the identification of other LysoPEth-species

Towards the implementation of a PEth Reference Measurement System – setting up a reference measurement procedure

Ververi Christina[1,2], Van Uytfanghe Katleen[1], Stove Christophe[1]

[1] Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Ghent University, Belgium
[2] Department of Chemistry, University of Turin, Italy

Introduction and aims
The direct alcohol biomarker phosphatidylethanol (PEth) is increasingly being applied in clinical and forensic contexts. Interpretation of PEth results is done against common decision limits (i.e 20 ng/mL in case of abstinence testing). This use of common decision limits is only valid when the outcome of the different analytical methods is comparable (even with different equipment, sample collection and sample preparation). This is currently not the case. For example, from the PEth-Net EQA scheme a difference of ~60% can be derived between the 2 most consistent deviating assays. To improve this inter-assay deviation, the implementation of a Reference Measurement System (RMS) is recommended. Such a RMS comprises: the definition of the measurand (compound to be measured) and the development of a higher order, highly sensitive, precise and accurate reference measurement procedure (RMP). Limits for precision and accuracy are preliminary set at 1/3 of those typically used in the application field (for PEth: 5% = 1/3 of 15%). The RMP is preferentially directly calibrated starting from a primary reference material in neat solvent and using gravimetry (in contrast to most routine procedures that include matrix-based calibrators and volumetry). We performed a feasibility study for the development of a RMP.

Methods
In this feasibility study, we implemented PEth 16:0/18:1 (the most abundant PEth homolog) in whole blood, as the measurand. PEth 16:0/18:1 in neat solvent is used for calibrators' preparation. The feasibility study included preliminary validation of the recovery, intra-day reproducibility between replicates and inter-day precision. In addition, native samples were processed with both this preliminary RMP and a routine procedure.
Calibrators were prepared in methanol by mixing a fixed absolute amount of PEth 16:0/18:1 and PEth-D_5. A straightforward sample preparation was implemented based on protein precipitation and solid phase extraction (using Phree Phospholipid Removal Solutions, Phenomenex). Native samples were processed based on this preliminary RMP, sampling a fixed absolute amount of analyte (5 ng) whereby the sampled volume is hence variable.

Results
The lowest concentration measured was 3 ng/mL, with a signal/noise ratio of 152 and a difference between duplicates of 4%, fulfilling the criteria needed for an RMP (high sensitivity and low CV <5% at LLoQ). An average internal standard-compensated recovery of 94% (5% CV) was obtained, starting from 12 independent blood samples, fortified at 204 ng/mL PEth 16:0/18:1. The intra-day reproducibility between duplicates (n=26), is optimal, with a CV of 2.6%. Inter-day precision was determined in 6 samples measured on 2 different days and was found to be within 7%. When measuring native samples, an absolute difference between the RMP and the routine procedure was still observed, the underlying cause being studied further.

Conclusion
The proposed limit of quantification, sample preparation and extraction procedure is proven reliable based on the S/N, recovery and precision studies and is the first step towards further validation of a full RMP. The possibility of insufficient recovery of native PEth from whole blood, in combination with correct calibration from neat solvent, should be considered and explored further.

Variability in phosphatidylethanol (PEth) formation rate in *ex vivo* whole blood

Stark Haidyn[1], Sanchez Jesus[1], Lopez-Cruzan Marissa[1], Hill-Kapturczak Nathalie[1], Roache John[1], Javors Martin[1], Simon Ted[1] and Ginsburg Brett[1]

[1] The University of Texas Health Science Center at San Antonio, Department of Psychiatry and Behavioral Sciences, San Antonio, Texas, 78229, USA

Purpose
Phosphatidylethanol (PEth) is a phospholipid used as a biomarker for ethanol consumption in humans. The interpretation of PEth measurement is limited due to inter-individual variability in detected PEth concentrations. Previous evidence from both in vivo and ex vivo alcohol exposure shows variability in PEth formation rate may contribute to variation in detected PEth levels between people. However, previous studies lack within subject assessment of multiple ethanol concentrations on PEth formation. Additionally, little is known of the consistency of detected PEth levels from the same person at different blood collection timepoints. This study assesses the extent of variability in PEth formation rate across a range of ethanol concentrations in ex vivo whole blood, and the similarity of detected PEth levels in each participant about one month apart to more accurately capture the dynamics of PEth formation.

