The 80-Hour Alcohol Test & More:
Novel Biomarkers of Alcohol Exposure

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Disclosures

- Nothing to disclose
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

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Categories of Alcohol Biomarkers

- Indirect Biomarkers
- Direct Biomarkers
Indirect Biomarkers

- Assesses alcohol effects on body systems
- Non-specific, insensitive, but cheap and readily available
- AST, ALT, GGT, MCV
  - Things other than EtOH abuse cause elevations
  - Some abusers do not have elevations - must be heavy users

Indirect Biomarkers

- Newest: CDT- Carbohydrate-deficient transferrin
  - First test approved by FDA for the monitoring of alcohol consumption over an extended period of time
  - Elevated after > 2 weeks of heavy EtOH abuse (>5 drinks/day, i.e., > 50 g/day)
  - Few other things cause elevations, can discriminate social EtOH use from an alcoholic
  - 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
  - Analytes are measured in the blood or urine.
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

Alcohol Metabolism

Unchanged in breath, urine, sweat

< 5%

Ethyl Glucuronide (EtG)

< 0.1%

UDP-glucuronosyltransferase

Ethanol

< 0.1%

Sulfotransferase

Ethyl Sulfate (EtS)

> 95%

ADH & AIDH

Acetaldehyde and acetic acid
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-glucurolyosyltransferase 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”

- Can be lower than expected with URI (EtS not affected)
**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

Ethyl Sulfate- EtS

- Minor EtOH metabolite
- Produced by sulfotransferase & breakdown by sulfatases
- Present in same fluids/tissues as EtG
- Advantages:
  - Not formed or broken down by microbes in urine
  - Stable for 6 days or more
  - When used with EtG, very good specificity & sensitivity using appropriate cut-offs
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

Winkler Int J Legal Med.
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)
Suggested EtG Cut-offs

- 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

- 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] @ 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
- EtS as discriminator at >250 ng/mL
<table>
<thead>
<tr>
<th>TEST(S) ORDERED</th>
<th>RESULTS</th>
<th>OTHER THERAPEUTIC USES</th>
</tr>
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<tbody>
<tr>
<td>TETRACYCLINE</td>
<td></td>
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The specimen was processed by Chromatography. Any positive result was confirmed by liquid chromatography with tandem mass spectrometry (LC-MS-MS). The following threshold concentrations were used for this analysis:

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<td>5.0 ug/mL</td>
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Alternative explanations should be considered for any positive result.

Please note that incidental exposure to tetracycline may result in detectable levels of its active form. The Centers for Disease Control and Prevention (CDC) advise caution in the interpretation and use of these results, as tetracycline levels may also be detected in the context of all available clinical and therapeutic information.

Ref: Centers for Disease Control and Prevention, "The Role of Tetracyclines in the Treatment of Allofasciatic Disease," Infection Control and Hospital Epidemiology, Volume 1, Issue 4, September 2000.

Certified by: (signature)

**Final Report**

Collected at 5189201004 MEDTAC facility site 5001 CENTER FOR OCCUPATIONAL HEALTH - GLASS PALACE GLASS PALACE, NY

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EtG in Hair Specimens

- Relatively high specificity- 80–95%
- And high sensitivity 70–90% for the detection of alcohol abuse
- Becoming more commonly used & commercially available
- May be more vulnerable to destruction by hair care products
Acetaldehyde Adducts

- First product of ethanol oxidation, is very reactive
- Forms adducts with hemoglobin, albumin, serum proteins, CYP450 2E1, RBC membrane proteins
- Some acetaldehyde-protein adducts last 3 weeks
- Hemoglobin adducts last 3 months (RBC lifespan)
- WBAA highly specific, precise, & extremely sensitive tool to test for heavy alcohol consumption
  - Sensitive to even a single high-dose binge (2g/kg) when conventional indices aren’t (MCV, GGT, etc.)
  - Can be used to monitor alcoholism abstinence
- Acet-prot adducts act as hapten, forms Ab
- Salivary IgA may be used to detect binge drinking

Fatty Acid Ethyl Esters (FAEE)

- From non-oxidative ethanol metabolism
- Conjugation product of fatty acyl chains (oleic, steric, palmitic) and ethanol
- Present in all tissues and fluids
- Accumulate preferentially in fat & hair
- FAEE in fat is a PM marker of EtOH use
- Long-term use monitoring in hair
Phosphatidyl Ethanol- PEth

