Poster 181

PETH IMMUNOASSAY: A SEMI-QUANTITATIVE ELISA FOR ASSESSMENT OF ALCOHOL CONSUMPTION

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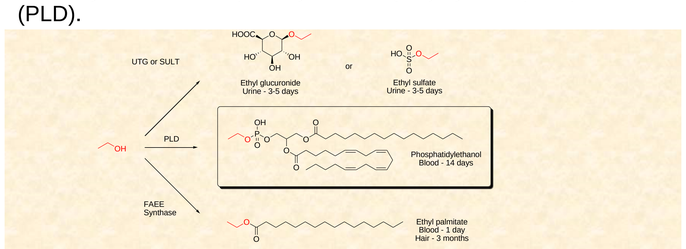
Overview

- Phosphatidylethanol (PEth) is accepted as a long-term direct biomarker for the detection and assessment of alcohol consumption.
- Utilizing a Semi-Quantitative PEth ELISA we analyzed a set of 120 clinical samples, compared four alcohol consumption categories, and identified a significant correlation between ELISA values and reported mass spectrometry (MS) values.
- Three blood collection devices were evaluated and confirmed to be compatible with the Semi-Quantitative PEth ELISA.

Introduction

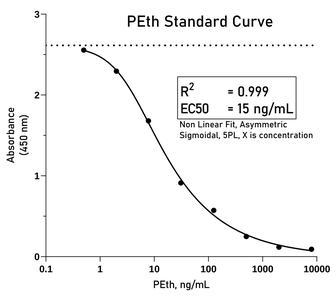
- Abuse and overuse of alcohol contributes to roughly 5% of the overall global health burden.
- Monitoring excessive binge and chronic drinking will benefit individual health outcomes as well as society at large.
- PEth is a phospholipid formed in the presence of EtOH by transacylation of Phosphatidylcholine (PC) by Phospholipase D (PLD).

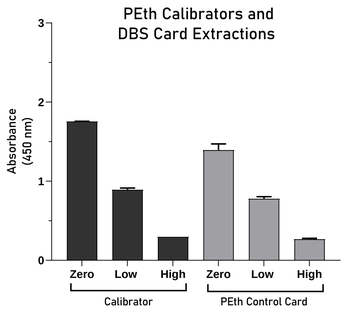


- PEth is quite stable when stored, extracted, and measured from dried blood, allowing for flexibility in the collection process.
- We present the first immunoassay that can measure PEth levels in a semi-quantifiable manner to supplement LC/MS analysis.
- Our goal is to further simplify clinical laboratory processes, reduce time & cost, and expand the available options for clinical analysis of PEth.

Semi-Quantitative PEth ELISA

- Competitive Semi-Quantitative Immunoassay utilizing a synthetic 16:0/18:1 PEth standard curve, allowing the user to assign samples a PEth concentration (ng/mL).
  - Free PEth in solution, either extracted from samples, controls, or provided as a standard, competes with the PEth ab against PEth bound to the wells of a 96 well plate.
  - Decreased PEth ab binding to the plate indicates the presence of PEth. A lower signal indicates higher levels of PEth.



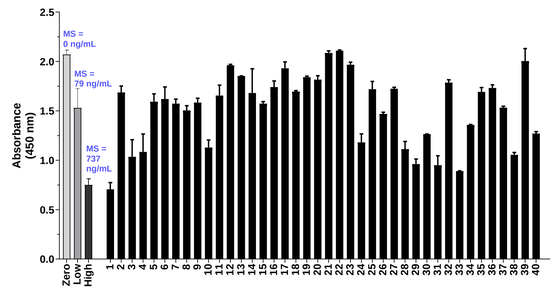


Sample Type	Reported LC/MS Value	Semi-Quantitative PEth ELISA (Interpolated Value)	
		Calibrator	DBS Card Extraction
Zero	< 8 ng/mL	7 ng/mL	13 ng/mL
Low	79 ng/mL	37 ng/mL	50 ng/mL
High	737 ng/mL	414 ng/mL	521 ng/mL