Methods
Whole blood samples from six participants were collected intravenously, and ethanol (0, 0.1, 0.5, 0.1, 0.15, 0.2, 0.25, or 0.3% BAC) was added to individual samples for each participant. Samples were incubated in a shaker oven at 37°C, and aliquots were taken every hour for five hours. PEth 16:0 18:1 concentration was measured using high performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS), and rate of formation (ng/mL/hr) was determined using a linear mixed effects model. Analyses were repeated with a second blood collection for each participant about a month later. Analysis of variance (ANOVA) of a linear mixed regression was used to determine differences in detected PEth concentration and PEth formation rates. Post hoc analyses were performed using Dunnett's test.

Results
A main effect of ethanol concentration on percent change in measured PEth concentration ($F(7,389) = 2.8$, $p < 0.05$) and rate of PEth formation ($F(7,74) = 2.19$, $p < .05$) was found, with the two highest ethanol concentrations yielding significantly different PEth formation rates than baseline of no added ethanol. No effects of blood collection timepoint or interactions were detected.

Conclusion
The results indicate PEth formation rate increases with ethanol concentration; and is similar between blood collection time points. Therefore, a single PEth measurement is likely reflective of recent alcohol consumption irrespective of physiological state of the individual. Further investigation of mechanisms of PEth formation – namely Phospholipase D – in the blood may highlight sources of variability in PEth formation rate.

Phosphatidylethanol, marker of alcohol uses, practical utility

Journe Bruno[1]

[1] Medical doctor, Addictologist, Paris, France

Context
Alcohol is responsible for behavioral disorders, accidents, and pathologies. A direct marker of uses, comparable to glycated hemoglobin, specific and proportional, could be a major asset for prevention and care. Phosphatidylethanol (PEth) is a specific direct marker of alcohol use. PEth is sensitive, its value is proportional to alcohol consumption in past weeks.

Objectives of the study
Propose the measurement of PEth to users in an outpatient consultation, assess the interest for the user, for the professional, in medical situations or in legal situations.

Methods.
The samples were taken during routine care, during a consultation. We recorded the clinical situations, the declared uses (number of alcohol units and AUDIT score). Capillary blood samples were taken with 10 µL volumetric absorption devices (VAMS). PEth 16:0/18:1 measurements are made by high-resolution liquid chromatography coupled with mass spectrometry detection.

Results
We gathered 102 PEth results, concerning 79 people. 23 had repeated measures, in the context of withdrawal, abstinence monitoring or legal needs. Sensitivity: the PEth measurement curves and user declarations (AUDIT) are consistent. The values follow abstinence, relapses and denials. Specificity: no interaction encountered with treatments or pathologies. Genders: PEth values are equivalent. Acceptability of sampling, convenience of VAMS, transportation, storage are excellent.

Discussion
Our study brings the availability of phosphatidylethanol into a consultation. We confirm the qualities of PEth: specific and proportional to the uses of ethanol. PEth is an objective measure of alcohol use. PEth allows an informed exchange with users. In legal or professional situations, PEth is an objective and clear measurement. PEth allows to debate with the patient about the place of alcohol use in his psychological history and his social life. PEth makes it possible to share information on the risks and harms of alcohol use. PEth is part of a long-term evolution of knowledge and prevention and care practices.

A comparison of PEth testing in combination with ethyl glucuronide in blood and ethyl glucuronide in hair in one-centimetre long hair sections

Thomas Tyson[1], Tsanaclis Lolita[1], Andraus Maristela[1], Nutt James[1], Bevan Sian[1]

