# Laboratory testing for biomarkers of alcohol exposure

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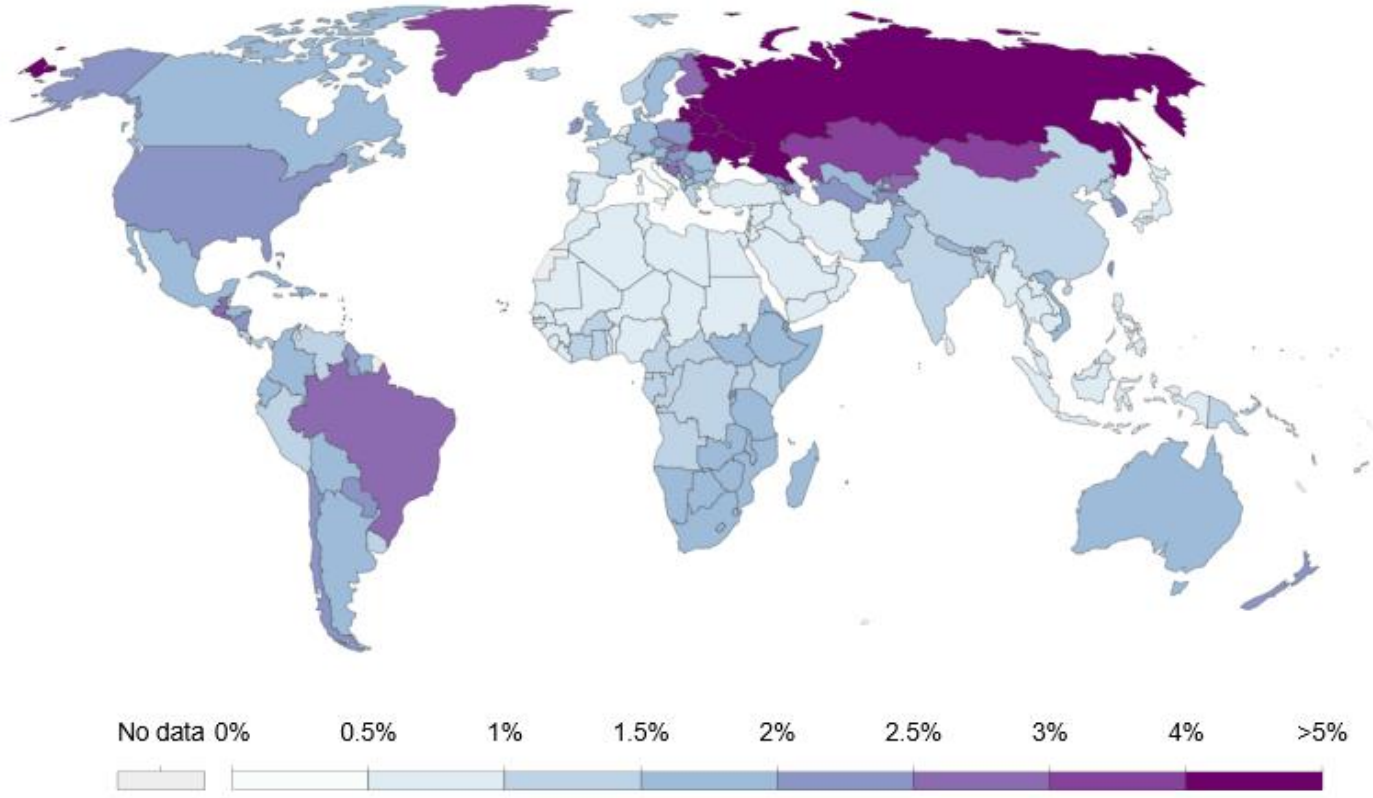
# Learning Objectives

- Describe the effects of ethanol on the human body
- Highlight the clinical need to test for alcohol exposure
- Review biomarkers commonly used to detect and monitor alcohol exposure
- Describe analytical methods to analyze alcohol biomarkers in biological matrices by LC-MS/MS
  - Urine
  - Whole blood
  - Serum
  - Meconium
  - Umbilical cord tissue

# 2017 – Alcohol Use Disorder - Worldwide

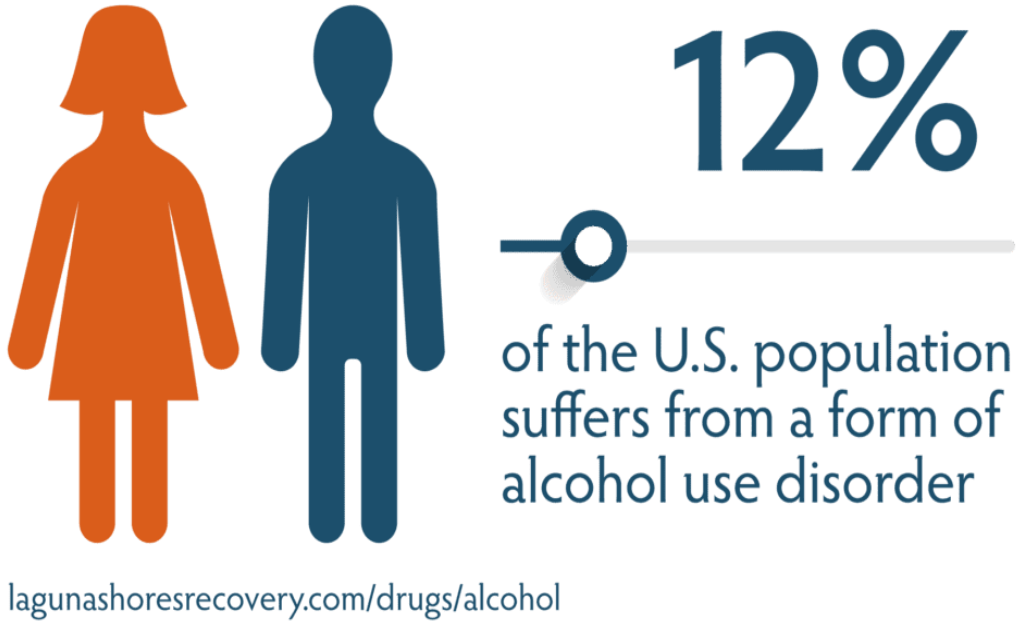
**1.4% (~107 million)** of the global population (~7.53 billion)
70% male (75 million) to 32 million females




https://ourworldindata.org/drug-use

# 2018 Alcohol (Ethanol) Facts and Statistics

- Alcohol use disorder (AUD)
  - 14.4 million adults ages 18 and older
  - 401,000 adolescents ages 12–17
- Leading cause of morbidity and mortality
  - 3rd Leading cause of preventable death
    - Ages: 15 – 45 yr




- 2018 – Centers for Disease Control and Prevention
  - Ethanol-induced deaths
    - > 88,000 deaths/ year
      - Ethanol and drug interactions – (cocaine, opiates, benzodiazepines, acetaminophen)
      - < 10,000 ethanol related traffic fatalities (all ages)
      - 39,921 deaths from alcohol liver disease (12 yr and older)

Natl. Inst. on Alcohol Abuse & Alcoholism – www.niaaa.nih.gov

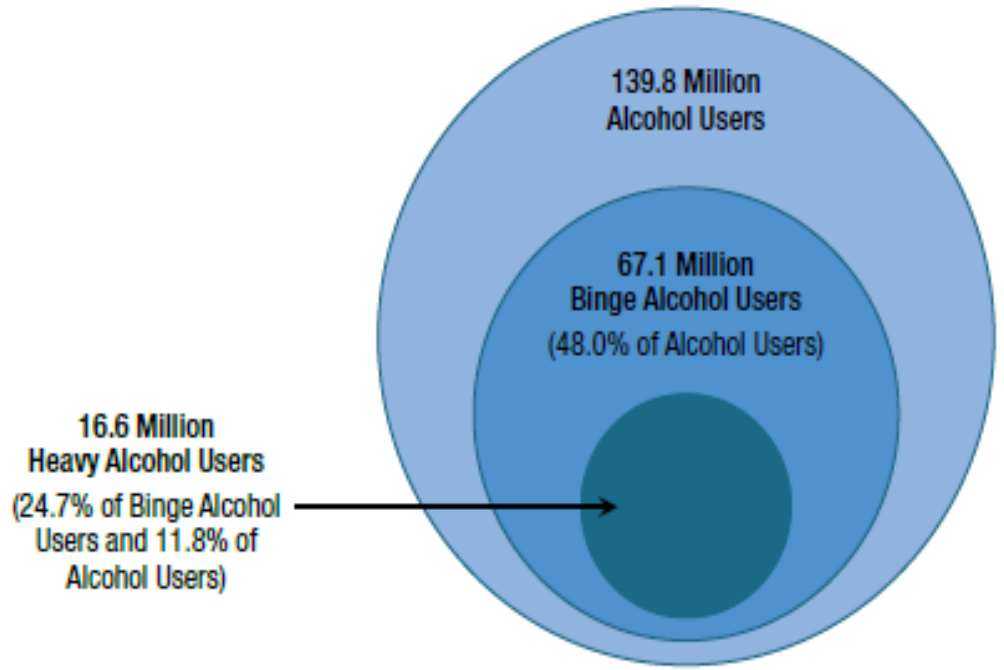
## 2018 National Survey on Drug Use and Health (NSDUH)