**The observed PEth ELISA concentrations (ng/mL) are correlative but are not equivalent to their LC/MS counterparts. This is likely due to differential matrix effects.*

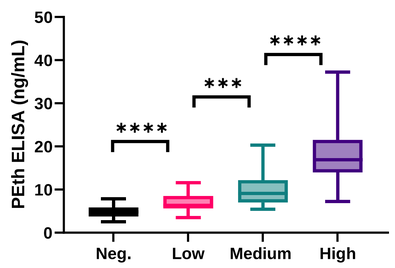
Analysis of 120 Clinical Samples

PEth ELISA Quantifies PEth levels in clinical samples

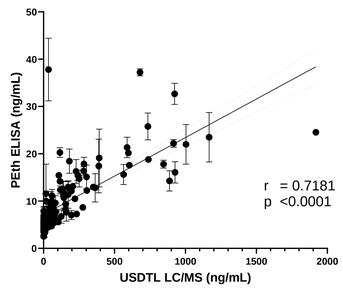


Drinking Category	n =	LC/MS
Negative	30	<10 ng/mL
Low	30	11 - 60 ng/mL
Medium	30	61 - 200 ng/mL
High	30	>200 ng/mL
Total	120	

Strong correlation between ELISA and MS PEth concentrations



Unpaired t test
*** = P < 0.001
**** = P < 0.0001

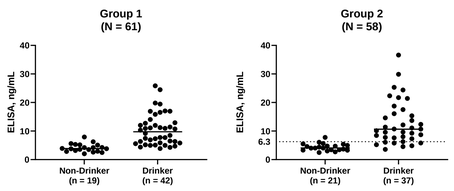


Sensitivity, Specificity, and Determination of Cut-Off Values

ELISA Cut-Off Values are contingent on specificity and sensitivity. Our goal was to define the ELISA limits for testing both Abstinence and Excessive alcohol use.

Approach:

1. We randomized half of the clinical samples (n=61) and created two groups, non-drinkers (n=27) & drinkers (n=34), defined by MS values less than or greater than 20 ng/mL for the first analysis. For the second we used 200 ng/mL.
2. Using ROC (receiver operating characteristics) and a maximized likelihood ratio to determine sensitivity and specificity, we established ELISA cut-off values of >6.3 ng/mL for the 20 ng/mL, and >12.4 ng/mL for the 200 ng/mL groups.
3. We analyzed the ELISAs performance using the second half of the samples (n=58) with the above determined cut-off values to define values for assay sensitivity, specificity, positive, and negative predictive values (PPV & NPV).

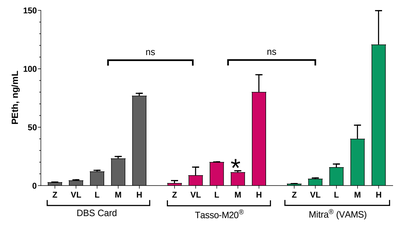


Assay Performance	n =	20 ng/mL ELISA cut-off >6.3 ng/mL	200 ng/mL ELISA cut-off >12.4 ng/mL
True Positive (a)	58	30	12
False Positive (b)	58	1	2
False Negative (c)	58	7	2
True Negative (d)	58	20	42
Sensitivity	58	81%	86%
Specificity	58	95%	95%
PPV	58	97%	86%
NPV	58	74%	95%

Comparison of Blood Collection Devices

Blood Collection Devices:	Whole blood equivalent	Sample Type
1. Whatman® 903 DBS Card	(15 µL, 5 x 3.2 mm punches)	Capillary and venipuncture
2. Tasso-M20® Device	(17.5 µL eq.)	Capillary and venipuncture
3. Mitra® Device (VAMS®)	(20 µL eq.)	Capillary and venipuncture

