

# PETH IMMUNOASSAY: AN ACCURATE OBJECTIVE BIOMARKER FOR SIGNIFICANT DRINKING

J. Johnson, F.V. Chan, C. Day, and P.O. Neilsen

Echelon Biosciences

675 Arapeen Drive, Suite 302

Salt Lake City, Utah 84108, U.S.A.

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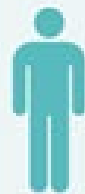
Orlando, Florida

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

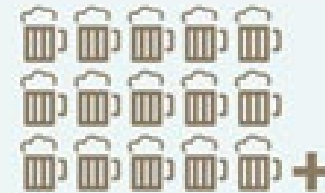


For men,  
binge drinking is  
**5 or more drinks**  
consumed on  
one occasion\*



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



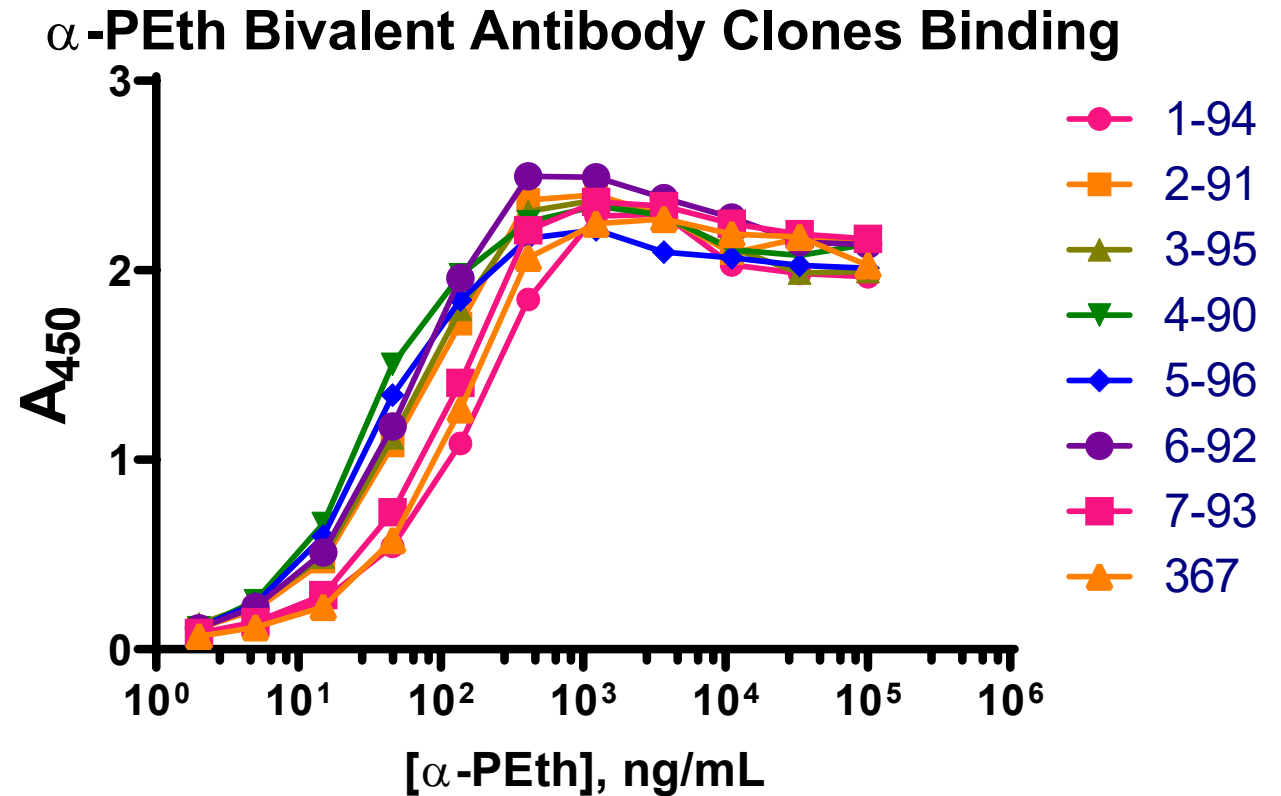
Any alcohol use by  
those **under age 21**



