Alcohol Biomarkers

- **Objective** measures that are helpful as:
  1. **Outcome measures** in studies
  2. **Screens** for possible alcohol problems in individuals with unreliable drinking histories
  3. **Evidence of abstinence** in individuals prohibited from drinking alcohol

These tests are complimentary to self-report assessments.
Categories of Alcohol Biomarkers

- Indirect Biomarkers
- Direct Biomarkers

Indirect Biomarkers

- Assesses alcohol effects on body systems
- Non-specific, insensitive
- AST, ALT, GGT, MCV
  - Things other than EtOH abuse causes elevations
  - Some abusers do not have elevations

Indirect Biomarkers

- Newest: CDL- Carbohydrate-deficient transferrin
  - Elevated after > 2 weeks of heavy EtOH abuse
  - Few other things cause elevations
  - Insensitive to bingeing
Direct Alcohol Biomarkers

- Analytes of alcohol or its metabolites
  - Measures alcohol directly in body matrices
  - Or alcohol adducts in body matrices
- Most common is BAC, BrAC

Direct Alcohol Biomarkers

- Alcohol Metabolites:
  - Most alcohol is oxidized by ADH and AlDH
  - A very small amount is broken down non-oxidatively, creating analytes that can be measured for a longer period than alcohol itself
  - Measured in the blood or urine.

Alcohol Metabolism

- Unchanged in breath, urine, sweat: < 5%
- Ethanol: < 1%
- Ethyl Glucuronide (EtG): > 95%
- Ethyl Sulfate (EtS): < 5%
- Acetaldehyde and acetic acid: ADH & AlDH
Direct Biomarkers

- Ethyl glucuronide (EtG), ethyl sulfate (EtS), and phosphatidyl ethanol (PEth).
- Usually measured in urine; detectable for days.
  - EtG and EtS tests become positive shortly after even low-level exposure to alcohol
  - PEth requires higher levels of ethanol use, detectable in blood for weeks

Ethyl Glucuronide- EtG

- EtG: Ethyl β-D-6-glucosiduronic acid
- Approx 0.02% of a dose of ethanol is metabolized by phase II conjugation with uridine 5'-diphospho-glucuronic acid (UDPGA) via UDP-glucuroligosyltransferase to form EtG

Ethyl Glucuronide- EtG

- The most studied and the most utilized long-term biomarker
- Can be measured in very [low]
- Detected for 4 days in urine after 1 drink- i.e., the “80-hour test”
Ethyl Glucuronide- EtG

- Cannot prove beverage alcohol as source
  - Hand sanitizers
  - Mouthwashes
  - Non-alcoholic beer

Ethyl Glucuronide- EtG

- Present in very low levels (<100ng/mL) even in abstainers and children
  - Endogenous ethanol by intestinal bacteria
  - Non-apparent dietary ethanol: old fruit juices, sauerkraut, old bananas

Ethyl Glucuronide- EtG

- Perfect for documenting abstinence
  - Underage patients
  - Military in combat zones
- Those requiring abstinence as condition of rehab or probation
  - Probation for EtOH-related crimes
  - Persons in court-ordered rehab as condition of release
  - Impaired professionals as condition of continued licensure
EtG, EtS Measurement Concerns

- Must confirm with LC/MS/MS
  - EIA unreliable, false positives
  - All EIA screen positives must be confirmed
- Varies with hydration, often corrected for creatinine
- Urine should be refrigerated or preserved if only EtG is analyzed
  - EtG produced from glucose fermentation
  - Not an issue for EtS
- Not FDA-approved medical test, not covered by health insurance

EtG and EtS Kinetics

Fig. 2. a, b Blood ethanol (BAc), serum EtS and EtG in two volunteers. a volunteer 1 (0.52 g EtOH/kg body weight), b volunteer 13 (0.78 g EtOH/kg body weight).