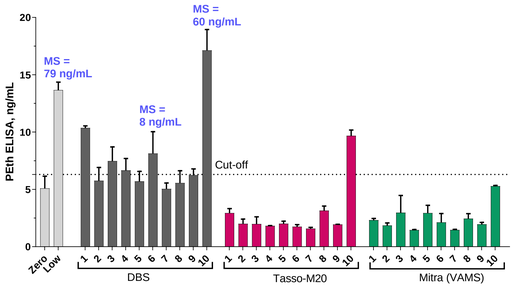
ELISA differentiates between five ex vivo generated PEth controls and displays no significant difference with blood collection devices



Sample Type	USDTL LC/MS (ng/mL)	Extracted DBS ELISA (ng/mL)	Extracted Tasso ELISA (ng/mL)	Extracted Mitra ELISA (VAMS) (ng/mL)
Zero	<8	3	2	2
Very Low	15	4	9	6
Low	66	12	20	16
Medium	188	23	11*	40
High	641	77	80	121

* Unexplained result

ELISA and MS analysis of Self-Reported Alcohol Use Samples are Comparable

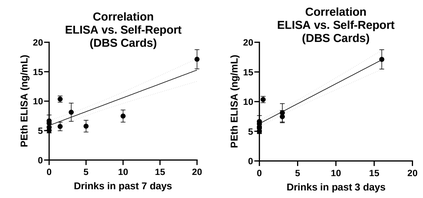


10 Self-Report Clinical Samples Tested:
- 4 Abstainers
- 6 Alcohol Users (Mostly light use)

Result:
1. PEth ELISA, using pre-defined cut-off, identified:
   a) 4 of 6 alcohol use samples with the DBS card.
   b) 1 of 6 alcohol use samples w/ Tasso.
2. LC/MS identified:
   a) 2 of 6 alcohol use samples
3. Neither ELISA nor LC/MS identified all of the lightly consuming subjects.

Clinical Samples	n =	Positive w/ DBS LC/MS	Negative w/ DBS LC/MS	Positive w/ DBS ELISA	Negative w/ DBS ELISA	Positive w/ Tasso ELISA	Negative w/ Tasso ELISA	Positive w/ Mitra ELISA	Negative w/ Mitra ELISA
Non-Drinking	4	0	4	1	3	0	4	0	4
Drinking	6	2	4	4	2	1	5	0	6
Total	10	2	8	5	5	1	9	0	10

ELISA Alone Correlates with Self-Report of Alcohol Use



Samples 1-10	P value (Significant Correlation)					
	7 days	<0.05		3 days	<0.05	
DBS	0.0028	Yes	**	0.0001	Yes	****
Tasso-M20® *	0.0026	Yes	**	<0.0001	Yes	****
Mitra® (VAMS) *	0.0101	Yes	*	0.0015	Yes	**

**Data not shown*

- The Tasso-M20® and Mitra®(VAMS®) further simplify the blood collection process and are compatible with the PEth ELISA.
- The PEth ELISA identified drinking in multiple samples with DBS Cards and in 1 of 6 drinkers in all three collection devices.
- As expected, ELISA results demonstrate stronger correlation to self reported drinking when drinking events are closer to the analysis date.

Conclusions, Future, & Acknowledgements

Conclusions

- The Semi-Quantitative PEth ELISA:
  - Differentiates between four alcohol consumption categories in a quantitative fashion.
  - Demonstrates significant correlation with reported LC/MS values.
  - Is compatible with the Tasso-M20® and Mitra®(VAMS®) collection devices.
- This 96-well immunoassay expands the availability of PEth analysis and is poised to become a valuable clinical tool in screening large numbers of samples in high-throughput workflows.

Future Directions:

- Explore additional extraction methods and assay formulations to refine assay sensitivity and specificity.
- Evaluate the stability of samples collected and stored in different conditions & increase assay ruggedness and robustness.

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