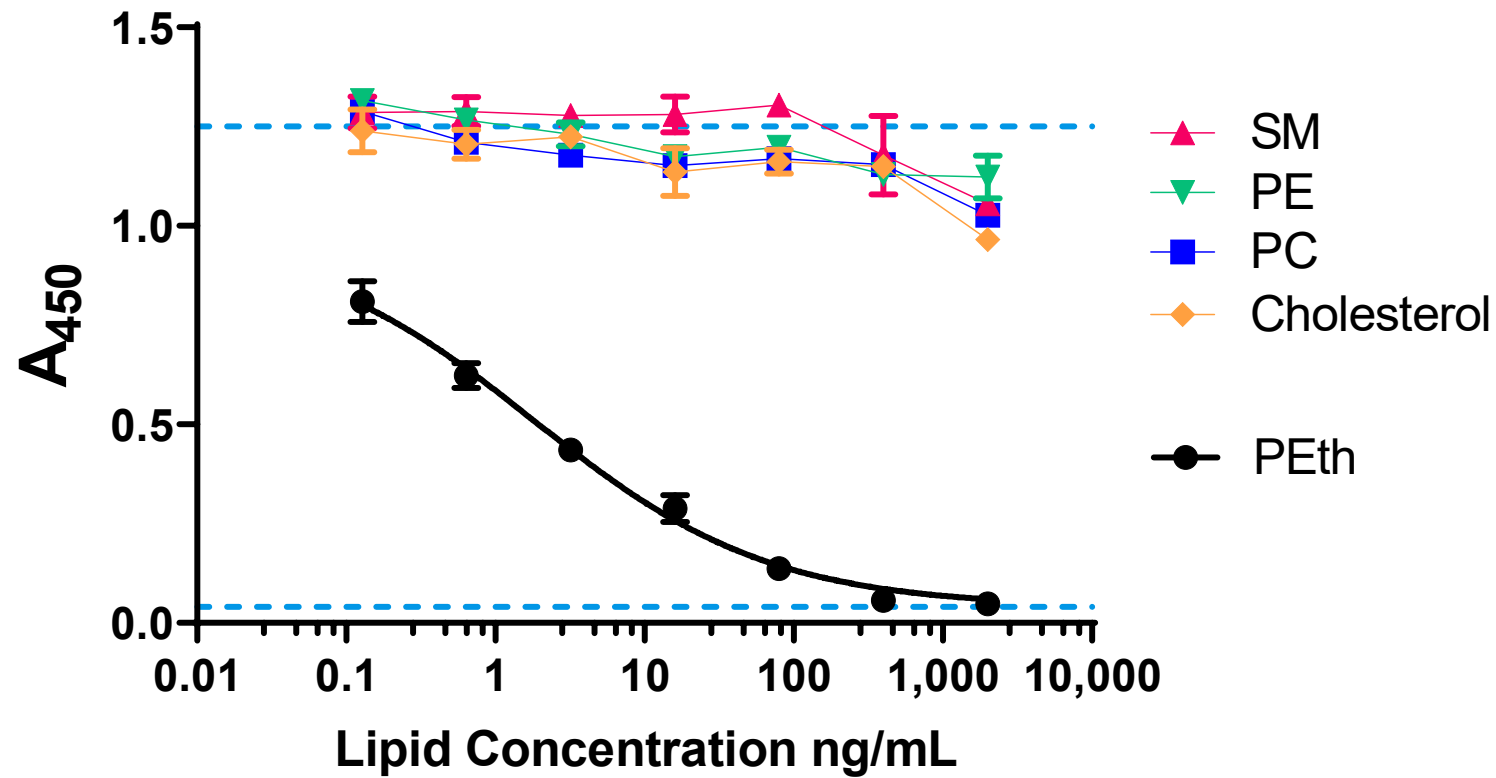
# ELISA Development: Antibody Clone Selection