[1] Cansford Laboratories Limited, Cardiff, Wales

Background and Aim
Hair ethyl glucuronide (H-EtG) testing allows the diagnosis of long-term abstinence and to differentiate between social and excessive chronic alcohol use. Hair testing does not cover the approximate week prior to sample collection. To overcome this, blood tests such as phosphatidylethanol (PEth) are employed in conjunction with H-EtG. PEth detects alcohol consumption for up to 28 days prior to sample collection[1].
The consensus of the Society of Hair Testing (SoHT) states, a concentration in the proximal scalp hair up to 6-centimetre of EtG ≥5 pg/mg strongly suggests repeated alcohol consumption and a concentration of >30 pg/mg strongly suggests chronic excessive alcohol consumption[2]. Segmented H-EtG alcohol testing can help provide additional information. The consensus recommends that when samples less than 3-centimetre are used, the results should be interpreted with caution with applying the cut-offs.
It is possible to obtain a negative H-EtG result from consuming small volumes of alcohol, a single event of heavy alcohol consumption or from using chemical hair treatments that can reduce the concentration of H-EtG. These results would be interpreted as suggestive of abstinence.
An additional marker, EtG in blood (B-EtG) can detect consumption minutes after drinking, with levels remaining detectable for hours [3].
The main aim of this study is to show real medico-legal cases and illustrate how the combined data of H-EtG, PEth and B-EtG can enable to improve the interpretation of a pattern of alcohol consumption.

Methods
250 cases were selected where 1-centimetre sections were tested for H-EtG with concurrent fingertip prick blood in DBS for PEth and B-EtG was analysed by UPLC/ESI-MS/MS using the following cut-offs: H-EtG=5 pg/mg; PEth=20 ng/mL and B-EtG=10 ng/mL.

Results
36% of the case showed H-EtG, PEth and B-EtG levels below cut-off, suggestive of abstinence, and 18% were above cut-off indicating continuous alcohol consumption. The remaining cases provided different patterns where H-EtG was negative in 9% of the cases along with B-EtG, whilst PEth was detected, suggesting alcohol use within the 2-4 weeks prior to sample collection. H-EtG was positive with PEth and B-EtG were negative in 5% of the case, indicating historic consumption and confirming recent abstinence. Only 1 case (0.4% of the cases) showed B-EtG positive with both H-EtG and PEth negatives, indicating recent alcohol use.

Conclusions
The results substantiate the value of testing PEth alongside current EtG testing to improve interpretation of alcohol consumption regardless of the length of hair.

[1] L. Tsanaclis, Drug Test Anal, 13(1), pp.203-207.
[2] Society of Hair Testing (SOHT): http://www.soht.org
[3] G. Høiseth, J Anal Toxicol, 34(6), pp.319-324

Pre-analytical considerations on PEth analysis

Neumann Jasna[1], Böttcher Michael[1]

[1] MVZ Medizinische Labore Dessau Kassel GmbH, Dessau-Roßlau, Germany

Introduction and Aim(s)
Phosphatidylethanol (PEth) analysis to assess an individual's alcohol consumption status is increasingly performed from capillary whole blood and with different collection devices. The aim of this study was to test different collection devices for the in vitro formation of PEth, in the presence and the absence of ethanol. In addition, the stability of PEth was investigated in EDTA whole blood obtained by venous puncture (i.v.).

Methods
Four different capillary whole blood collection devices were tested for in vitro formation of PEth: Mitra® (20 µL), Capitainer® (10 µL), Capitainer® Vanadate (10 µL) and glass capillary (20 µL, EDTA coated; Sarstedt) in isopropanol (GK). PEth measurement was performed with our DIN EN ISO 17025 and DIN EN ISO 15189 accredited UPLC-MS/MS method (LoQ: 9.1 ng/ml). Ethanol in plasma was quantified with the ADH method on an Olympus AU 680.

Results and Discussion
In none of the GK and the Capitainer® Vanadate but in 44% of the Mitra® samples and all Capitainer® samples filled with 32 different PEth and Ethanol negative i.v. EDTA whole blood samples, an in vitro PEth formation was observed in the absence of alcohol (Mitra®: 9.8 – 29.5 ng/mL; Capitainer®: 26.7 – 132 ng/mL).
The same experiment was conducted by fortifying aliquots of the same 32 blood samples with ethanol (1.6 g/L). In the presence of alcohol, the number of positive Mitra® samples increased to 81%. PEth values ranged from 11.4 to 84.4 ng/mL in the Mitra® device and from 66.8 to 432 ng/mL in the Capitainer® device. One GK sample and 28% of Capitainer® Vanadate samples (range: 18.3 – 50.6 ng/mL) showed an in vitro formation of PEth.
In the second part of the study, three different lots of Capitainer® Vanadate were tested for an in vitro formation of PEth by filling them with 33 different PEth and ethanol negative i.v. EDTA whole blood samples. No in vitro formation of PEth could be observed. According to the first study Capitainer® Vandate devices were then filled with ethanol spiked (1.6 g/L) aliquots of the 33 different whole blood samples. In all three lots 75 % of the samples showed an in vitro formation of PEth in the presence of alcohol (range 20.4 – 75.9 ng/mL)
In a second study we could demonstrate for 423 PEth positive but alcohol negative i.v. EDTA whole blood patient samples from routine that PEth is stable for at least 4 weeks when stored at 4°C. Ethanol positive i.v. EDTA whole blood patient samples from routine (0.11 – 3.12 g/L; n = 79) were reanalyzed after four weeks of storage at 4°C. No additional PEth formation in presence of ethanol could be observed.

