

PETH IMMUNOASSAY: AN ACCURATE OBJECTIVE BIOMARKER FOR SIGNIFICANT DRINKING

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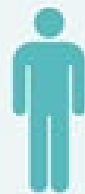
Orlando, Florida

June 25-29, 2022



Alcohol Causes Health Problems in U.S. & Worldwide

About 38 million adults in the US drink too much
Only 1 in 6 has talked about it with a health professional

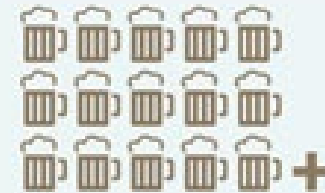


For men,
binge drinking is
5 or more drinks
consumed on
one occasion*



For women,
binge drinking is
4 or more drinks
consumed on
one occasion*

*One occasion = within 2 to 3 hours



For men –
15 or more drinks
on average per week

One Drink = 5-ounces of wine, 12-ounces of beer,
or 1 ½-ounces of 80-proof distilled spirits or liquor



For women –
8 or more drinks on
average per week

Drinking too much includes

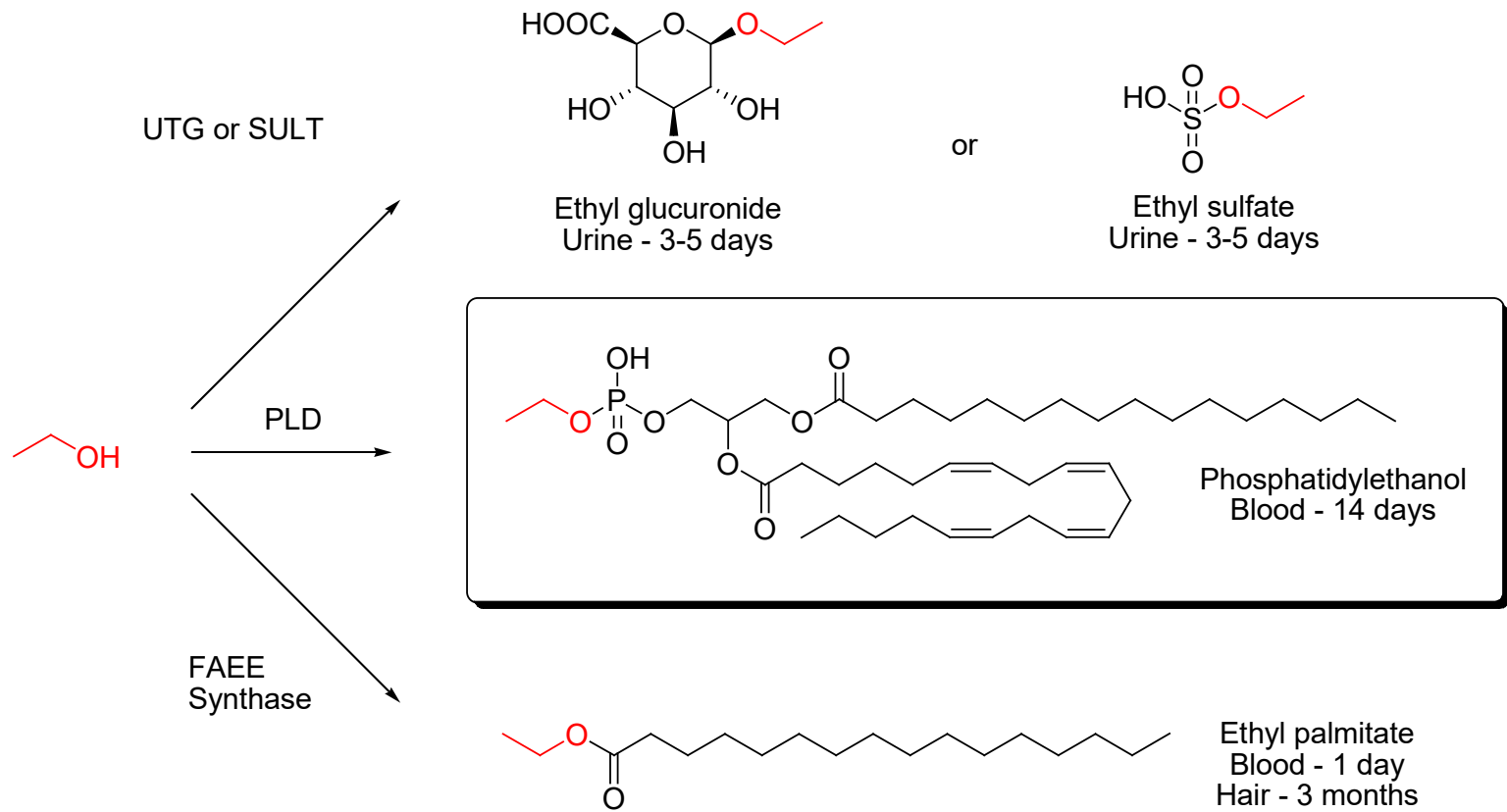
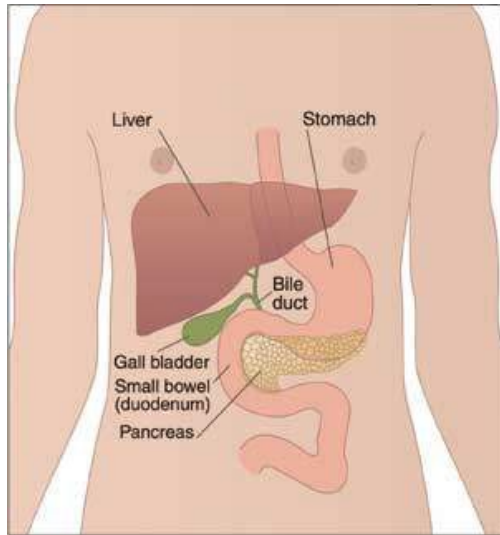