We screened dozens of antibody clones for PEth binding

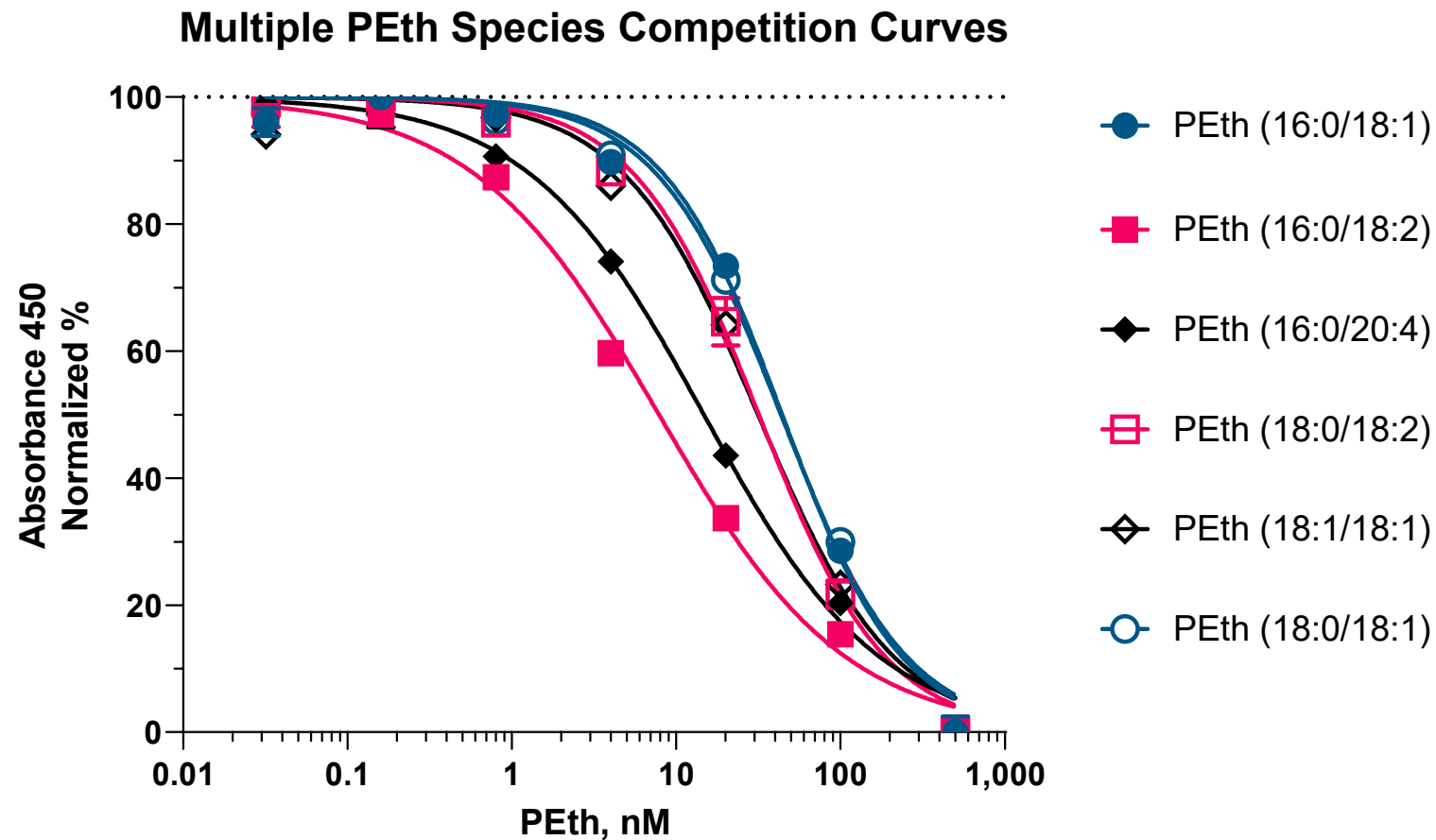
- PEth coated 96-well microtiter plates
- Standard ELISA conditions



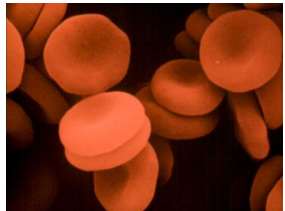
# ELISA Development: Antibody Specific for PEth