Conclusions
If a capillary blood sampling device is intended to be used for routine PEth analysis, it should be tested before for in vitro formation of PEth. The Phospholipase D inhibitor Vanadate reduces the in vitro formation of PEth but does not necessarily prevents this process completely in the presence of alcohol. In vitro formation of PEth in i.v. EDTA whole blood is negligible and PEth is stable if samples are stored at 4°C for four weeks.

Phosphatidylethanol measured on a buccal smear

Gaulier Jean-Michel[1], Hakim Florian[2], Journe Bruno[3]

[1] UF de Toxicologie - Centre de Biologie-Pathologie, CHU de Lille, France
[2] UF de Toxicologie - Centre de Biologie-Pathologie, CHU de Lille, France
[3] Medical doctor, Addictologist, Paris, France

Ethanol in contact with cell membranes produces an abnormal lipid, phosphatidylethanol (PEth). PEth is a direct marker of alcohol use. PEth is sensitive and proportional to the quantities of alcohol consumed in the past weeks.

Objectives of the preliminary study

-To propose a non-invasive sampling of the bucco-gingival sulcus,
-To research / quantify the presence of PEth in the mouth,
-To compare buccal and blood PEth measurements.

Material and method

The samples were taken from patients consulting for alcohol misuse. AUDIT scores and units of alcohol consumed (AU) were recorded. Buccal samples and capillary blood were collected with volumetric devices (VAMS Mitra 10 µL and 30 µL) and dried. They were sent by post to the laboratory. Measurements of blood and buccal samples were made by liquid chromatography and detected by tandem mass spectrometry (LC-MS/MS). One series measured PEth 16:0/18:1, PEth16:0/20:4 and ethyl glucuronide (EtG). Another series measured only PEth 16:0/18:1.

Results

The PEth 16:0/18:1, PEth 16:0/20:4, EtGs series involved 14 patients. It showed excellent sensitivity for PEth 16:0/18:1. It showed a lower performance for PEth 16:0/20:4 and EtG. The PEth 16:0/18:1 only series involved 34 people (including the previous 14). The average concentration ratio of buccal PEth/blood capillary PEth was 23%. The variations were from 6% to 98%. In the situation of withdrawal and abstinence that were observed (1 case), the decrease in blood PEth was 30% per week, buccal PEth was no longer detectable in the third week. The preservation of dried buccal and blood samples was excellent.

Discussion and conclusion

PEth is formed on the membranes of all cells; therefore it makes sense to look for it in the membranes of cells present in the buccal sulcus. Our results showed reliable detection of PEth in the buccal smear as soon as the blood PEth level reached a value greater than ≥200 ng/ml, a level suggestive of excessive consumption. Measuring PEth 16:0/18:1 on a smear of the jugo gingival (buccal) sulcus has two advantages: non-invasive, simple to perform. Buccal sampling has a place in alcohol prevention and risk reduction tools. For example, in situations where it is necessary to differentiate between isolated alcohol consumption and repeated alcohol consumption (measurement of exhaled alcohol and buccal PEth on the side of a road).

A PEth immunoassay for assessment of alcohol consumption

Johnson L. Jeff[1], Chan Fok Vun[1], Muyindike Winnie R.[2], Day Cameron[1], Hahn Judy A.[3] and Neilsen Paul O.[1]

[1] Echelon Biosciences, Salt Lake City, UT, USA
[2] Mbarara University of Science and Technology, Uganda
[3] University of California, San Francisco, CA, USA

Introduction & Aims
Phosphatidylethanol (PEth) is a phospholipid whole-blood biomarker for alcohol consumption that is remarkably stable as an analyte when stored, extracted, and measured from dried blood spot (DBS) cards. There are multiple options for collection, extraction, and analysis of PEth by mass spectrometry (MS). There are no accepted immunoassays (ELISAs) for PEth; and we sought to develop an ELISA and establish its utility using real-world DBS samples from HIV+ individuals. A second goal was to demonstrate the flexibility of the ELISA using multiple blood collection devices compared to standard DBS cards.