### Past Month Alcohol Use, Binge Alcohol Use and Heavy Alcohol Use among participants Aged 12 or older

- Moderate drinker – 1-2 drinks/ day
- Binge drinker – Males: ≥ 5 drinks

  Females: ≥ 4 drinks
- Heavy alcohol use – Binge drinking ≥ 5 days

**Figure 6. Current, Binge, and Heavy Alcohol Use among People Aged 12 or Older: 2018**

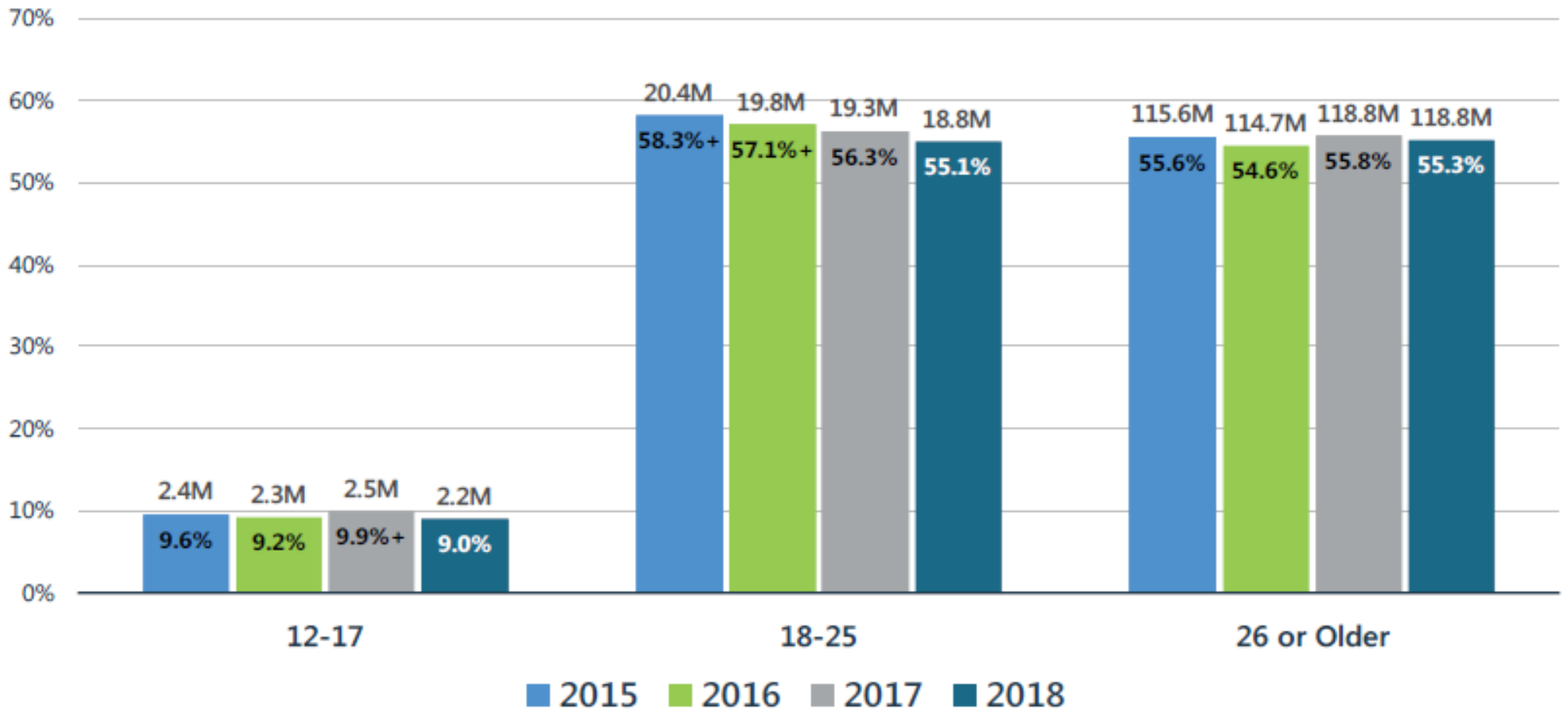



https://www.samhsa.gov/data/report/2018-nsduh-annual-national-report

Natl. Inst. on Alcohol Abuse & Alcoholism

# Alcohol Use

PAST MONTH, 2015-2018 NSDUH, 12+



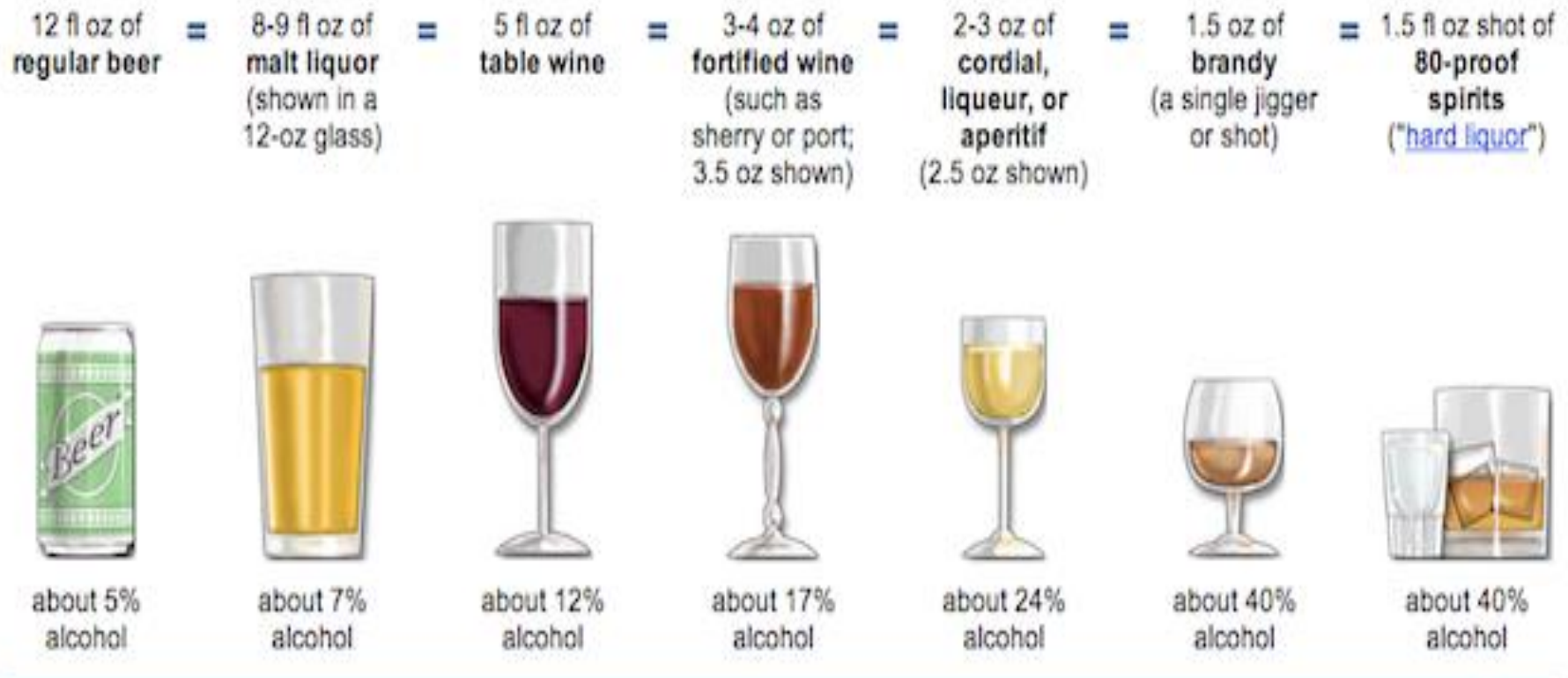

+ Difference between this estimate and the 2018 estimate is statistically significant at the .05 level.

SAMHSA
Substance Abuse and Mental Health Services Administration

https://www.samhsa.gov/data/report/2018-nsduh-annual-national-report

# What is a Standard Drink?

Standard Drink – 10-14 g of ethanol

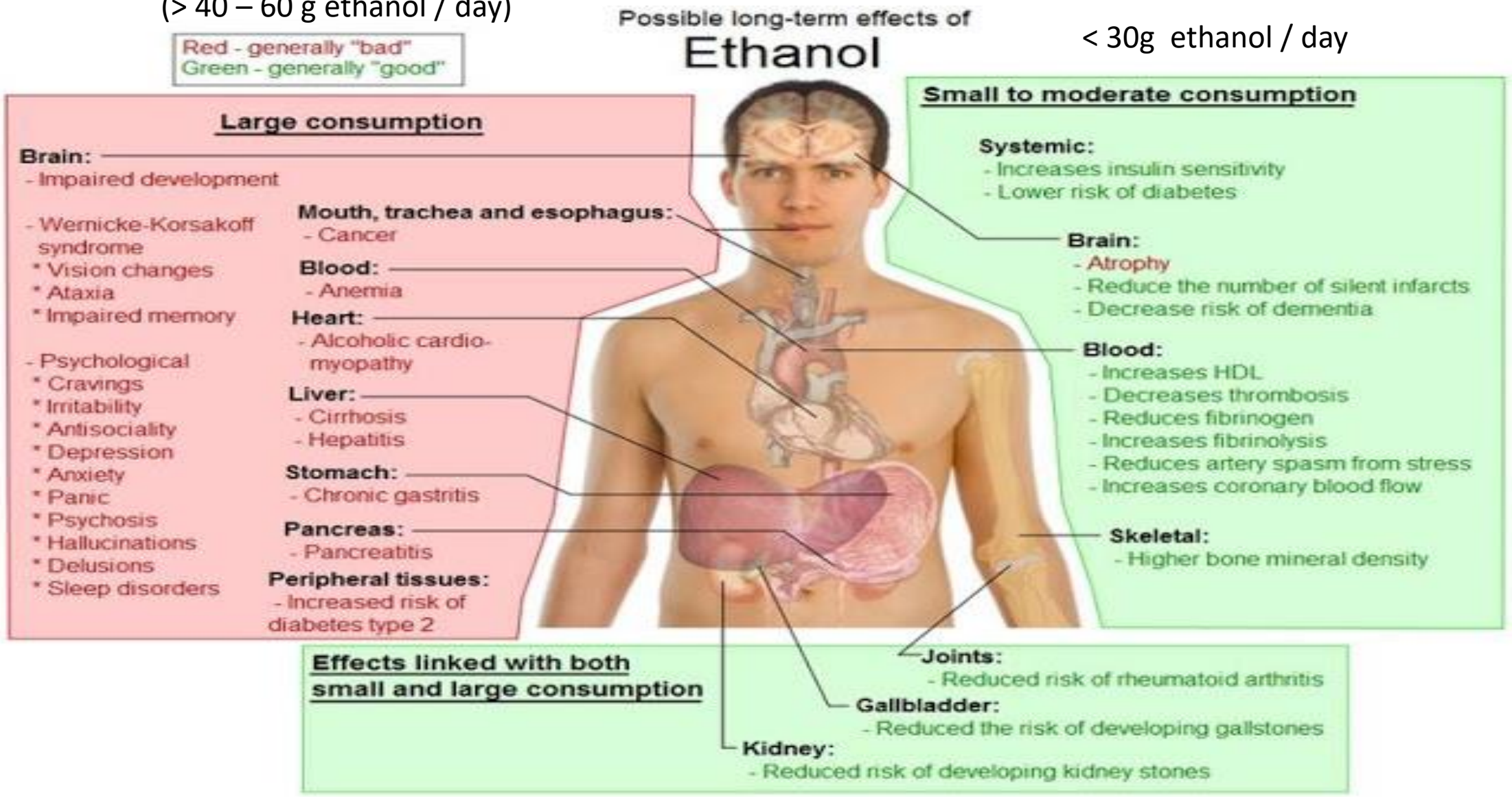



www.niaaa.nih.gov

# Health Benefits and Adverse Effects of Alcohol

(> 40 – 60 g ethanol / day)

< 30g ethanol / day



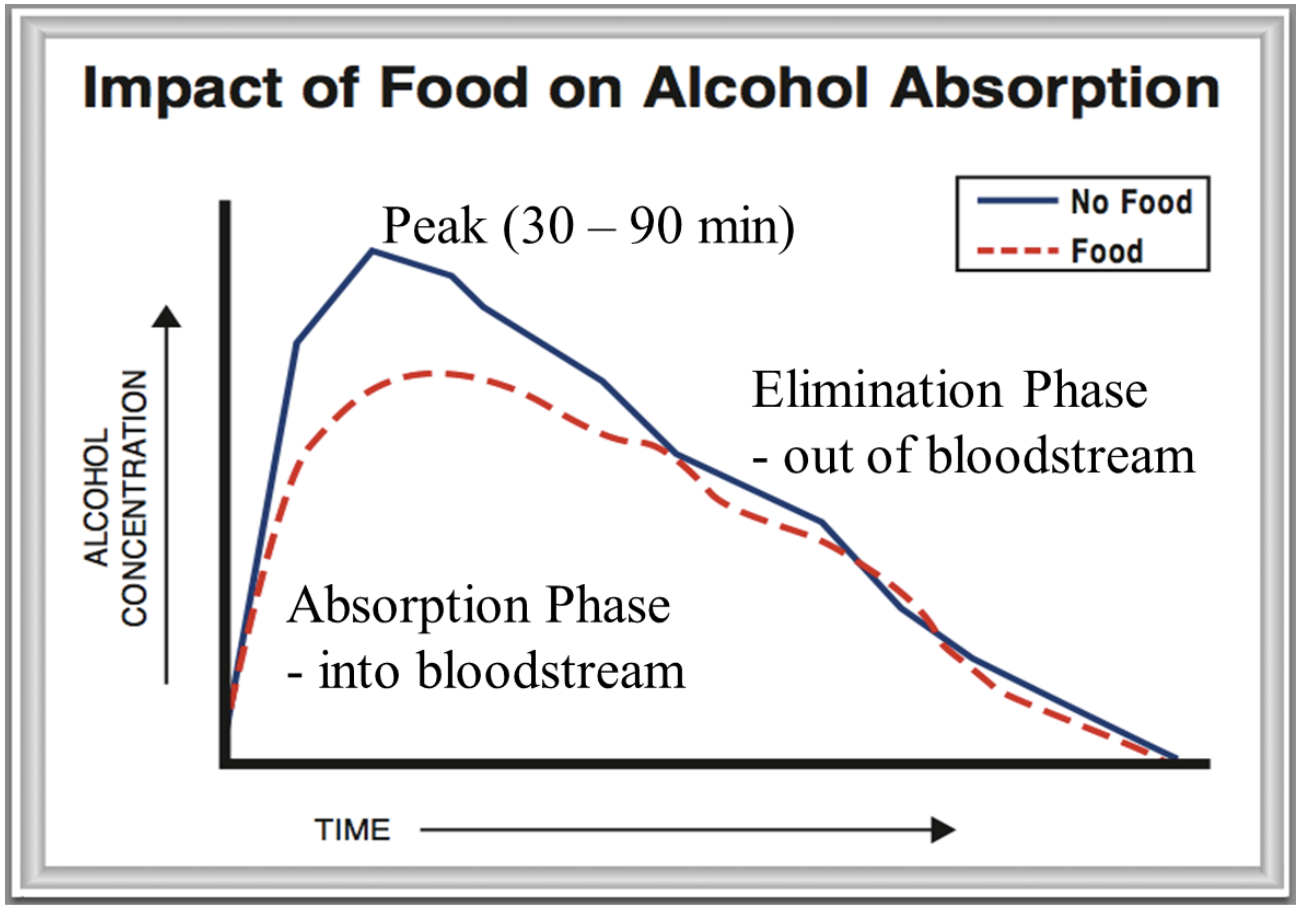

http://1.bp.blogspot.com/-2gTViGCbkOI/UQvxi7Qcv9I/AAAAAAAAAc8/hG-c2pHVKWY/s640/long+term+effects+alcohol.jpg

# Mechanism of Action

- CNS Depressant
  - Mechanism is not completely elucidated
  - Involves GABA-mediated inhibitory response
  - Inhibits neuronal NMDA and kainate receptors (Excitatory)