Any alcohol use by
pregnant women



Any alcohol use by
those **under age 21**

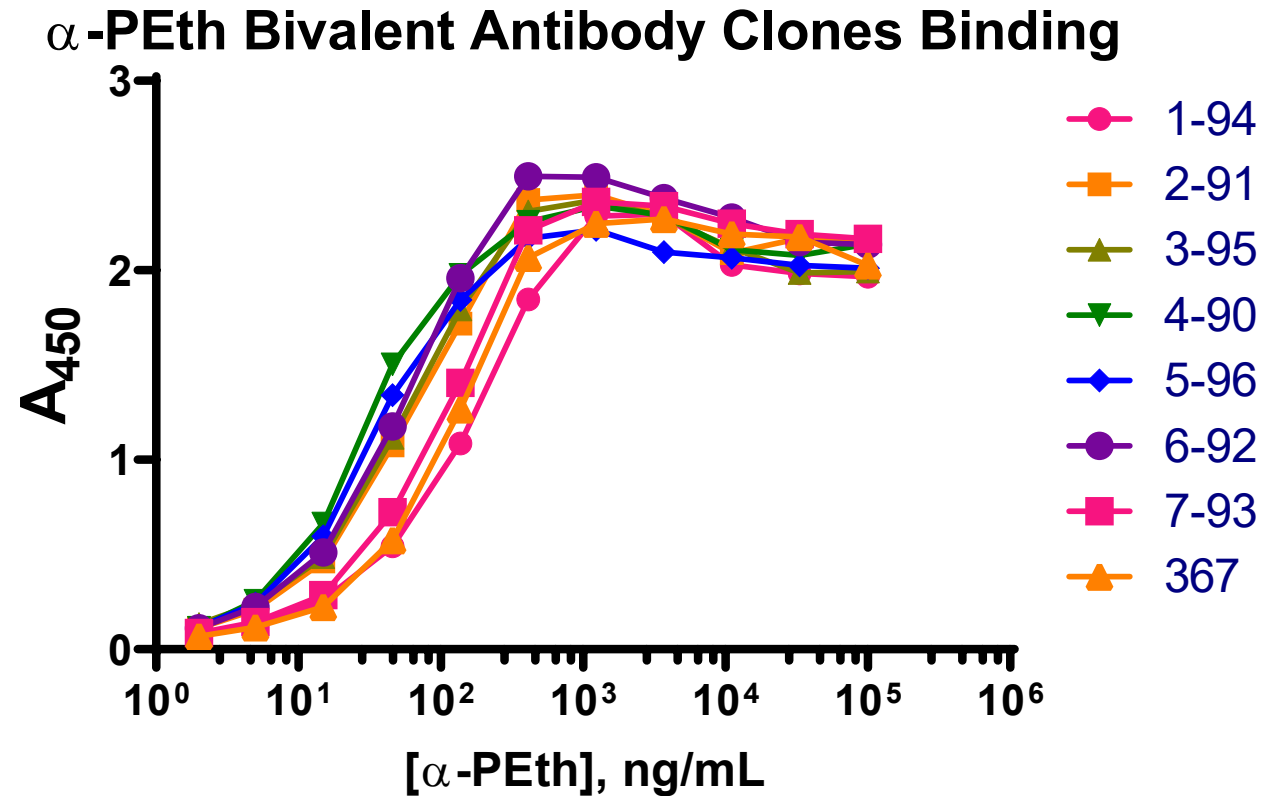