# 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

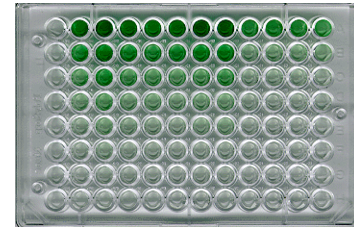


## Analyze

Mass Spec

Flow Cytometry, TLC

ELISA



# Ex Vivo PEth Generation

	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

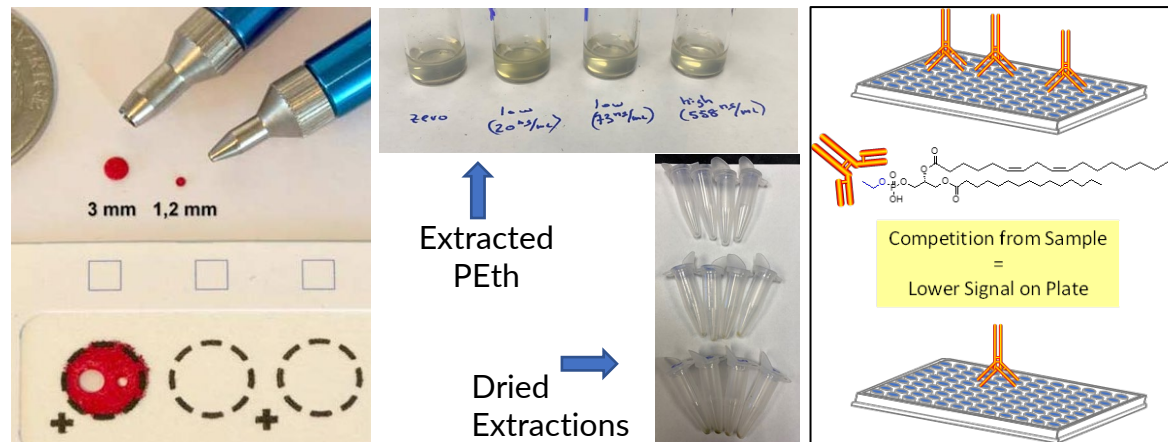
- Ratio of solvent to blood is maintained while scaling up volumes for consistent performance