- Heavy ethanol use
  - Cause up-regulation of NMDA receptors
  - Desensitization through phosphorylation of GABA and glutamate receptors
  - Physical abstinence syndrome from abrupt withdrawal

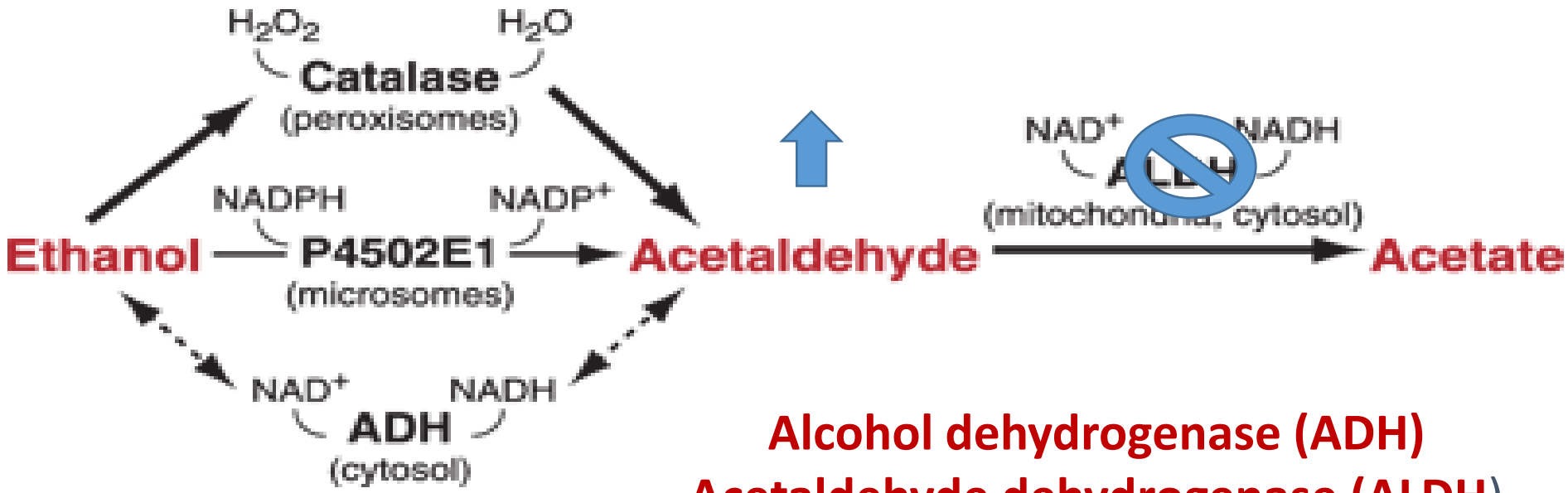
# Ethanol Pharmacokinetics




http://4.bp.blogspot.com/-x6j5SLAkam8/UkrXy2ow5VI/AAAAAAAAA8Y/k65S0vfyqQQ/s1600/Alcohol+curve.png

- Ethanol is transported to liver
  - First-pass metabolism - ~85% of alcohol
    - rate of ~10 g/hour (70 kg adult)
- Ethanol Elimination in Blood
  - 0.015 g/dL/hr or ~0.01%/hr
  - Alcoholics have increased elimination rates due to enzyme induction
- 10% Excreted in the urine, breath and sweat

# Ethanol Metabolism




**Alcohol dehydrogenase (ADH)**
**Acetaldehyde dehydrogenase (ALDH)**

Disulfiram - Symptoms of Elevated acetaldehyde

- increased flushing
- tachycardia (elevated heart rate)
- nausea, vomiting & hyperventilation

Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th edition

# Physiological Effects of Alcohol

- **Ethanol affects men and women differently**
  - Women have more body fat and less water than men
  - Women have 50% less gastric **alcohol dehydrogenase** (ADH)
  - Contribute to higher blood alcohol concentrations
  - More vulnerable to impairment due to alcohol consumption

(Marshall et al. 1983, Van Thiel et al. 1988).

# MEN
# KNOW YOUR LIMIT

**Approximate Blood Alcohol Content (BAC) In One Hour**

Source: National Highway Traffic Safety Administration

| Drinks | Body Weight In Pounds | | | | | | | | Influenced |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | |
| 1 | .04 | .03 | .03 | .02 | .02 | .02 | .02 | .02 | Possibly |
| 2 | .08 | .06 | .05 | .05 | .04 | .04 | .03 | .03 | |
| 3 | .11 | .09 | .08 | .07 | .06 | .06 | .05 | .05 | Impaired |
| 4 | .15 | .12 | .11 | .09 | .08 | .08 | .07 | .06 | |
| 5 | .19 | .16 | .13 | .12 | .11 | .09 | .09 | .08 | Legally Intoxicated |
| 6 | .23 | .19 | .16 | .14 | .13 | .11 | .10 | .09 | |
| 7 | .26 | .22 | .19 | .16 | .15 | .13 | .12 | .11 | |
| 8 | .30 | .25 | .21 | .19 | .17 | .15 | .14 | .13 | |
| 9 | .34 | .28 | .24 | .21 | .19 | .17 | .15 | .14 | |
| 10 | .38 | .31 | .27 | .23 | .21 | .19 | .17 | .16 | |

**Subtract .015 for each hour after drinking.**

# WOMEN
# KNOW YOUR LIMIT

**Approximate Blood Alcohol Content (BAC) In One Hour**

Source: National Highway Traffic Safety Administration

| Drinks | Body Weight In Pounds | | | | | | | | Influenced |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | |
| 1 | .05 | .04 | .03 | .03 | .03 | .02 | .02 | .02 | Possibly |
| 2 | .09 | .08 | .07 | .06 | .05 | .05 | .04 | .04 | |
| 3 | .14 | .11 | .11 | .09 | .08 | .07 | .06 | .06 | Impaired |
| 4 | .18 | .15 | .13 | .11 | .10 | .09 | .08 | .08 | |
| 5 | .23 | .19 | .16 | .14 | .13 | .11 | .10 | .09 | Legally Intoxicated |
| 6 | .27 | .23 | .19 | .17 | .15 | .14 | .12 | .11 | |
| 7 | .32 | .27 | .23 | .20 | .18 | .16 | .14 | .13 | |
| 8 | .36 | .30 | .26 | .23 | .20 | .18 | .17 | .15 | |
| 9 | .41 | .34 | .29 | .26 | .23 | .20 | .19 | .17 | |
| 10 | .45 | .38 | .32 | .28 | .25 | .23 | .21 | .19 | |

**Subtract .015 for each hour after drinking.**

http://www.businessinsider.com/drinks-before-driving-if-bac-is-05-2013-5

# Intoxication Effects correlated to Blood Alcohol concentrations

| **Blood Alcohol Concentration** | **Acute Intoxication Effects** |
|---|---|
| 100 mg/dL = 0.10%W/V = 0.1 g/dL | |
| 0.02–0.05 g/dL<br>20–50 mg/dL | Most individuals experience **mild euphoria** but may have no obvious signs. Diminished fine motor function occurs. |
| 0.05–0.15 g/dL<br>50–150 mg/dL | Euphoria with reduction in judgment, motor function and reaction time. |
| 0.15–0.30 g/dL<br>150–300 mg/dL | Obvious signs of intoxication including impaired balance, speech, reaction time, emotional stability, vision and comprehension. |
| >0.25 g/dL<br>>250 mg/dL | Marked loss in motor function, impaired consciousness may be associated with death in uncomplicated adult cases. |
| >0.40 g/dL<br>>400 mg/dL | Respiratory depression. Blood concentration at which most fatalities occur. |

# Why Should we Test for Ethanol Exposure?

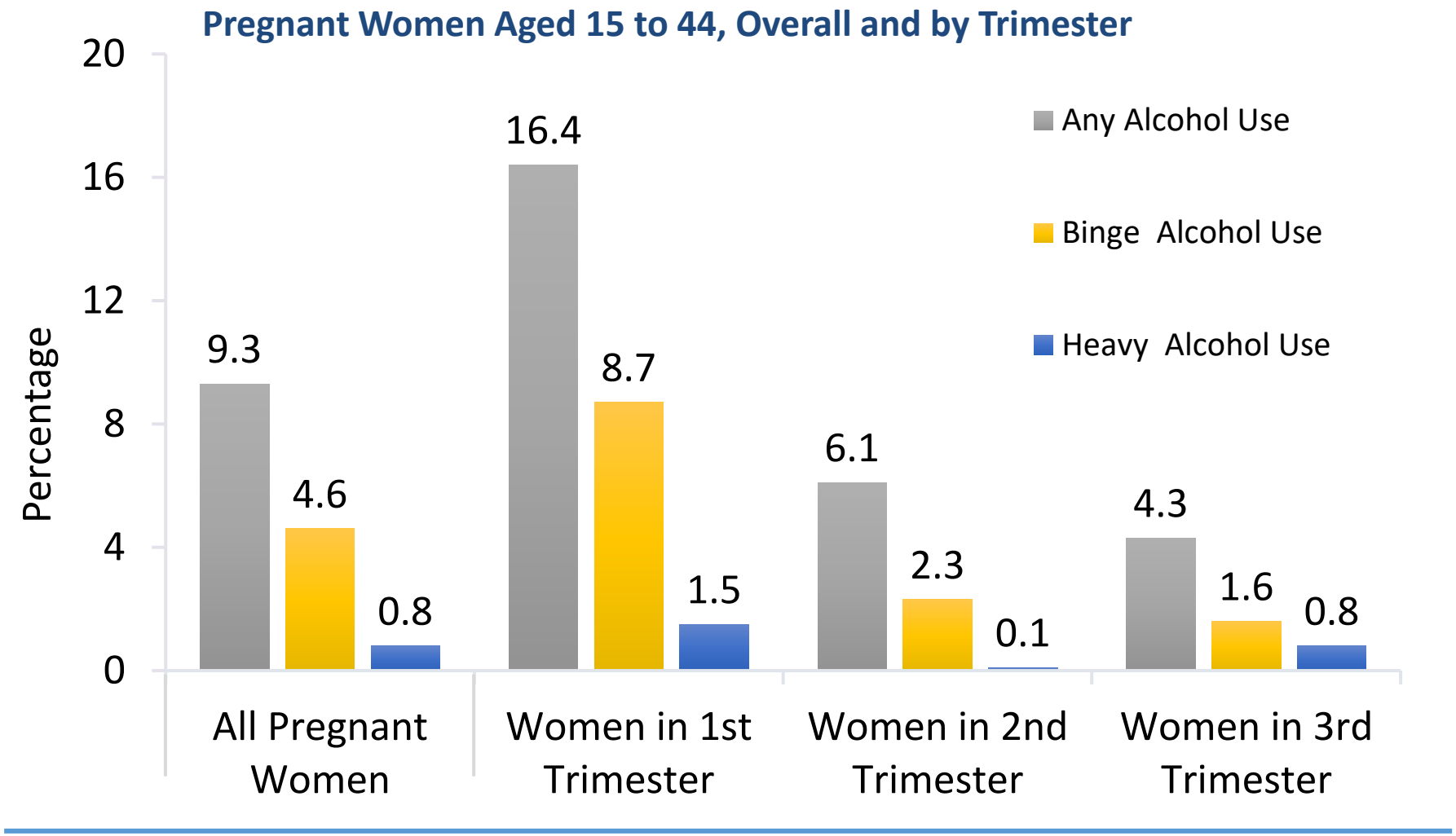
- Trauma Centers/Emergency Department
- Department of Transportation/Highway Patrol
  - DUI and related-fatalities
- Child/Family Services
  - > 10% of children live with parent with AUD
  - Underage drinking
    - 7.1 million people ages 12–20
- Adherence Testing
  - Pain management
  - Addiction recovery
  - Alcohol abstinence
- Transplant Clinics
  - Surgery Prequalification testing
- Prenatal Care /Neonate Management
  - Neonatal abstinence syndrome