Non-Oxidative Alcohol Metabolites



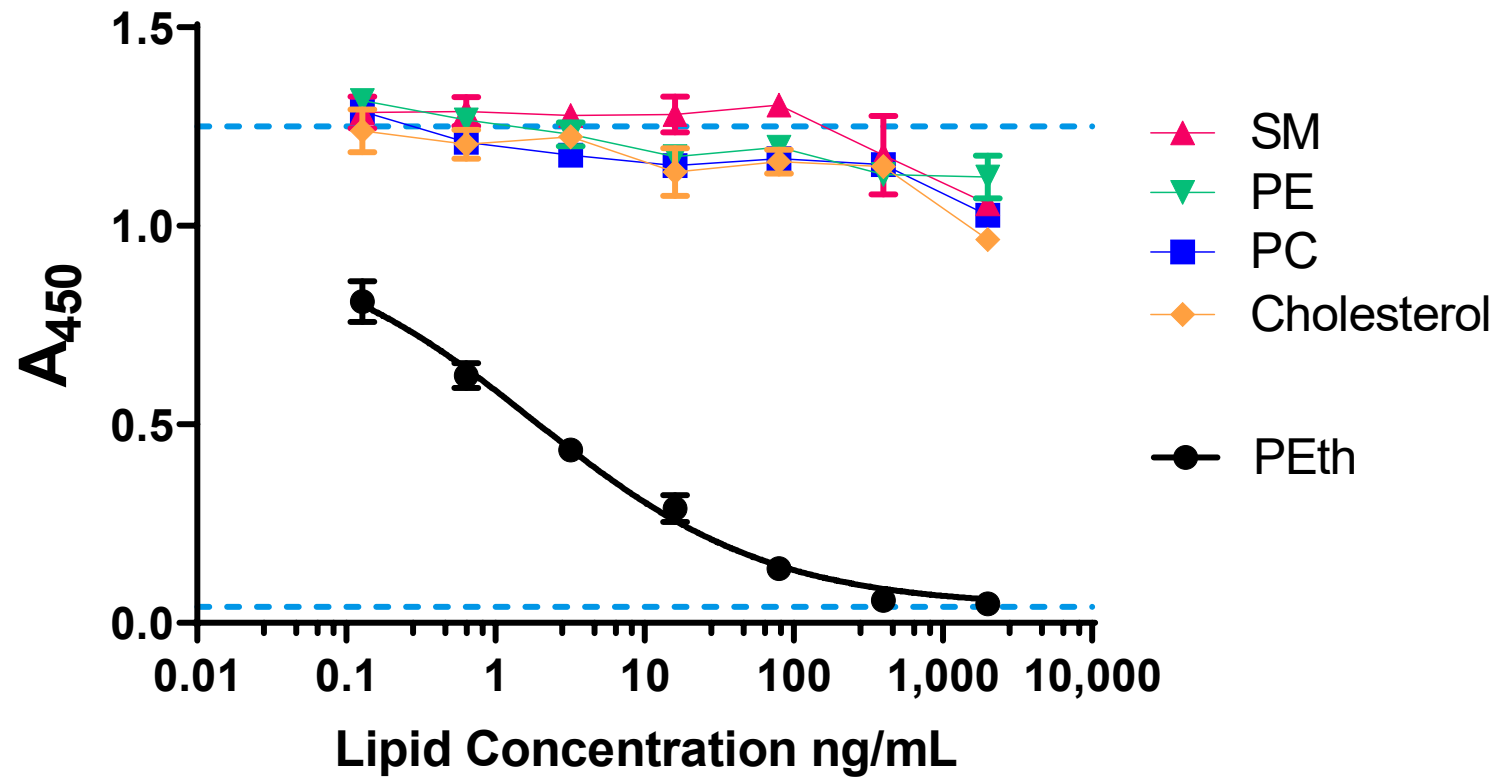
ELISA Development: Antibody Clone Selection