Methods
We screened multiple monoclonal antibodies in a competitive ELISA format to identify antibodies specific for the PEth headgroup. We employed simple extraction and sample prep methods to test venous blood pipetted onto DBS cards, as well as alternative Mitra® and Tasso-M20® devices. We grouped samples into four alcohol consumption categories based on literature MS values: abstainer (< 10 ng/mL), low (10 to 99 ng/mL), moderate (100 to 250 ng/mL), and high (> 250 ng/mL). We used 5-parameter non-linear regression analysis on GraphPad software to determine best-fit curves with r2 values > 95%. We compared ELISA PEth values to values determined with established LC/MS methods at United States Drug Testing Labs, Desplaines Illinois, and the Biological Psychiatry Analytical Laboratory at UT Health San Antonio, Texas.

Results & Discussion
We built a quantitative ELISA testing several types of standards: (i) synthetic PEth analogs including the abundant 16:0/18:1 PEth homolog and (ii) authentic whole-blood, ex-vivo samples with confirmed MS values. We used both types of standard curves to interpolate PEth values from 120 whole-blood DBS samples from an HIV-positive drinking cohort in Uganda. We found that the ELISA method identified abstainer/low from moderate, and high categories in a qualitative fashion; and that the measured ELISA values have significant correlation with MS values (Pearson, r = 0.72) and an LOD below 40 ng/mL. We measured quantitative different PEth values by ELISA and MS due to differential matrix effects; and discovered that the matrix effects decreased and the ELISA accuracy improved when using an ex-vivo PEth-generated standard curve. Finally, we determined the ELISA is compatible with multiple blood collection devices and demonstrates sensitivity and specificity useful for certain clinical applications.

Conclusions
The Echelon Total PEth 96-well ELISA expands the availability of PEth analytical methods, can differentiate between multiple categories of alcohol consumption; and is poised to become a valuable tool in screening large numbers of samples in high-throughput workflows in conjunction with MS testing.

The availability and quality/purity of PEth reference materials: addressing regioisomer interferences and enhancing quantification accuracy

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Phosphatidylethanol (PEth) has emerged as a promising and widely used alcohol biomarker in recent years. Unlike traditional alcohol markers, PEth is a direct biomarker of alcohol consumption and offers several advantages, including higher specificity, a wider detection window, and independence from age, gender, other ingested substances, or pathological conditions.
From a structural perspective, PEth is not a single molecule but a family of phospholipids that share a common polar phosphoethanol head and differ in the length and composition of their fatty acid chains. These chains range from 14 to 22 carbons and can contain up to six carbon-carbon double bonds. Over 40 PEth homologs have been identified, with the most abundant being PEth 16:0/18:1 and PEth 16:0/18:2.

Accurate identification and quantification of PEth homologs present a significant analytical challenge, exacerbated by the limited availability of reference standards. Various analytical techniques have been employed to detect and quantify total PEth in biological matrices. LC-MS/MS-based methods have gained prominence in recent years, enabling quantification and molecular species determination in human blood samples.

The primary objective of this study was to develop chemical synthesis strategies for the production of a broader range of PEth reference materials and stable isotope-labeled internal standards. This effort was intended to improve the availability, quality, and purity of reference materials while addressing the potential impact of regioisomers and other impurities on qualitative and quantitative analysis.
In this project, chemical synthetic methods were developed for the synthesis of individual PEth homologs and stable isotope-labeled PEth internal standards, particularly for those containing one or more double bonds in their fatty acid chains at the sn-2 position. Selective synthetic approaches were employed to minimize the presence of regioisomers and other impurities.
Both unlabeled, deuterium-labeled, and 13C-labeled PEth standards were synthesized as free acids or ammonium salts and evaluated against patient blood samples. The synthesis of these standards addressed the concern regarding the impact of regioisomers on routine analysis of PEth by LC-MS/MS. Moreover, the study investigated the accuracy of PEth homolog quantification in the presence of regioisomer contamination in reference materials.