Natl. Inst. on Alcohol Abuse & Alcoholism – www.niaaa.nih.gov

# Alcohol Use in Pregnancy

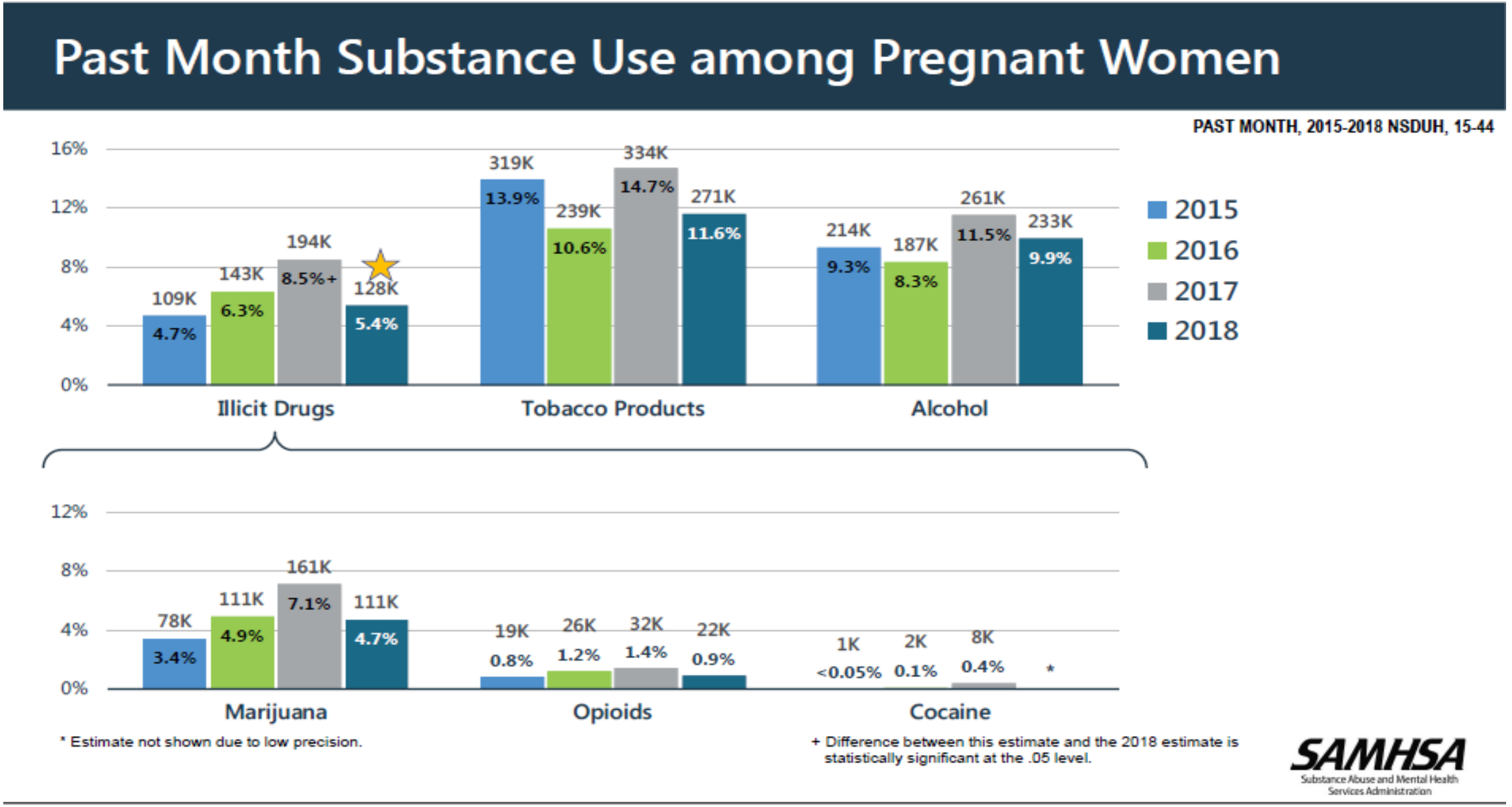
- About 1 in 9 pregnant women
- About 1/3 of pregnant women who consumed alcohol engaged in binge drinking
- Pregnant women who reported binge drinking in the past 30 days reported an average of 4.5 binge-drinking episodes during that same time period

# 2015 National Survey on Drug Use and Health




https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015.pdf

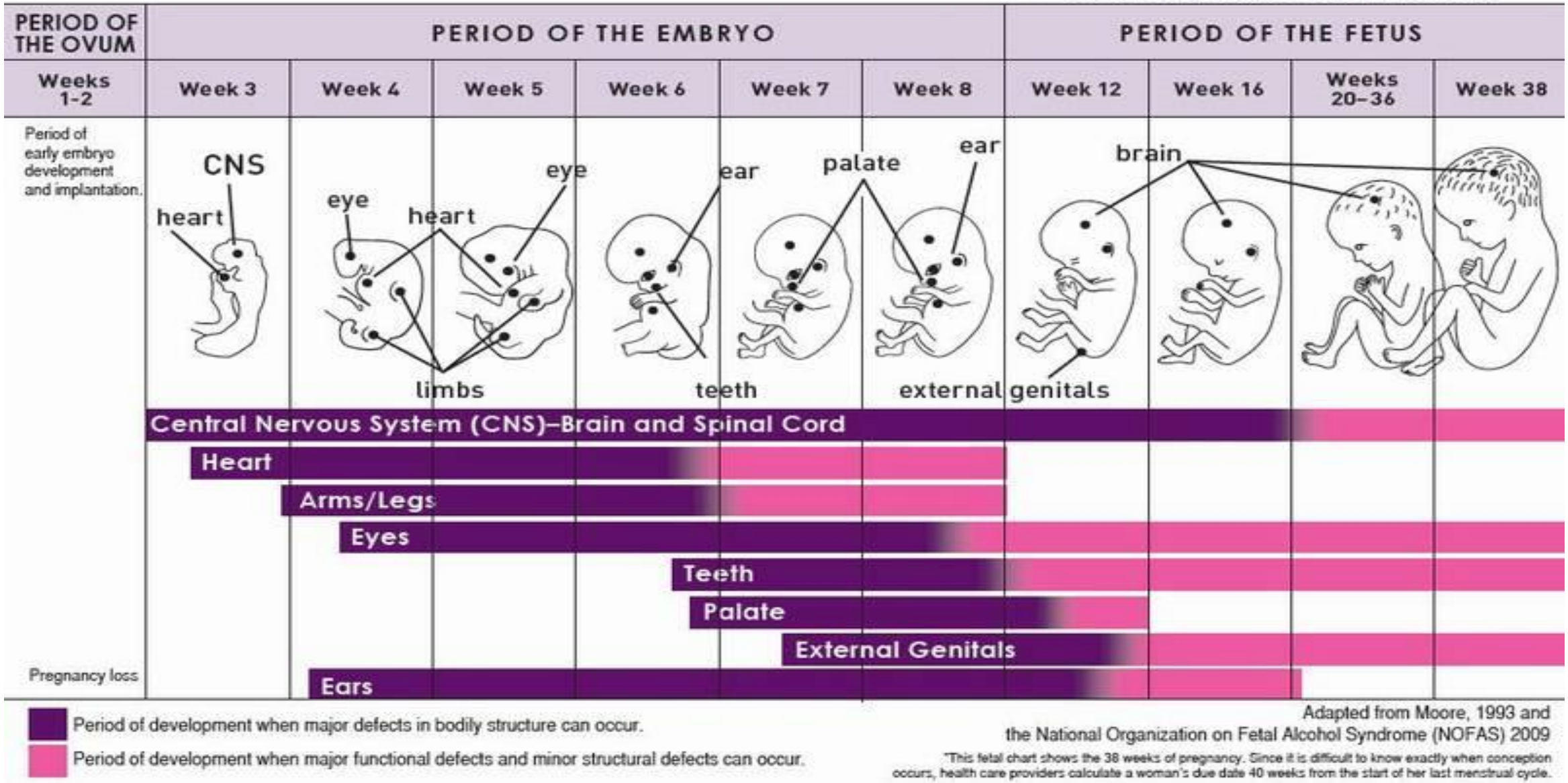
# Statistics on Neonatal Drug Exposure



# FETAL DEVELOPMENT CHART

This chart shows vulnerability of the fetus to defects throughout 38 weeks of pregnancy.*

• = Most common site of birth defects




Adapted from Moore, 1993 and the National Organization on Fetal Alcohol Syndrome (NOFAS) 2009

*This fetal chart shows the 38 weeks of pregnancy. Since it is difficult to know exactly when conception occurs, health care providers calculate a woman's due date 40 weeks from the start of her last menstrual cycle.

https://www.cdc.gov/media/dpk/alcohol/alcohol-pregnancy/dpk-vs-alcohol-pregnancy.html

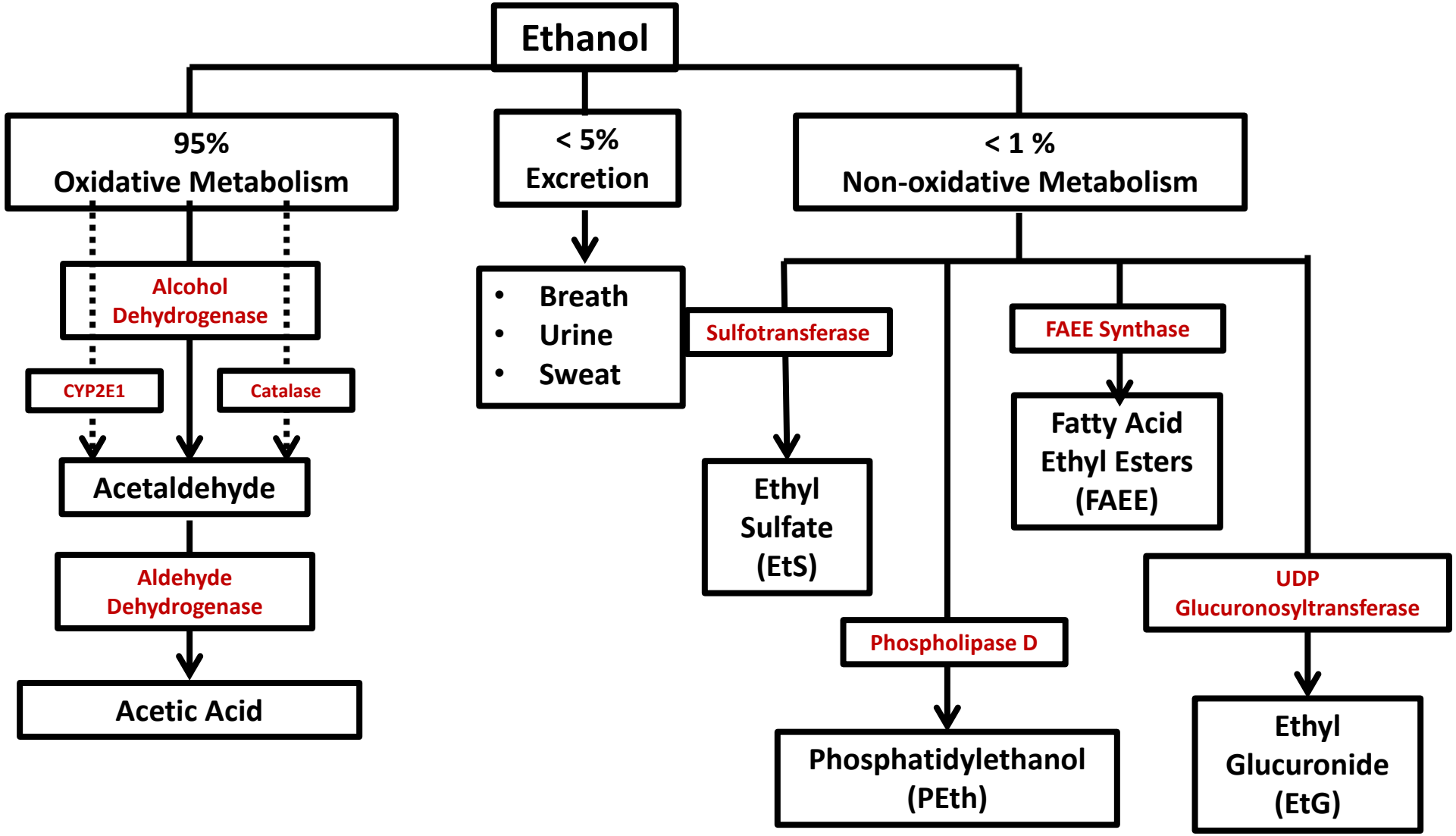
# Fetal Alcohol Spectrum Disorders

- Alcohol-related birth defects
  - Facial abnormalities
- Growth retardation
  - Low birth weight
- CNS impairment
  - Learning disabilities
  - Behavioral abnormalities