We screened dozens of antibody clones for PEth binding

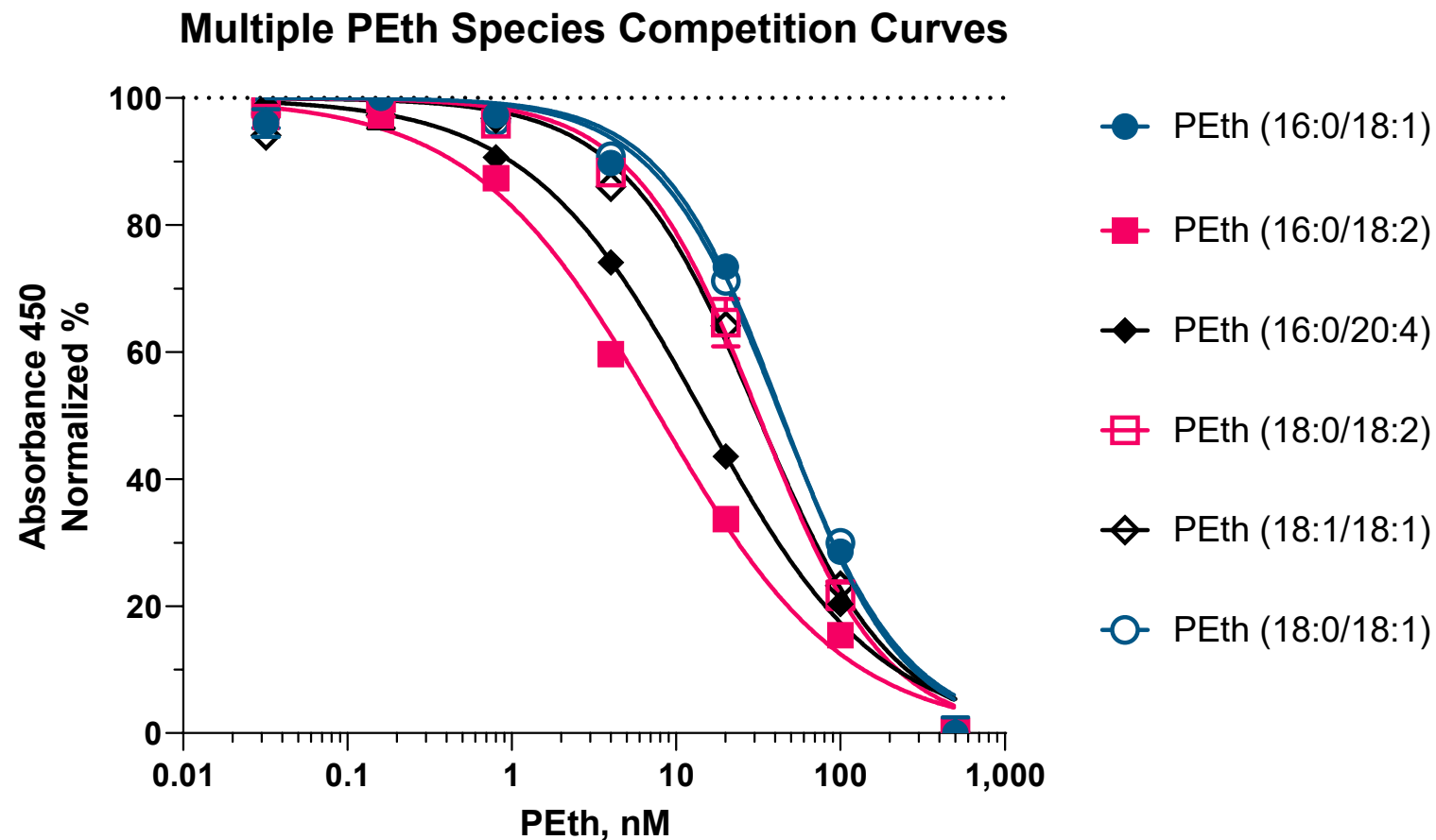
- PEth coated 96-well microtiter plates
- Standard ELISA conditions