- PEth, a group of membrane glycerophospholipid homologues
- Formed exclusively in the presence of ethanol via the action of phospholipase D
- Found primarily in the RBC membranes
- Long detection time- 120 d- RBC lifespan
  - Depends on concentration & duration of EtOH
  - (think Hgb A1c for DM monitoring)

Phosphatidyl Ethanol- PEth

- PEth is a direct blood-based biomarker
- 48 species of PEth identified
- Persists in blood for as long as 3 weeks
  - After a few days of moderately heavy drinking (>about four drinks per day)
  - Insensitive to a single use episode
  - Insensitive to mouthwash, hand sanitizer use
- Perfect for detecting binge drinking
- Not readily available, being heavily 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 >5nmol/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
Newer Biomarkers Under Investigation

- β-Hexosaminidase (β-HEX) - lysosomal enzyme for carbohydrate metabolism
  - Elevated in alcoholics, bingers
- Plasma Sialic Acid Index of Apolipoprotein J (SIJ)
  - Long-term ethanol intoxication decreases amount of sialylation of plasma Apo J
- Total sialic acid (TSA) and free sialic acid (FSA)
  - Alcoholics have elevated levels in blood, urine, saliva
- Cholesterol ester transfer protein (CETP) - hepatocyte glycoprotein
  - Decreased in alcoholics
- 5-hydroxytryptophol (5-HTOL) - serotonin metabolite
  - Acetaldehyde, affects serotonin metabolism, increasing urine 5-HTOL concentration
  - Used in conjunction with a ratio with 5-hydroxyindole-acetic acid (5-HIAA)
- Circulating cytokines
  - Certain cytokines elevated in alcohol abuse
- Proteomics - analysis of many or all of the proteins in a sample
  - Structure, function, and expression of serum proteins are affected by alcohol consumption

Summary characteristics of traditional alcohol biomarkers - Jastrzebska 2016

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glutamyl transferase (GT)</th>
<th>Aspartate aminotransferase (AST)</th>
<th>Alanine aminotransferase (ALT)</th>
<th>Mean corpuscular volume (MCV)</th>
<th>Carbohydrate-deficient transferrin (CDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of drinking characterized</td>
<td>Probable at 5 drinks/day for several weeks</td>
<td>Unknown, but heavy and lasting for several weeks</td>
<td>Unknown, but heavy and lasting for several weeks</td>
<td>Unknown, but heavy and lasting for at least a few months</td>
<td>Probable at least 5 drinks/day for 2 weeks</td>
</tr>
<tr>
<td>Time to return to normal levels</td>
<td>14–21 days</td>
<td>2–4 days of abstinence (≤144 h)</td>
<td>2–4 weeks of abstinence (≤ 214 h)</td>
<td>4–6 weeks of abstinence (≤ 273 h)</td>
<td>2–3 weeks of abstinence (≤ 168 h)</td>
</tr>
<tr>
<td>Sensitivity for detecting excessive alcohol consumption</td>
<td>37–49%</td>
<td>25–60%</td>
<td>15–40%</td>
<td>40–50%</td>
<td>55–90%</td>
</tr>
<tr>
<td>Specificity</td>
<td>88–99%</td>
<td>71–80%</td>
<td>51–73%</td>
<td>80–90%</td>
<td>90–97%</td>
</tr>
<tr>
<td>Relapse sensitivity</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>20%</td>
<td>15–30%</td>
</tr>
<tr>
<td>Current clinical use</td>
<td>Identifying chronic alcohol abuse, Screening for heavy drinking</td>
<td>Identifying chronic alcohol abuse, Screening for heavy drinking</td>
<td>Identifying chronic alcohol abuse, Screening for heavy drinking</td>
<td>Screening for heavy drinking</td>
<td>Screening for alcohol dependence, Screening for heavy drinking, Identifying relapse (especially to heavy drinking)</td>
</tr>
<tr>
<td>Strengths in clinical use</td>
<td>High specificity in patients with suspected alcohol abuse, Effective marker for patients with low drinking, binging</td>
<td>Highly sensitive and specific for alcohol-induced liver damage, Effective marker for patients with heavy drinking, binging</td>
<td>Highly sensitive and specific for alcohol-induced liver damage</td>
<td>Accuracy similar in male and female subjects, Indicates chronicity of drinking, Effective laboratory tool</td>
<td>High specificity for alcohol use, High sensitivity in distinguishing alcoholics from social drinkers, Confirmatory test for patients suspected of alcohol abuse, Marker of relapse and alcoholism from binging</td>
</tr>
<tr>
<td>Limitations in clinical use</td>
<td>Many factors cause false positives, Poor screening tool in general population due to low sensitivity, Poor marker of relapse</td>
<td>AST seems to be less sensitive than ALT, Elevates only after periods of heavy drinking, Elevates secondary to liver damage at hepatocellular level</td>
<td>ALT seems to be less sensitive than AST, Elevates only after periods of heavy drinking, Elevates secondary to liver damage at hepatocellular level</td>
<td>Many factors cause false positives, Poor screening tool for alcohol abuse (due to low sensitivity), Poor marker of relapse</td>
<td>Low sensitivity, more suitable to confirm that total alcohol drinking, Poor screening tool for alcohol use in general population, Cost and low availability of testing</td>
</tr>
</tbody>
</table>
Factors affecting the serum level of traditional alcohol biomarkers - Jastrzebska 2016