Prevalence – 1-2 cases per 1000 live births

https://www.medlife.com/blog/fetal-alcohol-syndrome-symptoms-causes-prevention/

# Biomarkers to Assess Alcohol Exposure



# Specimens to test biomarkers for Alcohol Exposure

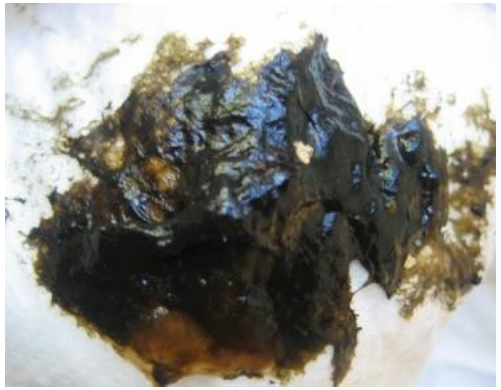
Urine

Breath

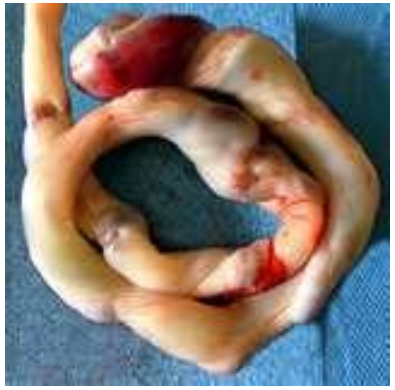
Saliva


Hair

Whole blood/Serum/Plasma/DBS



Meconium

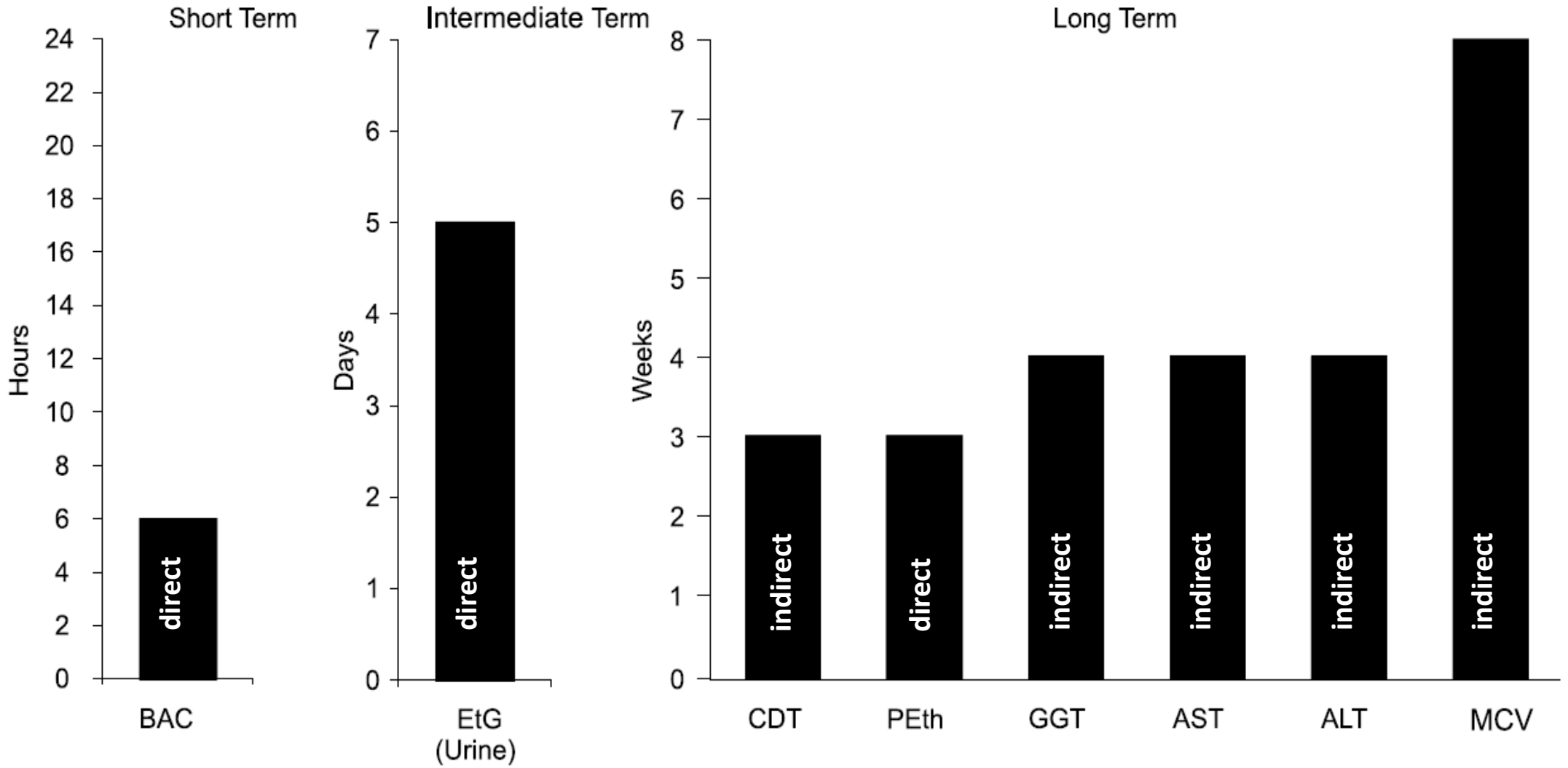


Umbilical Cord

# Alcohol Concentrations in Different Specimen Types

- Alcohol is distributed throughout the body in proportion to the water content of the body fluid
  - Plasma and serum -concentrations – 12-18% higher than whole blood
  - Saliva alcohol concentrations – 7% higher than whole blood
  - Urine alcohol concentration may be 30% higher than whole blood
- The laboratory report must indicate the specimen type

# Detection Window – Alcohol Biomarkers




The Role of Biomarkers in the Treatment of Alcohol Use Disorders. *Substance Abuse Treatment Advisory*. Volume 5, Issue 4, September 2006.

# Limitations for Ethanol Detection

- Half-life (blood): 2-14 h
- Half-life (urine): <24h
- Unintentional exposure
  - Mouthwash, hand sanitizer
- Evaporation from specimen

- False Positives
  - Bacterial/Yeast fermentation of glucose in sample
  - Postmortem ethanol formation
- False Negatives
  - Dilute urine
  - Diuretics
  - Microbial consumption

# Specificity of Indirect Ethanol Biomarkers

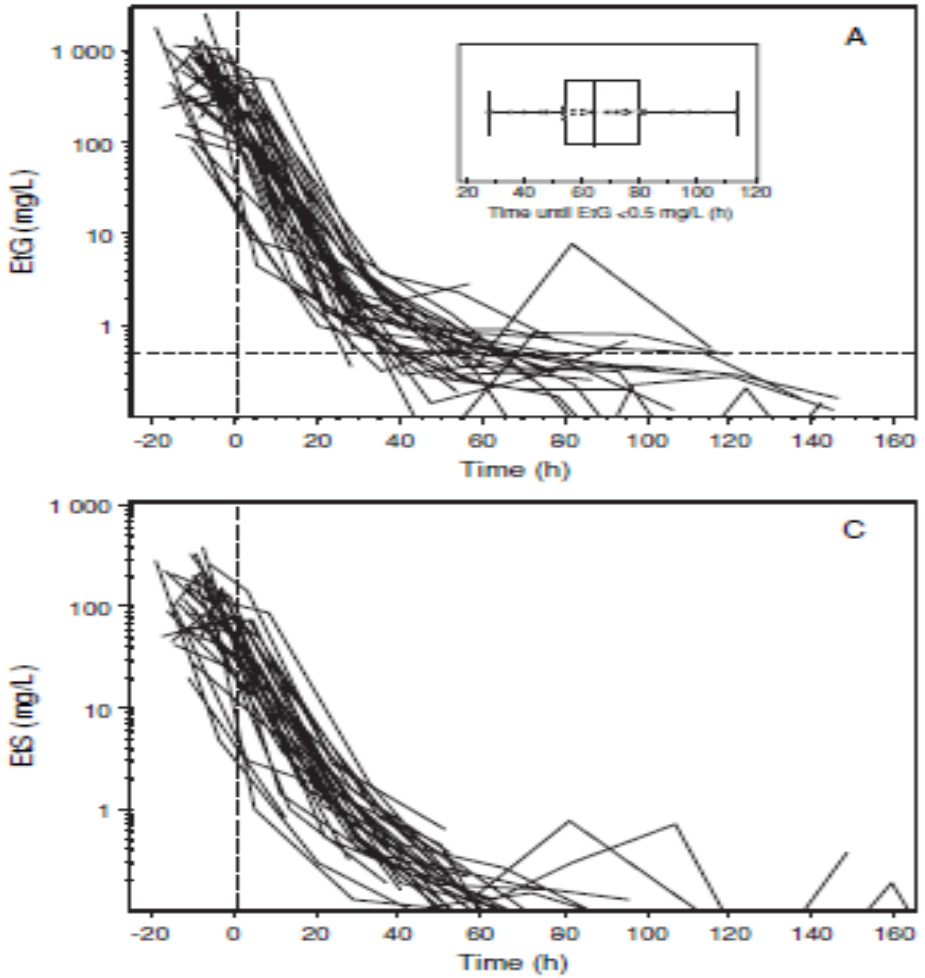
| Marker | Sensitivity/ Specificity (%) | Clinical Use | Potential for False Positives | General Comments |
|---|---|---|---|---|
| Gamma-glutamyl transferase (GGT) | 64% / 72%<br><br>Cutoff: 30 U/L | Heavy Alcohol Use | Not specific – liver, diabetes, biliary disease, obesity, and medications can increase enzymes | Elevations caused by excessive drinking (100g/day) for up to 14 – 26 days |
| Alanine Aminotransferase (ALT)<br><br>Aspartate Aminotransferase (AST) | 32% / 90%<br>Cutoff: 35 U/L<br><br>68% / 80%<br>Cutoff: 35 U/L | Chronic Alcohol Abuse | Not specific – liver, biliary disease, obesity and medications can induce increase in enzymes | AST/ALT > 2.0 suggests ethanol-related liver disease |
| Mean corpuscular volume (MCV) | 44% / 98%<br><br>Cutoff: 96 fL | Heavy alcohol use | Not specific – hemolysis, anemia, liver disease, Vitamin B12 and folate deficiency, medications, leukemia, monoclonal gammopathies | MCV increases with excessive ethanol intake Caused by drinking 60g/day for > 2 wk |
| Carbohydrate-deficient transferrin (CDT) | 84% / 92%<br><br>Cutoff: 2.4% | Heavy alcohol use Indicator of relapse | Not specific – iron deficiency, fulminant HCV, Inborn Errors of Glycogen metabolism, pregnancy, oral contraceptives, immunocompromised patients | Altered form of iron transport protein when drinking is continued for >2 weeks. Caused by drinking 60g/day for > 2 wk |

# Carbohydrate Deficient Transferrin

- Serum marker of long-term, heavy alcohol use (≥40 g/day for up to 2 weeks) or relapse.
- Concentrations correlate with an individual's drinking pattern, especially during the preceding 30 days
- Useful indirect marker for long-term abstinence monitoring.
- Factors that affect CDT levels include body mass index (BMI), female sex, and smoking.
- CDT testing cannot be used in individuals suspected of having congenital glycosylation disorders.

# Ethyl Glucuronide / Ethyl Sulfate

- Non-volatile, water-soluble, direct minor metabolite of ethanol
- Excreted in the urine
- Appears 1 h after ethanol ingestion
  - Window of detection – up to 120 hr
- Detects recent ethanol ingestion
  - EtG Concentration range: up to 300 mg/mL
  - EtS concentration range: up to 61.0 mg/mL
- Stable marker of recent ethanol ingestion
  - Not produced from bacterial formation
  - Not sensitive to bacterial hydrolysis




Helander A et al 2009 Alcohol & Alcoholism 44(1):55-61.