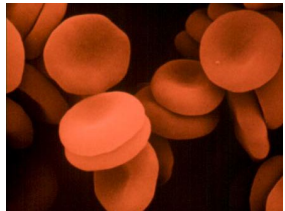
ELISA Development: Antibody Specific for PEth



ELISA Dev: Ab Recognizes Lipid Headgroup



ELISA Testing: Generating & Analyzing PEth in Blood



Generate

Drinking

De novo: PMA + EtOH

Exogenous
Incorporation



Extract

Acetone, Methanol
or Butanol

+/- Column

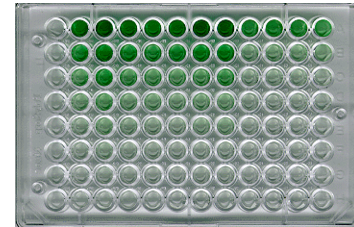


Analyze

Mass Spec

Flow Cytometry, TLC

ELISA



Ex Vivo PEth Generation: MS Analysis

	EoE, 23°C	Fold Increase	Shaking, 37°C	Fold Increase
48 hr Control	145		123	
EtOH, 24 hrs	184	1.3	314	2.6
EtOH, 48 hrs	203	1.4	449	3.7
EtOH+PMA, 24 hrs	791	5.5	1,313	10.7
EtOH+PMA 48 hrs	961	6.6	1,738	14.1

Table values are PEth 16:0/18:1 (ng/mL) measured by MS

ELISA Testing: Calibrators & Samples

Calibrators are bulk extracted from DBS paper

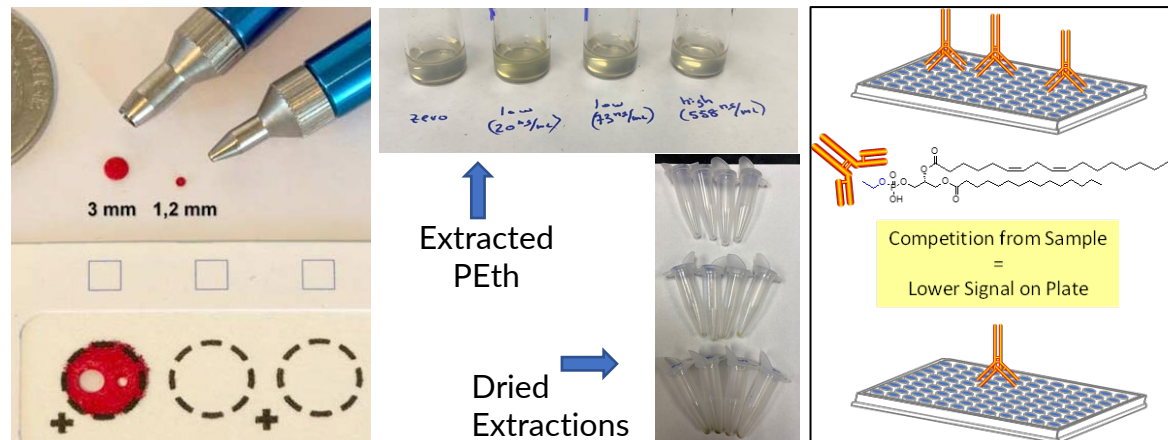
- Ratio of solvent to blood is maintained while scaling up volumes for consistent performance

Samples are individually extracted from micro punches of DBS cards

- Simple 2-hour procedure
- There is some variability inherent with lipid extractions, improves with practice