## 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



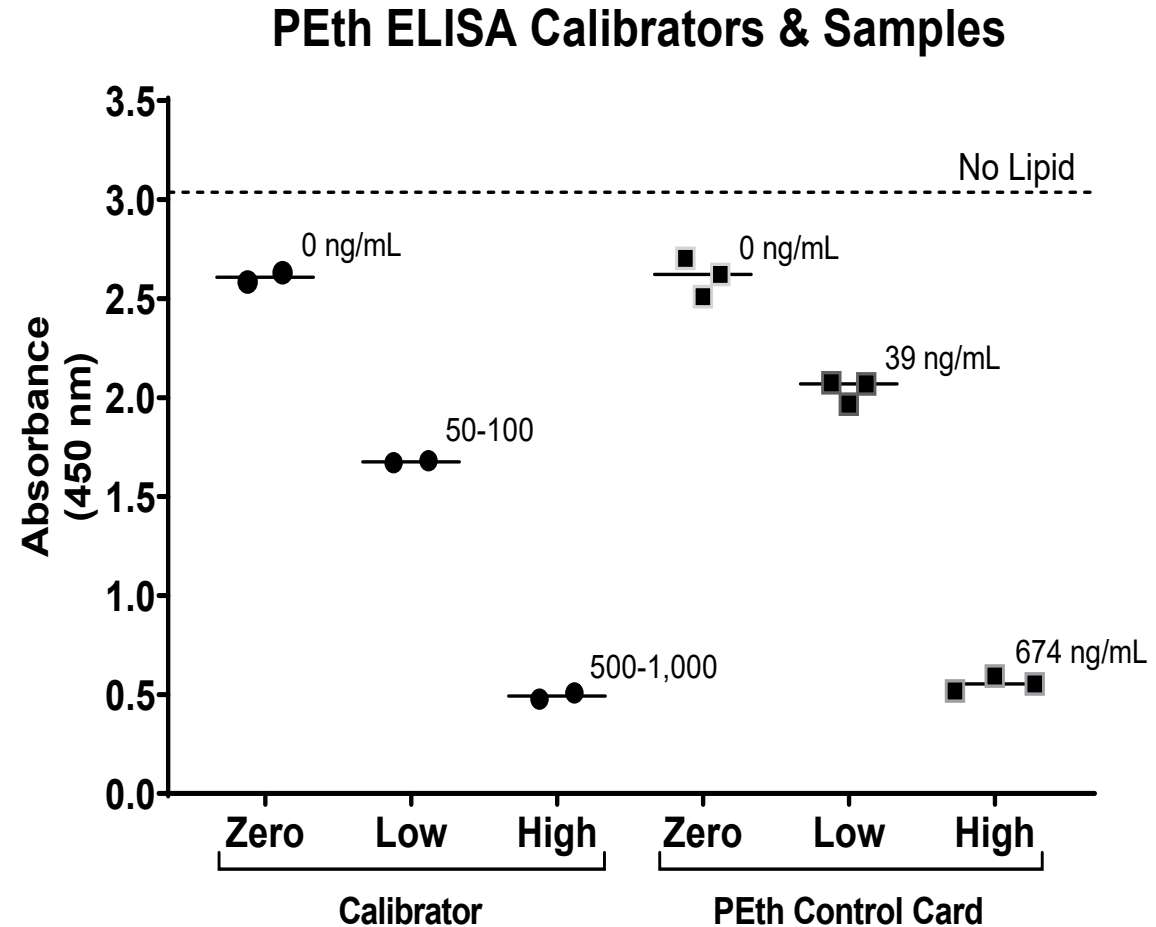
K-5500 PEth ELISA Data Sheet



# 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



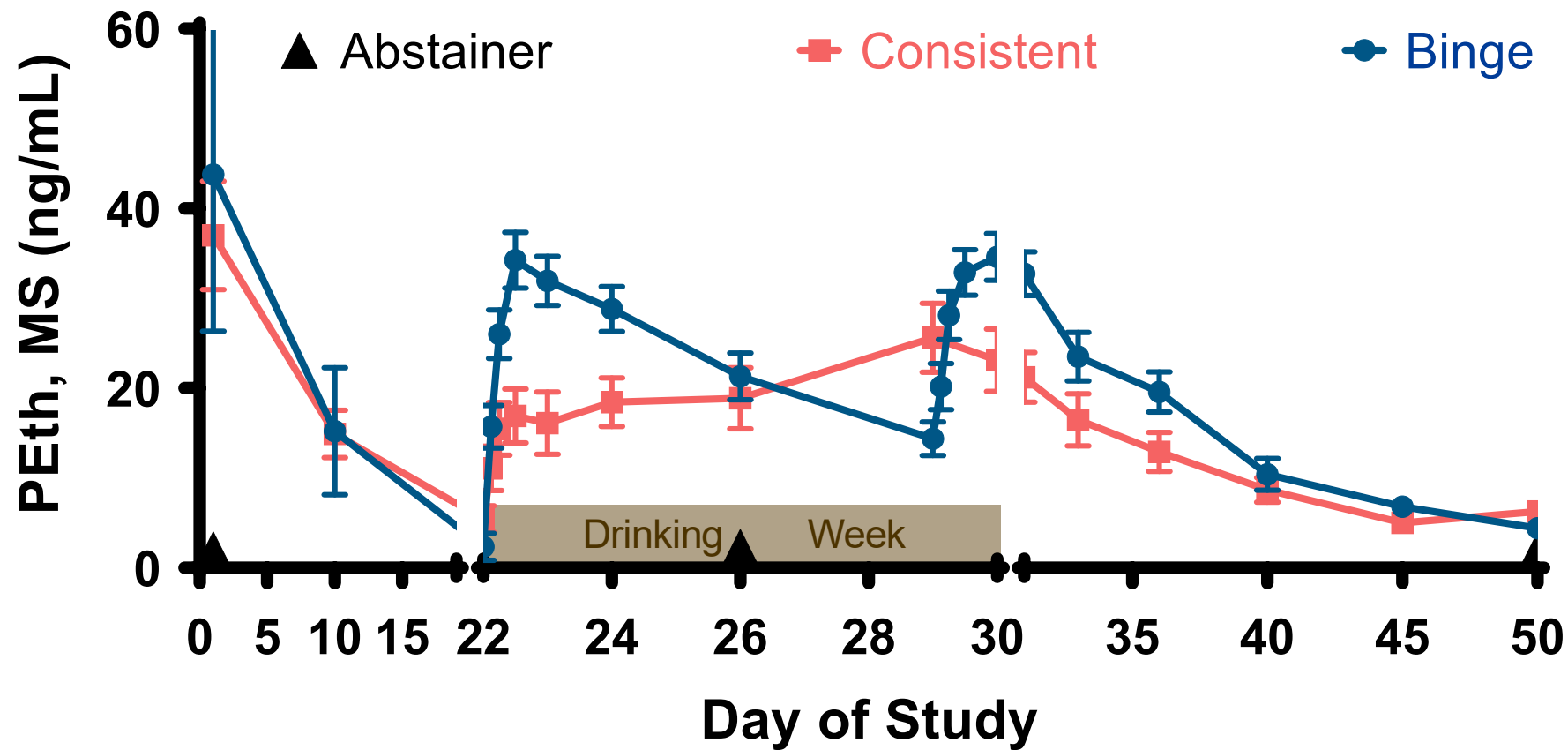
# Biomarker Clinical Validation Study

- **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  $\alpha$ -PEth-IgG (ELISA), **680 total blood samples**
  - 2) Compare w/ EtG/EtS, **306 total Urine**, and %CDT, **255 total Serum**
- **Outcomes:**
  - 1)  $\alpha$ -PEth-IgG ELISA useful as a screening assay?
  - 2) Does drinking pattern influence PEth?