# Detection of EtG and EtS for Recent Exposure

EtG and EtS – present after recent use

| | EtS Positive | EtS Negative |
|---|---|---|
| EtG Positive | 86<br><br>92.5% (both) | 3<br><br>3.2%<br>(only EtG) |
| EtG Negative | 4<br><br>4.3%<br>(only EtS) | 261 |

Helander and Beck, 2005

## Single Detected Metabolites

- Genetic polymorphisms
  - UDP-glucuronosyltransferases (UGT1A)
    - Gilbert's Disease
    - Criglar-Najar Syndrome
  - Sulfotransferases (SULT)

Reisfield et. Al, 2011

- False Negatives (EtG)
  - Bacterial hydrolysis
    - β-glucuronidase (*E. coli*)
- False Positives (EtG)
  - Bacterial production (*E. coli*)

Helander and Dahl, 2005

# Confirmation testing by LC-MS/MS

- Presence of both EtG and EtS
  - Accurate indicators of recent ingestion
- Quantitative
  - Distinguish between high and low ethanol exposure
- AMR range:
  - 100 – 10,000 ng/mL
  - Consumption of >24 g ethanol
    - > 10,000 ng/mL EtG (w/i 24h)

Urine Concentrations

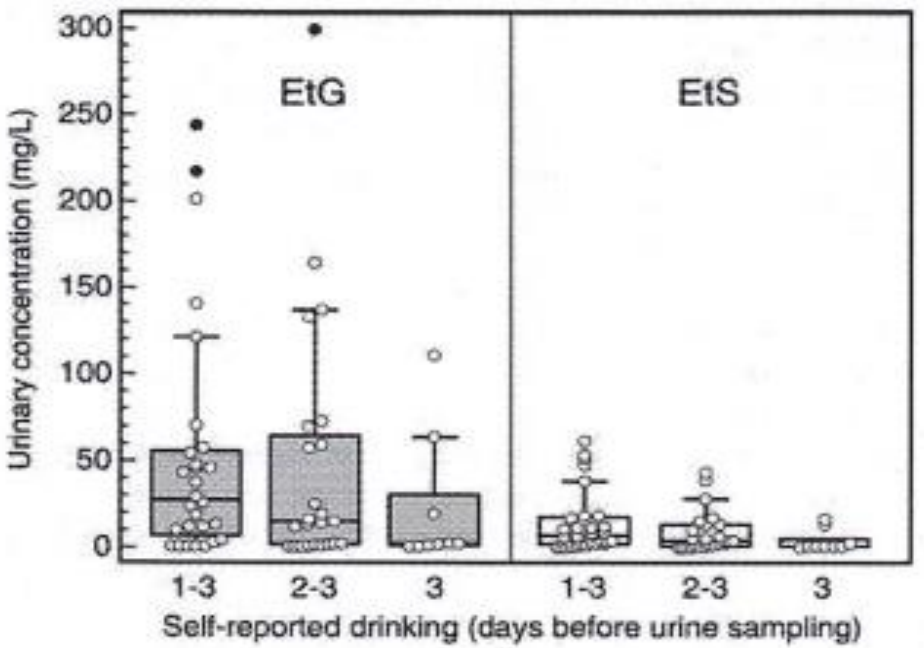



Fig. 3. Box-and-whisker plot showing the distribution of positive urinary EtG (reporting limit ≥ 0.5 mg/l) and EtS (≥0.1 mg/l) concentrations in cases where patients had admitted alcohol consumption in the past 1–3 days prior to urine sampling. The EtS concentrations in the 1–3-days back and 3-days back groups were significantly different ($P = 0.0455$).

Dahl et al., 2011

# Hand Sanitizer Exposure

**Table IV. Summary of Literature Reports of EtG and/or EtS After Passive Exposure**

| Reference | Agent (ethanol) | Max EtG (ng/mL) (per g creatinine) | Max EtS (ng/mL) (per g creatinine) | Max Ethanol |
|---|---|---|---|---|
| Rosano and Lin (8) | Avagard D (61%) | 114 | NM* | NM |
| Rohrig et al. (5) | Germ-X (62%) | 62 | NM | ND* |
| Helliker (7) | Purell (62%) | 770 | NM | NM |
| Jones et al. (9) | Purell (62%) | 713 (799) | 51 (29) | ND |
| This report | Purell (62%) | 2001 (1998) | 83 (94) | ND |

* NM, not measured and ND, not detected.

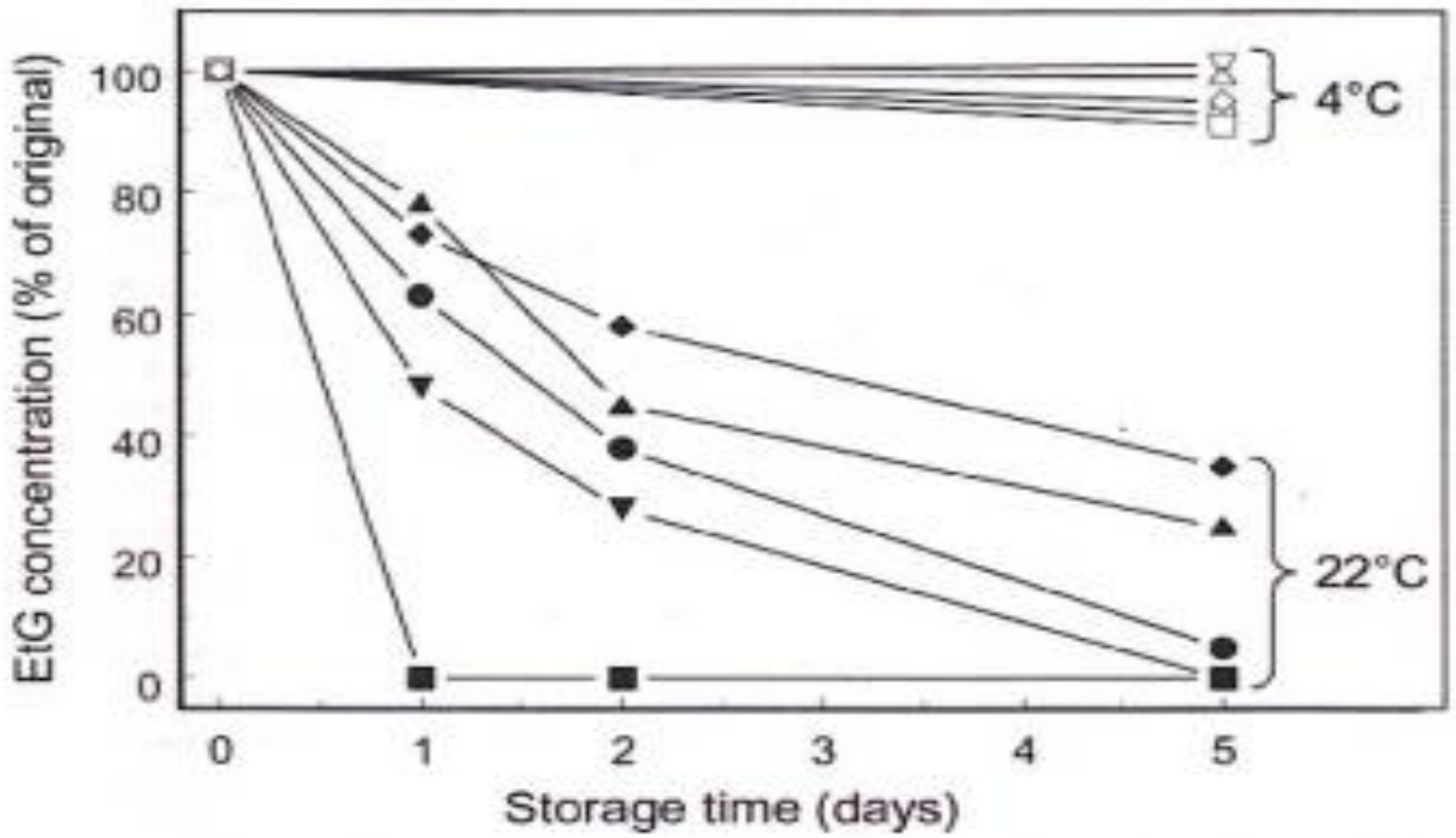
LC-MS/MS Detection

EtG – cutoff 500 ng/mL

EtS – cutoff 100 ng/mL

Reisfield et al. 2011

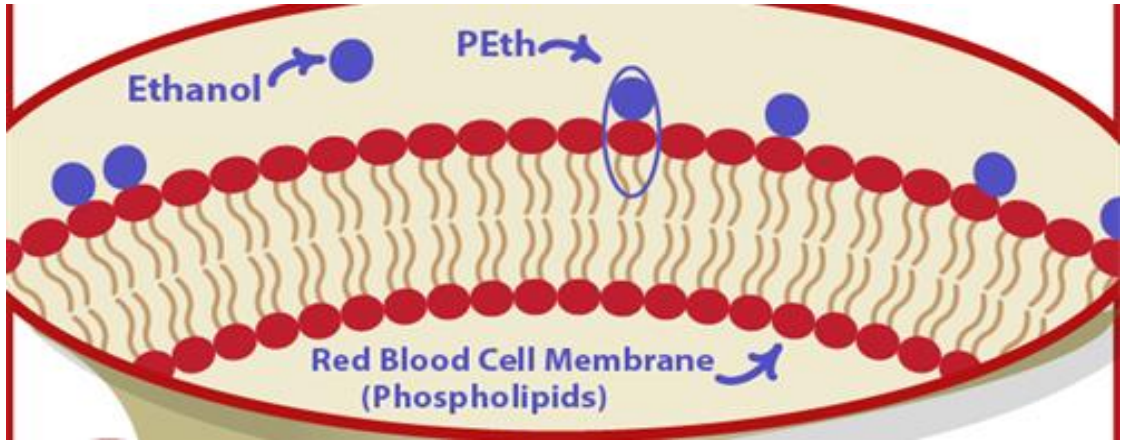
# Hydrolysis of EtG by E. coli in urine specimens



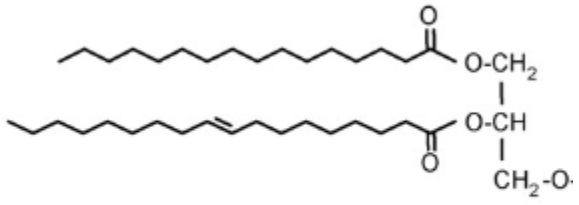

Helander and Dahl, 2005

# Phosphatidylethanol

- Long-term direct biomarker of alcohol use
- Formed in the presence of ethanol
- PEths are incorporated into the membrane of erythrocytes
- PEth half-life of 4±0.7 days
- Specificity: ~100%

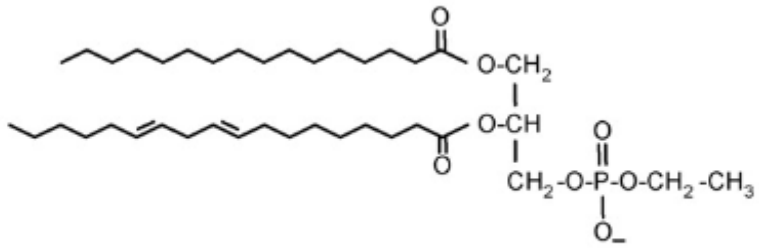



www.usdtl.com




PEth-16:0/18:1*

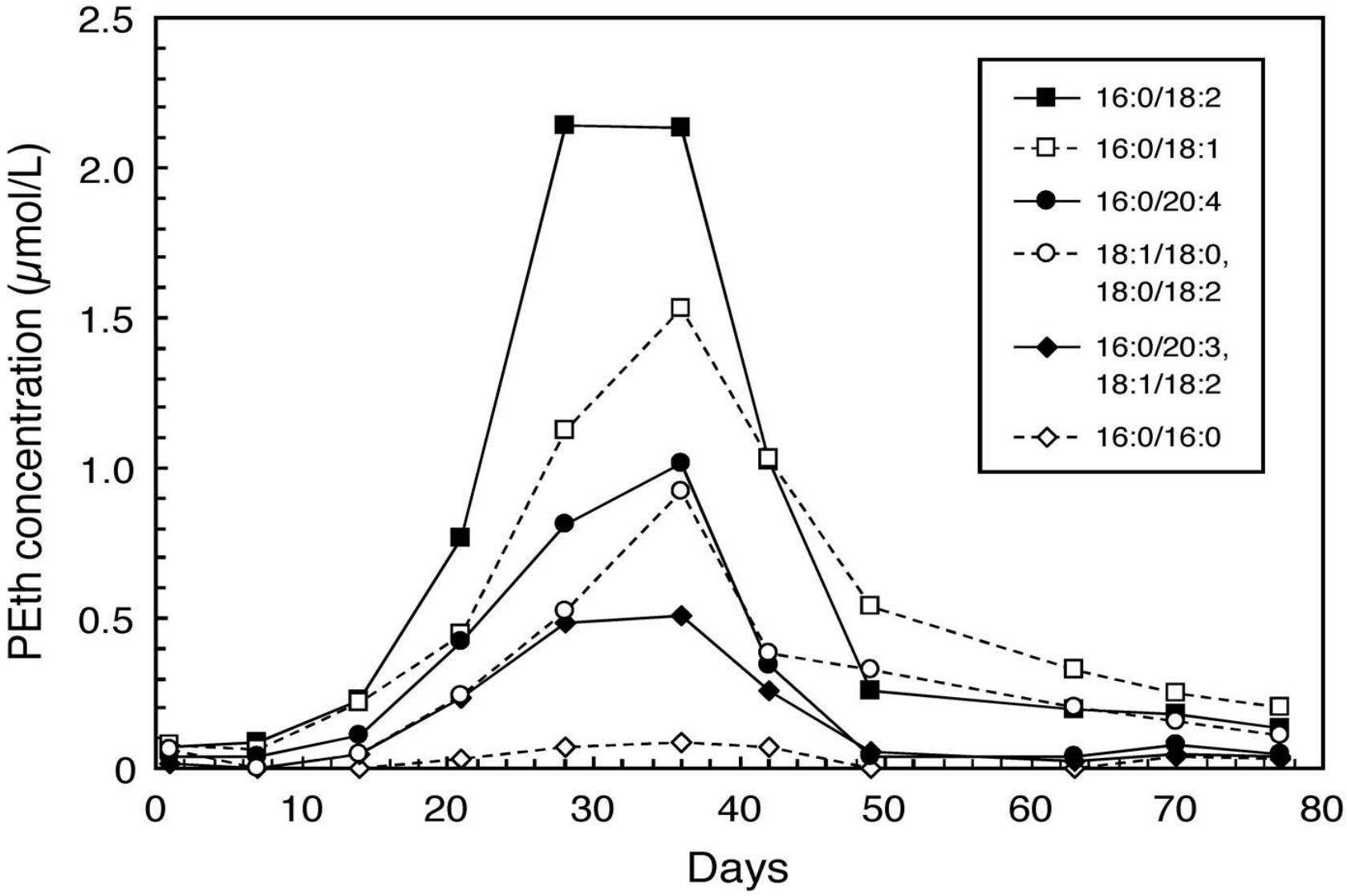
1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol (POPEth)