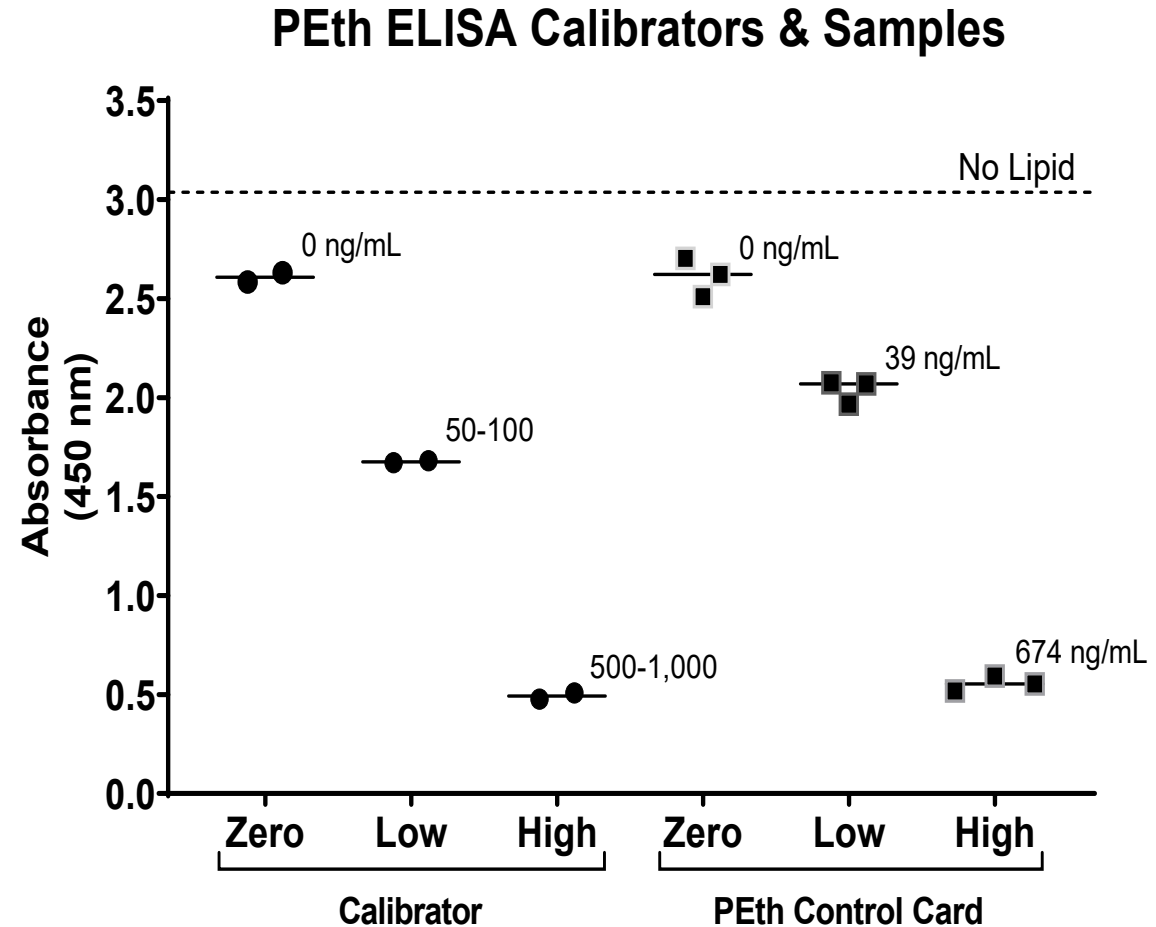
K-5500 PEth ELISA Data Sheet



ELISA Testing: Results with DBS replicates

Qualitative Results

- Calibrators & Sample measured by MS
- ELISA Absorbance values show consistent competition corresponding with increasing PEth amounts
- Interpolation from PEth standard curves gives quantitative values that sometimes correspond with MS values