The regioisomers of PEth possess identical molecular weights and exhibit similar chromatographic and spectral properties, making them difficult to separate from PEth in LC-MS/MS analysis. This raises the question of whether it is necessary or feasible to account for regioisomers in routine PEth analysis using LC-MS/MS. Further investigation is required to determine the impact of regioisomer contamination on the accuracy of PEth homolog quantification.

Increasing harmonization of PEth measurements across the world using an interlaboratory comparison

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Aims: Phosphatidylethanol (PEth) has become an important marker for abstinence monitoring and drinking behaviour assessment. Despite the alcohol biomarker is being used more and more frequently, standardization and knowledge on the robustness and comparability of the methods used are still limited. In 2022 the first consensus for the use of the alcohol biomarker phosphatidylethanol was published. To obtain a more experience-based foundation for further harmonization, a total of three rounds of interlaboratory comparisons with authentic blood samples were carried out in the years 2022 and 2023.

Methods: Participating laboratories have been invited to send their dried blood spot (DBS) sampling devices to a central laboratory. There, authentic fresh blood samples from routine forensic and clinical cases as well as a lyophilized sample were applied to these sampling devices and sent back to the laboratories. For each round, four samples with concentrations covering the decision limits according to the 2022 Consensus of Basel were used. The results were evaluated and compared by the central laboratory according to standards proposed by Horwitz and the Society of Toxicological and Forensic Chemistry (GTFCh). The most abundant analogue, PEth 16:0/18:1 was analysed and reported. The concentrations tested ranged from 16 to 480 ng/mL.

Results and Discussion: Five different microsampling devices and five different reference material manufacturers were used by the participants. Most of the laboratories used DBS card-based devices, followed by Capitainer®, Mitra® VAMS, and HemaXis devices. Analysis of the results according to Horwitz revealed that more than 85% of all values were within the acceptance criteria for successful participation (|z-score| < 2): In the first round, 16 laboratories participated with an overall success rate of 97%. In the second round, 19 laboratories participated with an overall success rate of 86%. In the third round, 20 laboratories participated with an overall success rate of 89%.

Conclusion: The three rounds of interlaboratory comparison using authentic blood samples showed that, for at least one sample, more than 90% of the participating laboratories were within the ranges of the calculated PEth concentrations. It has been shown that there is good comparability of the results in a large concentration range covering both lower and upper decision limit for most laboratories. There are still challenges in terms of calibration range and reporting limits.

Implementation of PEth to optimize AUD treatment

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Introduction and aims: Alcohol use disorder is a chronic recurrent disease that may last over lifetime. Treatment is usually needed for different clinical manifestations of the disease like for alcohol withdrawal, rehabilitation therapy and maintenance of abstinence. Recent clinical studies have yielded that PEth may be a useful clinical tool that can be applied in each stage of the treatment process. This talk aims at providing a comprehensive review of clinical applications for PEth alongside each of the mentioned clinical situations.

Methods: Significance of PEth will be demonstrated by citing research findings in the different settings of AUD diagnostics, treatment of alcohol withdrawal and in the consecutive rehabilitation.

Results and conclusion: PEth may be a game changer for certain aspects of AUD diagnostic and treatments (e.g. treatment decision concerning alcohol withdrawal) but further research is needed to establish PEth in the clinical setting.

Blood phosphatidylethonol 16:0/18:1 and urinary ethyl glucuronide levels during a virtual contingency management intervention for alcohol use disorder

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Background. This study measured weekly changes in blood phosphatidylethanol (PEth) and urinary ethyl glucuronide (uEtG) levels to determine abstinence from alcohol during a virtual contingency management intervention for alcohol use disorder (AUD). PEth and uEtG are metabolites of ethanol and have mean half-lives of ~7 days and 7 hours during abstinence, respectively. This analysis evaluated the relationship between time since last drink or number of drinks consumed at the last drinking episode and alcohol biomarker results.

Methods. Community-dwelling participants (N=15) with AUD and baseline PEth levels ≥20 ng/mL were recruited. Blood and urine samples were collected weekly for 4 weeks, then biweekly for a month. Blood samples were self-collected using the TASSO-M20 device on camera then shipped for analysis via HPLC/MS/MS. Urine samples were collected off camera and then uEtG detected on camera using a uEtG dipstick (negative uEtG <300ng/mL). At each visit participants reported the hours since their last drink and the number of standard drinks consumed during that drinking episode. PEth, uEtG, and self-reported drinking measures were correlated to assess agreement across the measures of alcohol use.