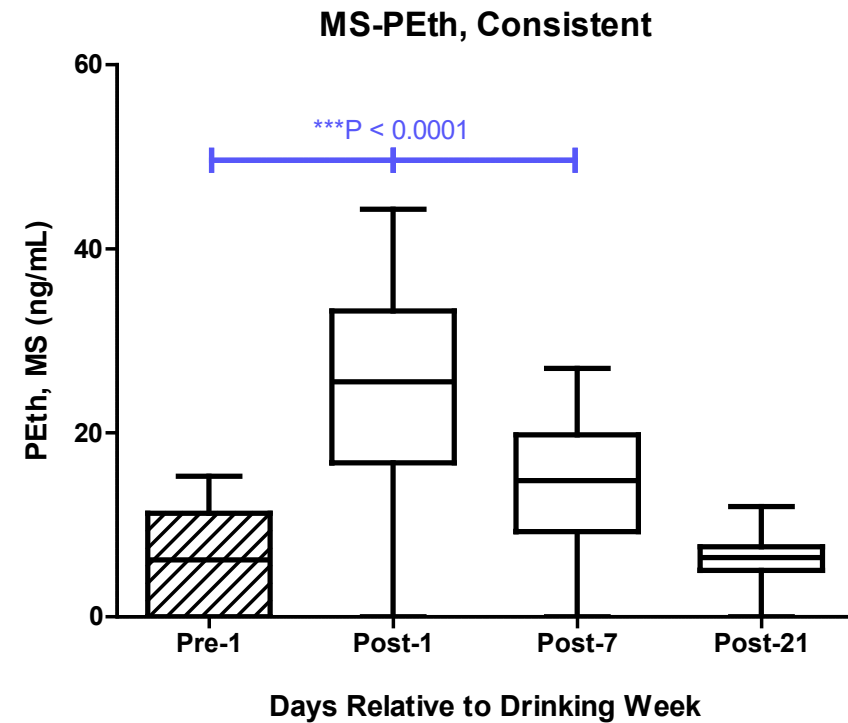
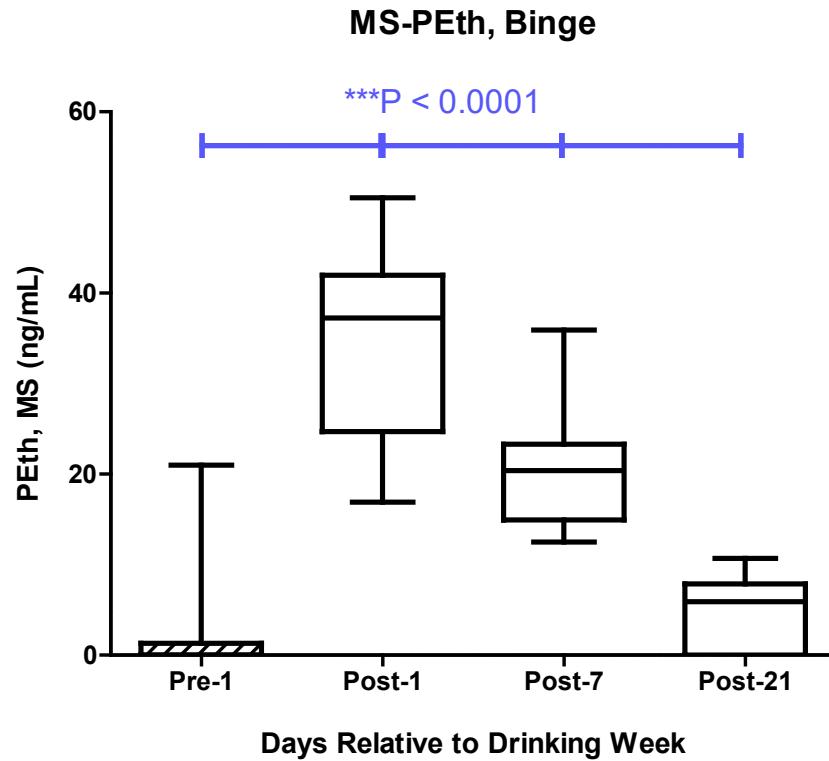
# Enrollment Details

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 <sup>st</sup> 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 me with any questions:

Paul O. Neilsen, Ph.D. MBA  
VP BD/R&D Echelon Biosciences  
Tel 801.588.0455 x 304

[pneilsen@echelon-inc.com](mailto:pneilsen@echelon-inc.com) [www.echelon-inc.com](http://www.echelon-inc.com)



Additional Information  
PEth Biomarker Blog