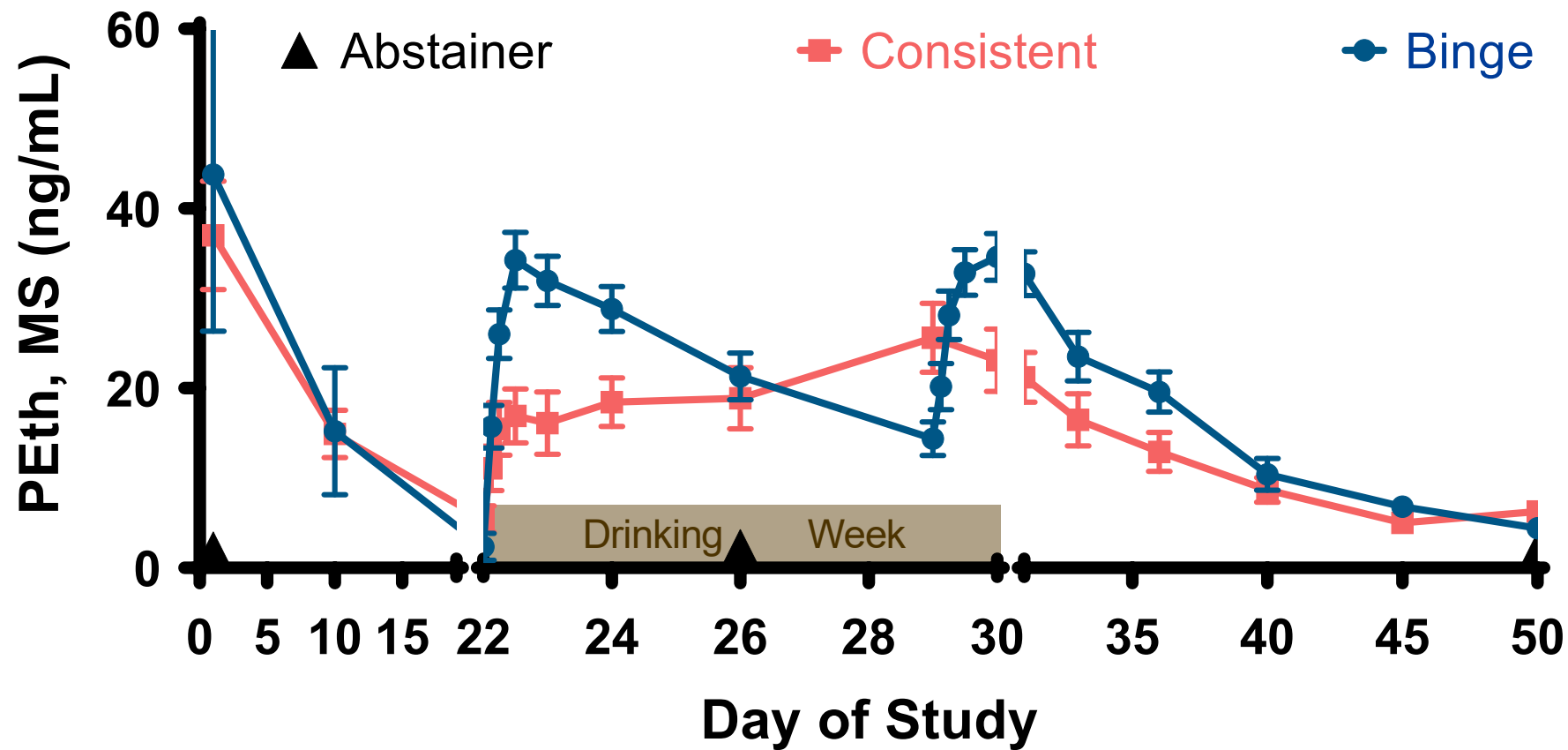
Biomarker Clinical Validation Study: MS measurement

- Design:
 - 1) Single Site, Matched (3 Groups) X (n of 17) = 51
 - 2) Continuous Monitoring, 50 Days, 14 Subject Visits w/ two O/N stays during “drinking week”
- Analyze:
 - 1) PEth(MS) and α -PEth-IgG (ELISA), 680 total blood samples
 - 2) Compare w/ EtG/EtS, 306 total Urine, and %CDT, 255 total Serum
- Outcomes:
 - 1) α -PEth-IgG ELISA useful as a screening assay?
 - 2) Does drinking pattern influence PEth?