PEth-16:0/18:2

1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanol (PLPEth)

# Detection Window for PEth Homologs




Clinical Chemistry 55:7 1395–1405 (2009)

# Alcohol Biomarkers in Neonatal Specimens

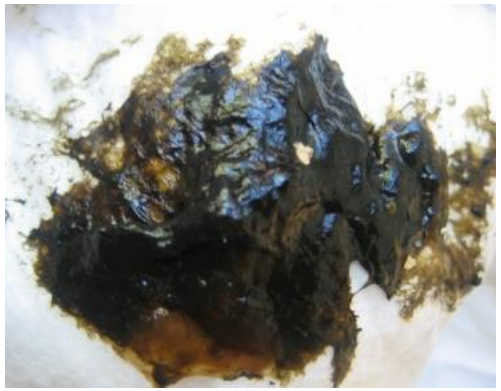
- Ethyl glucuronide (EtG) and ethyl sulfate (EtS)
  - Umbilical cord tissue

- Phosphatidylethanol (PEth)
  - 1-Palmitoyl-2-oleoyl-sn-glycerol-3-phosphoethanol (POPEth)
  - Whole blood
  - Dried blood spots

Jones et al, *Am J Analy Chem* 3:800-10, 2012; Lange et al, *BMC Public Health* 14:127, 2014; McQuire et al, *Pediatrics* 138, 2016

# Alcohol Biomarkers in Neonatal Specimens

- Fatty Acid Ethyl Esters (FAEE)
  - Direct marker of heavy alcohol use
  - Chemical reaction of fatty acids and alcohol by FAEE synthase
  - Formed in Liver and Pancreas and released into circulation

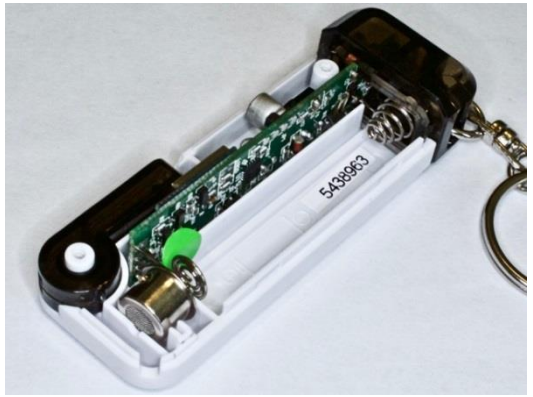


- Specimens for testing
  - Hair Cutoff: 0.5 ng/mg
  - Meconium Cutoff: 500 ng/g
  - Blood – up to 24 hr after last drink

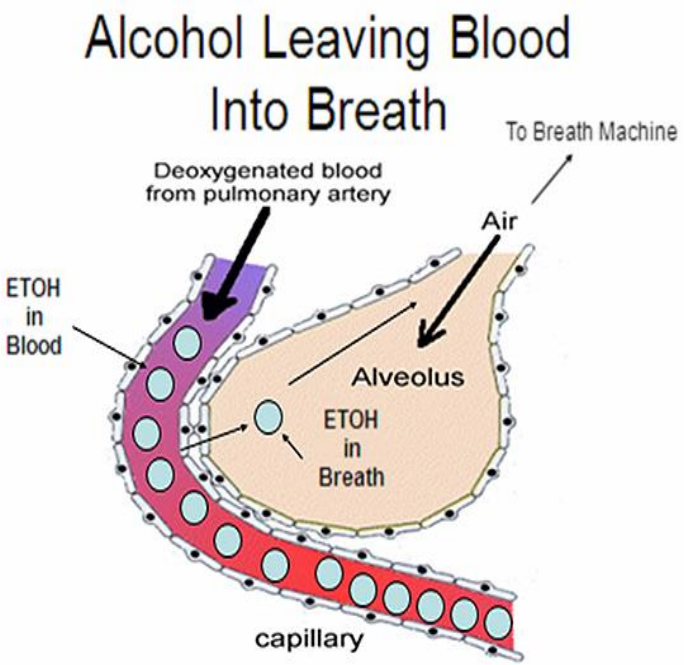
- Sensitivity: >90% / Specificity: >90%

# Analytical methods – Breathalyzer

- Based on Alcohol Blood:Breath partition ratio
  - 2100 mL breath : 1 mL of blood
- Platinum electrode in Fuel Cell – oxidizes alcohol → acetic acid, $H^+$, $e^-$
- Electrons flow through wire from electrode to current meter
- The more alcohol present → the greater the electrical current



https://www.duiease.com/wp-content/uploads/2015/11/breathalyzer.jpg




http://www.norcalduidefense.com/images/alcohol.jpg

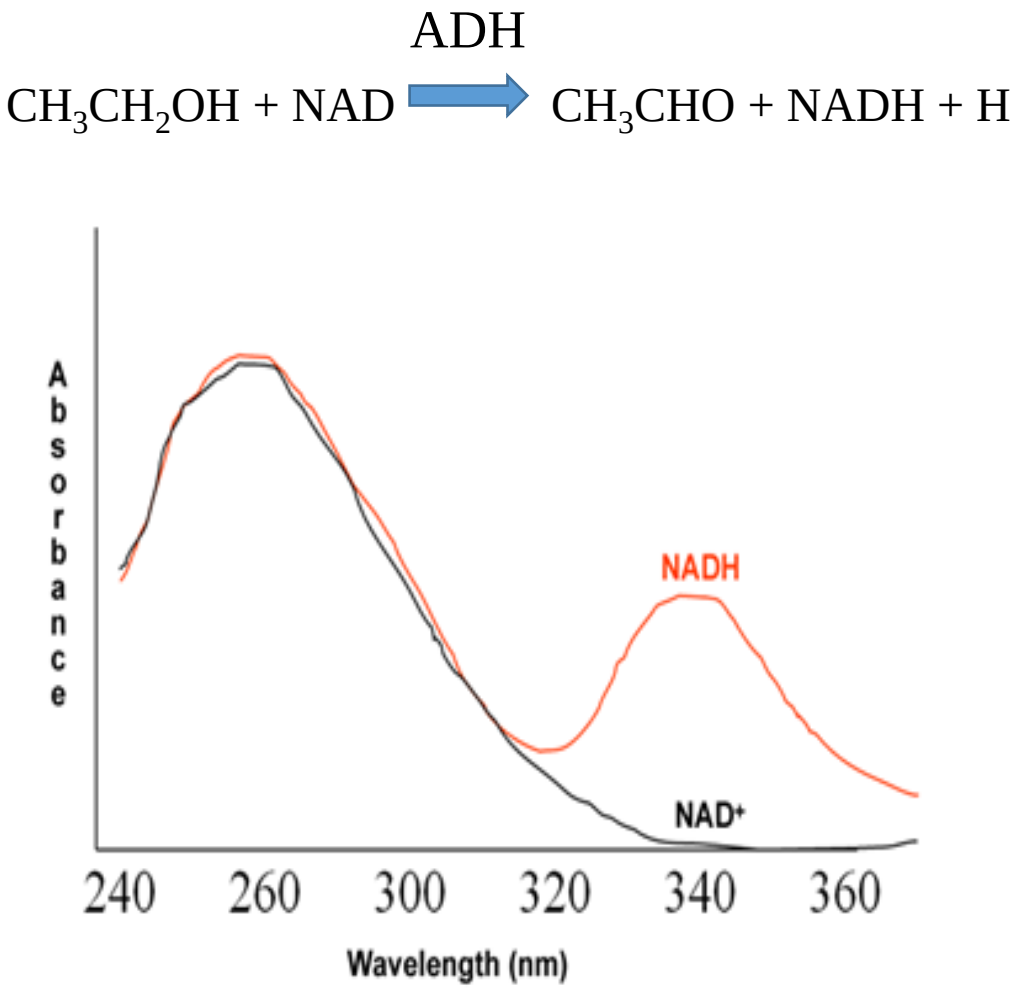
# Analytical methods – Enzymatic Assay

**Advantages**

- Rapid, easy to use kits
- Quantitative
- Stat testing within 1 hr
- Specimens -serum/plasma, urine

**Disadvantages**

- Not able to detect methanol and isopropanol overdoses



# Enzymatic Oxidation Assay

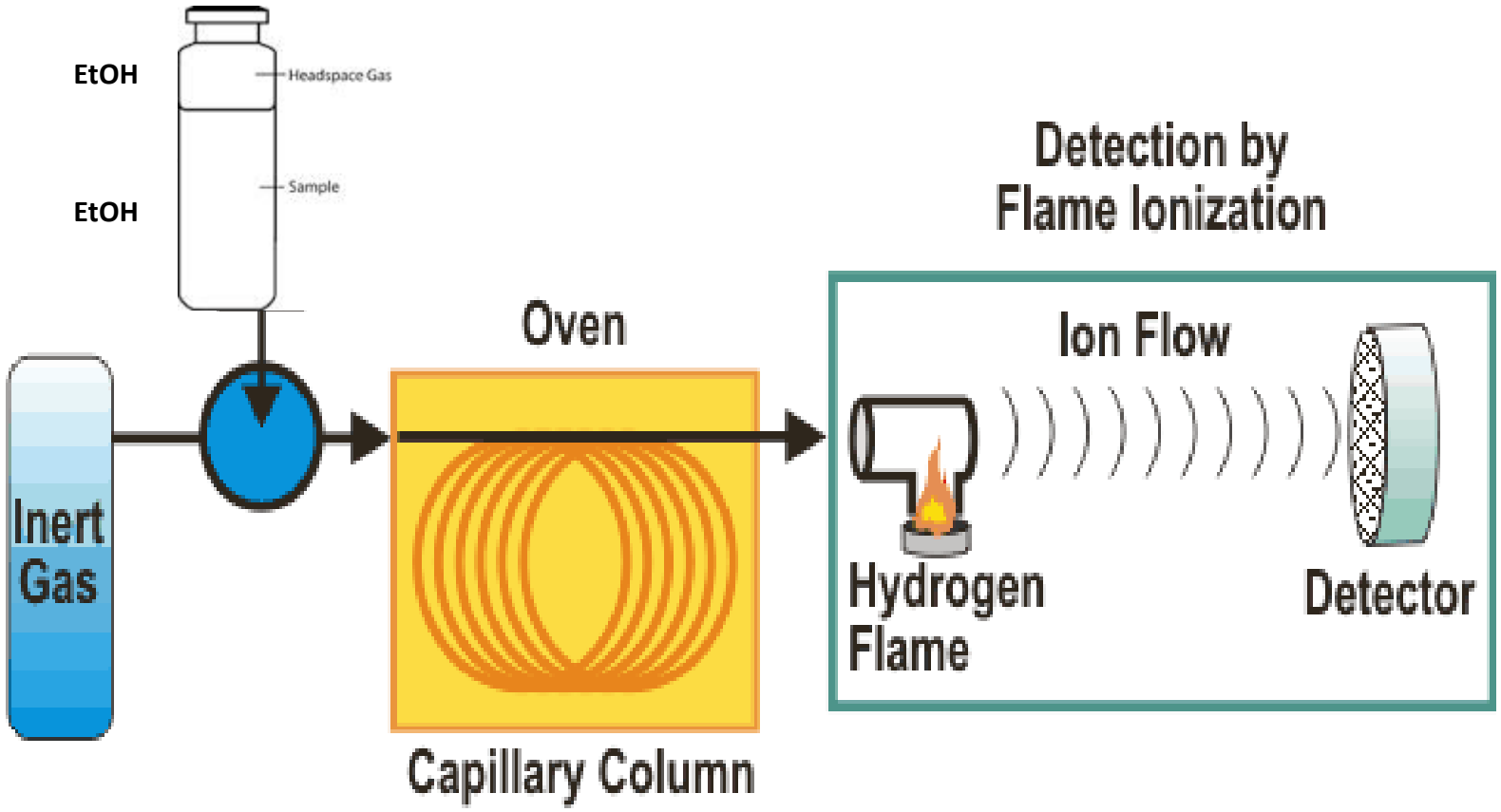
**Advantages**

- Rapid, easy to use kits
- Quantitative
- Stat testing within 1 hr
- Widely used (95% of labs from CAP survey)
  - Kits can be adapted to various chemistry analyzers
- Testing for serum/plasma and urine