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Biomarker</th>
<th>γ-Glutamyl transpeptidase (GGT)</th>
<th>Aspartate aminotransferase (AST)</th>
<th>Alanine aminotransferase (ALT)</th>
<th>Mean corpuscular volume (MCV)</th>
<th>Carbohydrate-deficient transferrin (CDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Less likely in subjects &lt; 30 years of age and possibly in subjects &gt; 70 years of age</td>
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<td>Less likely in subjects &lt; 30 years of age and possibly in subjects &gt; 70 years of age</td>
<td>Less likely in subjects &lt; 30 years of age and possibly in the elderly</td>
<td>Less likely in subjects &lt; 30 years of age</td>
<td>Less likely in subjects &lt; 30 years of age</td>
</tr>
<tr>
<td>Gender</td>
<td>More likely in men</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>More likely in women</td>
<td>Less likely in women</td>
</tr>
</tbody>
</table>

- Medical conditions: (†) Liver and biliary disorders, obesity, hypertriglyceridemia, diabetes, pancreatitis, hypercholesterolemia, hypertension, heart diseases, kidney diseases, venous embolism and thrombosis, serious injuries
- Medications: (†) Liver microsomal enzymes inducers, e.g. antidepressants, anticonvulsants, non-steroidal anti-inflammatory drugs, barbiturates, benzodiazepines, tricyclic antidepressants

Window of Assessment for Various Alcohol Biomarkers

![Graph showing window of assessment for various alcohol biomarkers](image)

- **BAC** = Blood alcohol concentration
- **EIS** (Urine) = Ethanol in Urine
- **EIS** (Urine) = Ethanol in Urine
- **PEth** = Protein Ethanol
- **CDT** = Carbohydrate Deficient Transferrin
- **GGT** = γ-Glutamyl Transpeptidase
- **AST** = Aspartate Aminotransferase
- **ALT** = Alanine Aminotransferase
- **MCV** = Mean Corpuscular Volume
## 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>
</tr>
</thead>
<tbody>
<tr>
<td>CDT</td>
<td>✓</td>
<td>✓</td>
<td>2-3 weeks</td>
<td></td>
</tr>
<tr>
<td>EtG, EtS</td>
<td>✓</td>
<td></td>
<td>1-3 days</td>
<td>✓</td>
</tr>
<tr>
<td>GGT</td>
<td>✓</td>
<td></td>
<td>2-4 weeks</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>✓</td>
<td></td>
<td>Several months</td>
<td></td>
</tr>
<tr>
<td>PEth</td>
<td>✓</td>
<td></td>
<td>2-4 weeks</td>
<td></td>
</tr>
<tr>
<td>BAT</td>
<td>✓</td>
<td></td>
<td>hours</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>✓</td>
<td></td>
<td>2-4 weeks</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>✓</td>
<td></td>
<td>2-4 weeks</td>
<td></td>
</tr>
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### Bibliography

1. **The Role of Biomarkers in the Treatment of Alcohol Use Disorders, 2012 Revision**
   - SAMHSA Advisory
3. Rohrig TP. Et al. Detection of ethylglucuronide in urine following the application of Germ-X. *Journal of Analytical Toxicology*, 30(9):703-4, 2006