Results. 1) A decrease/increase of PEth levels correctly matched the decrease/increase of total drinks self-reported for the previous week at 76% of visits. 2) Participants with negative uEtG results reported a mean of 2.9 (SD=2.4) drinks consumed 53.5 (SD=40.2) hours prior to the study visit, while those with positive uEtG results reported a mean of 4.9 (SD=3.8) drinks consumed 16.6 (SD = 16.4) hours prior to the study visit. At least 13.1 hours of no drinking was required to observe a negative uEtG result. 3) The mean PEth level was 856 (SD=801) ng/mL when uEtG tests were positive and was significantly lower when uETG tests were negative (Mean PEth = 91.2 (SD 186) ng/mL; $p<0.01$). 4) Change in uEtG results (positive or negative) closely matched the direction of PEth results at 83% of visits. 5) 52 of 55 negative uEtG tests had detectable PEth levels (PEth ≥20 ng/mL).

Conclusions. We observed strong agreement between PEth and uEtG results from visit to visit. The few discrepancies that occurred likely resulted from the significant differences in the half-lives of the two biomarkers. Repeated within subject measurements at regular intervals enabled identification of changes in drinking. Observed blood collection with TASSO devices during virtual visits, and subsequent PEth analyses, were successful in detecting recent drinking from week-to-week and will be used in future studies.

Virtual incentives for alcohol treatment (VITA): results of a feasibility study and methods for a definitive trial

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Introduction/Aims: In contingency management (CM), individuals receive a tangible reinforcer when they submit a biomarker consistent with alcohol abstinence. We will describe two studies focused on developing and testing a virtual CM treatment for alcohol use disorders that uses phosphatidylethanol (PEth) to confirm alcohol abstinence. Our aims are to (1) determine the acceptability, feasibility, and initial efficacy of our virtual PEth-based CM intervention (Study 1- completed) and (2) conduct a definitive trial of this intervention (Study 2- ongoing).

Methods: Study 1: Sixteen adults with an alcohol use disorder who submitted a blood sample with PEth ≥20 ng/mL were randomized to receive 6 months of (1) reinforcers regardless of their PEth levels (control condition) or (2) reinforcers for submitting blood samples consistent with PEth-defined alcohol abstinence (CM condition). Participants self-collected blood samples using the TASSO-M20 device. All research procedures were conducted over Zoom. Intervention acceptability and satisfaction were assessed with quantitative and qualitative methods. The primary efficacy outcome was PEth-defined abstinence (Weeks 1-4: week-over-week reduction in PEth 16:0/18:1, Weeks 5-26 PEth 16:0/18:1 <20 ng/mL). Study 2: 200 adults with an alcohol use disorder who submit a blood sample with PEth ≥20 ng/mL will be randomized to receive a 6 month (1) virtual cognitive behavioral therapy and reinforcers for submitting blood samples (no abstinence required- control group) or (2) virtual cognitive behavioral therapy and reinforcers for submitting blood samples consistent with PEth-defined alcohol abstinence (CM group). The primary outcome will be PEth-defined abstinence as defined above and our secondary outcome will be PEth-defined excessive drinking (≥200 ng/mL). Participants will be followed for 12 months after treatment. Analyses will investigate predictors of treatment response using the Addictions Neuroclinical Assessment model and evaluate the economic impact of the intervention.

Results/Discussion: Study 1: Mean CM retention was 18.6±8.8 weeks and satisfaction was high (Mean= 30.3±1.5). 72% of PEth samples from CM participants were consistent with abstinence versus 34% for controls (OR=5.0, p<0.05). Other measures of alcohol use (PEth ≥200 ng/mL; urine ethyl glucuronide, self-report) were also lower in the CM condition, but differences were not significant. Study 2: Funding was received and research review approval was obtained. Recruitment for this study will begin in March 2024.

Conclusion: In a feasibility trial we were able to substantiate PEth as a measure of alcohol abstinence in a virtual CM intervention for alcohol use disorders. An ongoing trial will test the efficacy of our virtual approach.

Sponsoring

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