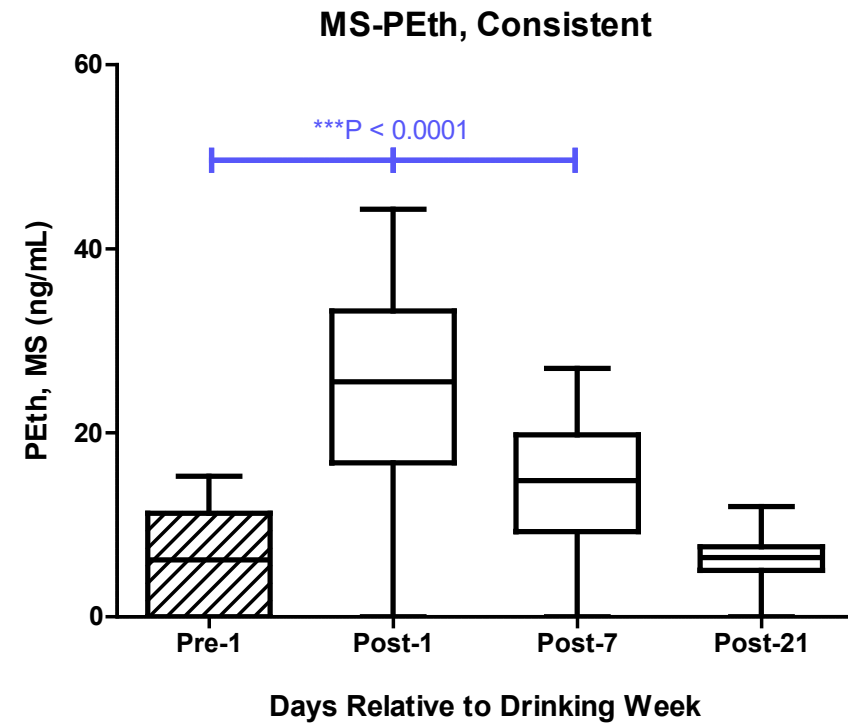
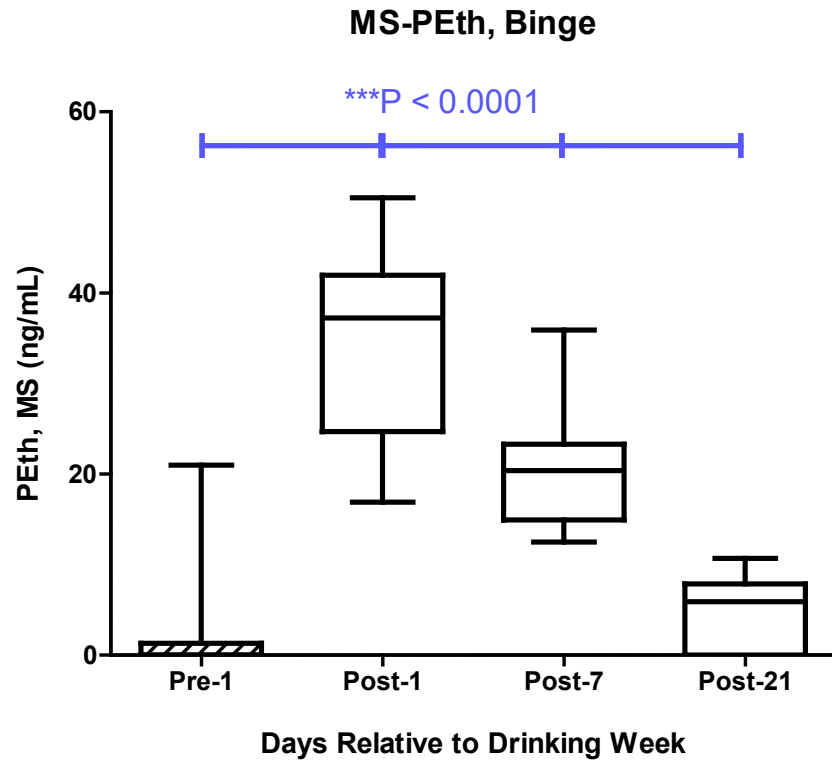
Enrollment Details

Table 1. Final Study Enrollment N/A = not applicable	GROUP			
ENROLLMENT & DEMOGRAPHICS	Binge	Consistent	Teetotaler Control	Total
# Female	6	6	6	18
# Male	9	9	12	30
# Enrolled	15	15	18	48
Ave Age, Years (Range)	24.9 (21-32)	32.5 (24-58)	30.2 (22-49)	29.3 (21-58)
Ave Weight, kg (Range)	89.1 (59-132)	80.3 (56-140)	87 (48-129)	85.5 (48-140)
BMI (Range)	28.5 (20-41)	26.7 (19-39)	27.9 (18-38)	27.7 (18-41)
DRINKING DETAILS				
Ave # drinks consumed on 1 st drinking day (Range)	7.1 (4-10)	4.6 (2-8)	0	N/A
Peak BrAC (%) Measured (Range)	0.145 (0.11-0.20)	0.093 (0.075-0.17)	N/A	N/A
Ave # drinks reported over 7 day drinking period (Range)	13.7 (8-21)	19.1 (12-33)	N/A	N/A

PEth Generated in Hours, Remains Elevated for Weeks



Binge v. Consistent: 3-Weeks Post Drinking



Easy & Accurate PEth ELISA for Alcohol Ingestion

Please contact us with any questions:

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Additional Information
PEth Biomarker Blog