EtG and EtS Kinetics

Fig. 3. Concentrations of EtG (a) and EtS (b) in urine after consumption of 0.30–0.78 g EtOH/kg body weight.
Suggested EtG Cut-offs

- EtG > 1,000 ng/mL indicates:
  - Heavy drinking in past 1-2 days
  - Light drinking the same day (or the night before)

- EtG between 500–1,000 ng/mL indicates:
  - Heavy drinking previous 1–3 days
  - Light drinking past 24 hours
  - Intense "extraneous exposure" within 24 hr or less

- EtG positive, above LOQ but <500 ng/mL indicate:
  - Previous heavy drinking (1–3 days).
  - Previous light drinking (12–36 hours).
  - Recent "extraneous" exposure.
EtG and Hand Sanitizer Use
Rosano & Lin *J Anal Tox* 2008
- 9 adults, used ethanol skin sanitizers 20x/day
- EtG levels + but < 120 ng/mL in first morning specimens
- EtG accumulation with repeated dermal ethanol did not occur

EtG and Hand Sanitizer Use
- 11 adults, used Purell® (62% EtOH) q 5’ for 10 hours (120 uses each)
- Urine specimens end of each day
  - Mean [EtG] conc. @ end of Days 1, 2, and 3 were 493, 601, and 542ng/mL respectively
  - Range of 0–2001 ng/mL
- EtS may be a good discriminator
  - Very few had + EtS
  - All EtS values < 100ng/mL

EtG and Mouthwash Use
- 10 adults gargled with Listerine® (27% EtOH), 20mL, 30 sec. each, 4 X/d
- Only one subject had + urine EtG
  - 173ng/mL
  - 2 hr post-gargle
- No one had +EtG specimens at first void of each day
- Several + EtS in 7 subjects
  - Maximum EtS value 104ng/mL
- EtS cut-off of 250- 500ng/mL seems reasonable
Suggested EtG Cut-offs

- **EtG >1,000 ng/mL** indicates:
  - Heavy drinking in past 1-2 days
  - Light drinking the same day.
- **EtG between 500–1,000 ng/mL** indicates:
  - Heavy drinking previous 1–3 days
  - Light drinking past 24 hours
  - Intense "extraneous exposure" within 24 hr or less
- **EtG positive, above LOQ but <500 ng/mL** indicate:
  - Previous heavy drinking (1–3 days).
  - Previous light drinking (12–36 hours).
  - Recent "extraneous" exposure.
Phosphatidyl Ethanol- PEth

- PEth, a group of glycerophospholipid homologues
- Formed exclusively in the presence of ethanol via the action of phospholipase D
- Found primarily in the RBC membranes
- Long detection window – weeks- due to life of the RBC
Phosphatidyl Ethanol- PEth

- Phosphatidyl ethanol (PEth) is a direct blood-based biomarker.
- 48 species of PEth identified:
  - PEth 16:0 and PEth 18:1 seem to be the most abundant species.
- Persists in blood for as long as 3 weeks:
  - After a few days of moderately heavy drinking (>about four drinks per day).
- Perfect for detecting binge drinking.
- Not readily available, being studied.

Phosphatidyl Ethanol- PEth

Kwak et al – Clin Tox 2012

- Monitored 2 groups of pregnant women:
  - Group 1- 26 women-No ethanol use: Negative for PEth.
  - Group 2- 13 women- 2.5- 20 drinks/wk:
    - Positive for PEth at >5 nmol/L.
    - Detectable for up to 4 wks.

Comparison of biomarkers with PEth in blood and urine

Winkler- Int J Legal Med. 2012 Dec 29

- Studied the correlation between PEth and other biomarkers (ethyl glucuronide, ethyl sulfate, CDF, GGT).
- 18 alcohol-dependent patients in withdrawal therapy monitored for up to 19 days.
- No correlation between the different markers.
- PEth showed an initial rapid decrease:
  - Then a slow decline after the first few days.
  - Could still be detected after 19 days of abstinence.
Window of Assessment for Various Alcohol Biomarkers

Alcohol Biomarkers and their Usefulness

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Screen for Heavy Drinking</th>
<th>Identifying Relapse</th>
<th>Time to Return to Normal</th>
<th>Monitoring for Abstinence</th>
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</thead>
<tbody>
<tr>
<td>CDT</td>
<td>✓</td>
<td>✓</td>
<td>2-3 weeks</td>
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<tr>
<td>EtG, EtS</td>
<td>✓</td>
<td>✓</td>
<td>1-3 days</td>
<td>✓</td>
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<tr>
<td>GGT</td>
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<td>2-4 weeks</td>
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<tr>
<td>MCV</td>
<td>✓</td>
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<td>Several months</td>
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<tr>
<td>PEth</td>
<td>✓</td>
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<tr>
<td>BAT</td>
<td>✓</td>
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<tr>
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<tr>
<td>ALT</td>
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<td></td>
<td>2-4 weeks</td>
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</table>

Bibliography
1) The Role of Biomarkers in the Treatment of Alcohol Use Disorders, 2012 Revision - SAMHSA Advisory.