**Disadvantages**

- Not specific for ethanol
  - Cross-reactivity with n-Propanol
- Not able to detect methanol and isopropanol overdoses

# Analytical Methods – Gas Chromatography - Flame Ionization Detector (GC-FID)




https://blogreu.files.wordpress.com/2015/06/11.gif

# Gas Chromatographic Techniques

**Advantages**

- Specificity for ethanol
- Quantitative assay
- Testing for serum, plasma, blood and urine
- Ability to quantify
  - Methanol
  - Isopropanol
  - Ethylene glycol

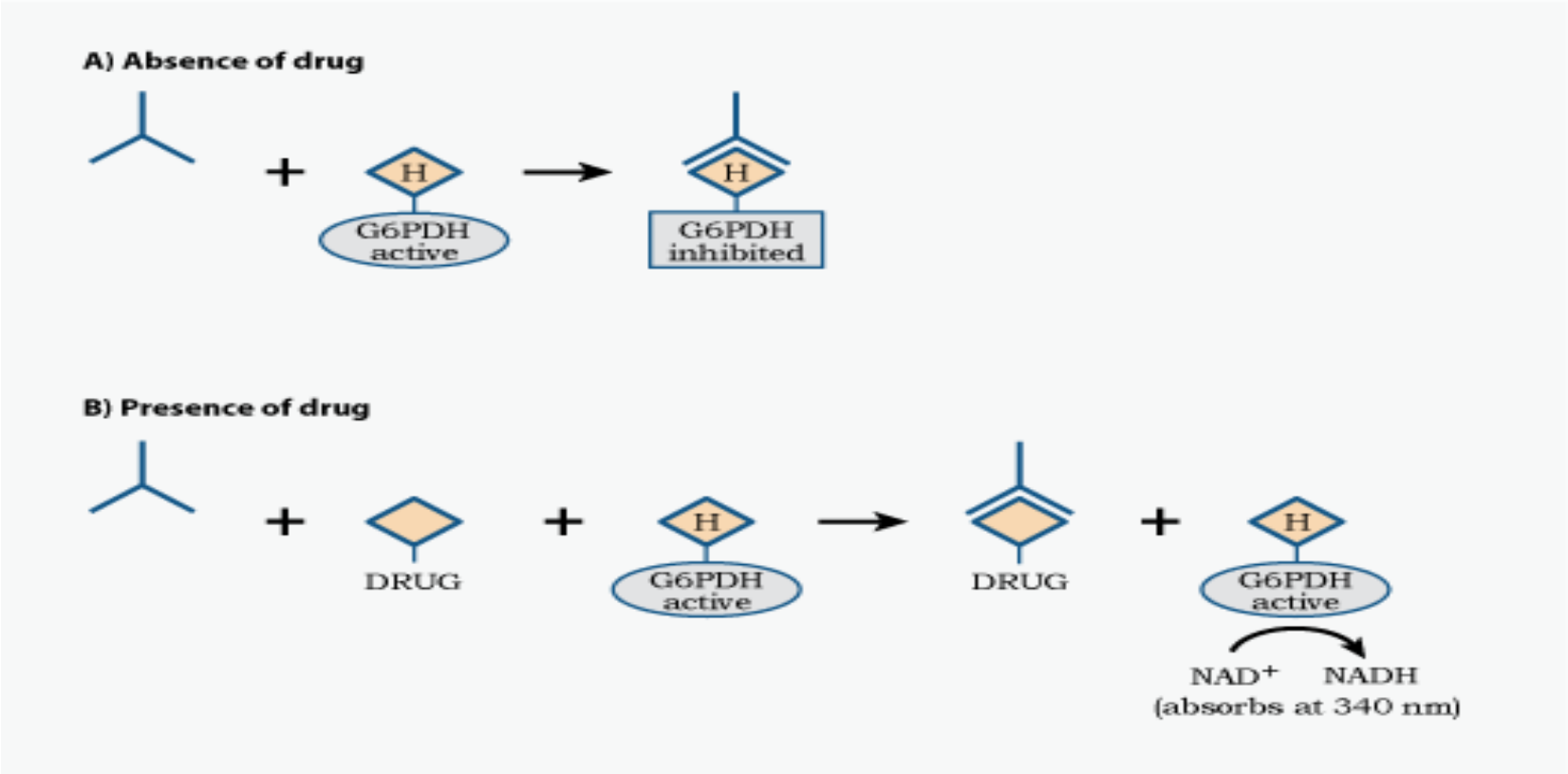
**Disadvantages**

- Equilibration time
  - (15–30 min) delays turnaround time
- Requires specialized instrumentation (GC)
- Requires highly trained technical staff
- Analysis slower than enzymatic assay

# Commercial EtG Screening Assay

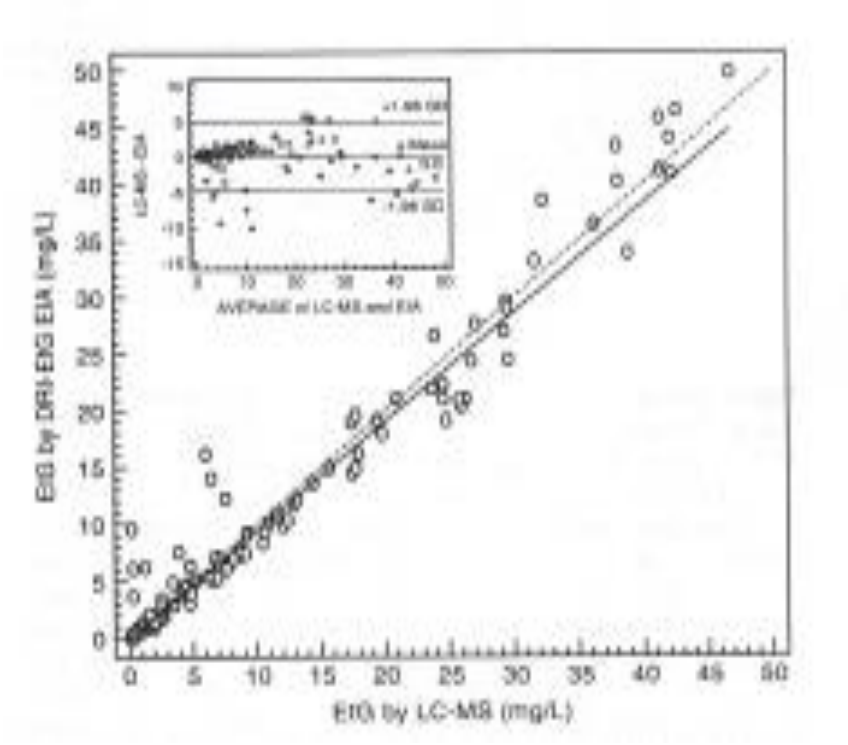
- Cutoff – 500 ng/mL

No Cross-reactivity to EtS



# EtG EIA vs LC-MS/MS

**Y = 0.96 (LC/MS/MS) - 0.104**



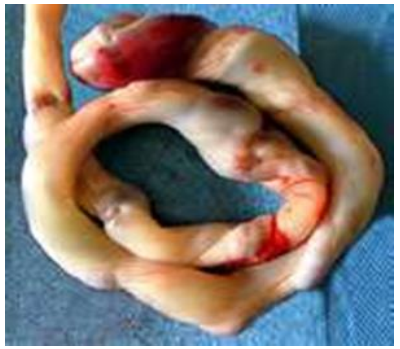

**EIA cutoff: 500 ng/mL**

| | | LC/MS | |
|---|---|---|---|
| | | + | - |
| EIA | + | 151 | 5 |
| | - | 2 | 242 |

Bottcher et al. 2008

# Liquid Chromatography Tandem Mass Spectrometry

Umbilical Cord
Testing for
EtG and EtS



Urine
Testing for
EtG and EtS

Whole blood
Testing for
Phosphatidylethanol

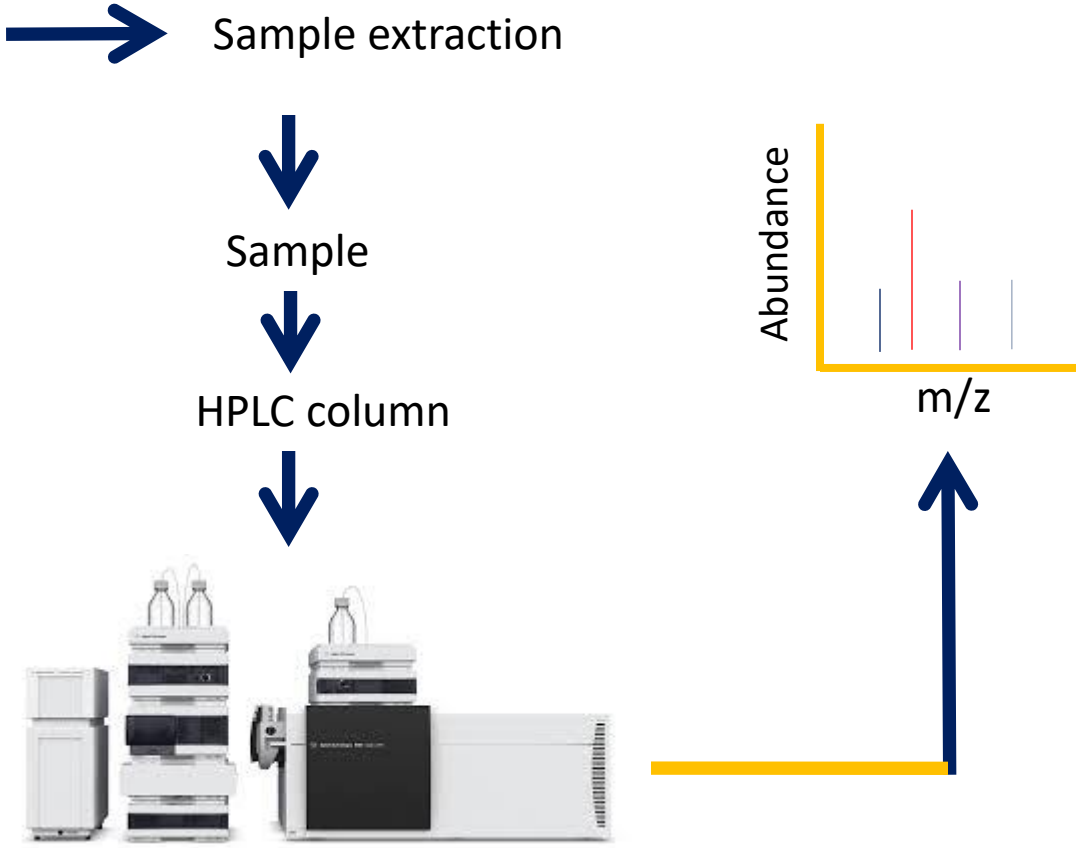



*Photo: ARUP ClinTox3 Protocol*

# What do these results mean?

**Ethyl glucuronide and Ethyl sulfate**

- EtG > 10,000 ng/mL  EtS > 10,000 ng/mL
  - Recent exposure (w/I 24h)
- EtG: 1359 ng/mL  EtS: < 100 ng/mL
  - Not considered to be recent exposure (>24h)
  - Possible hand sanitizer exposure
- EtG: < 100 ng/mL  EtS: 187 ng/mL
  - Possible bacterial degradation of EtG

**Phosphatidylethanol**

- **<20 ng/mL**
  Abstinence or light drinking (< 2 drinks per day for several days a week)
- **20–200 ng/mL**
  Moderate drinking (to 4 drinks per day for several days a week)
- **>200 ng/mL**
  Heavy drinking (at least 4 drinks per day several days a week)

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# Summary

- Testing for alcohol exposure is important to manage the health of patients with alcohol use disorders

- Testing for alcohol biomarkers provides opportunities to identify and evaluate alcohol use and exposure
  - Direct markers are preferred over indirect markers
  - Phosphatidylethanol has high specificity for acute and chronic alcohol exposure

- Testing for alcohol biomarkers should be aligned with clinical needs/expectations