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

ACMT Alcohol Academy
San Juan, PR
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Center for Occupational Health of Glens Falls Hospital
Glens Falls, NY
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
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 AIDH
  - 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
- Ethyl Glucuronide (EtG) < 1%
- Ethanol ADH & AIDH > 95%
- Ethyl Sulfate (EtS) < 1%, Sulphotransferase

Acetaldehyde and acetic acid
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-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”
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 3 (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 0.50–0.78 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)
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
Suggested EtG Cut-offs

- 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.
LABORATORY REPORT

Account #: 51637
DM: K. MICHAEL HOLLAND
WILSON, NY 12991

General Information
Date Collected: 12/18/2012
Date Received: 12/29/2012
Date Reported: 1/16/2013

TEST(S) REQUESTED
ALCOHOL BIOMARKERS

RESULTS
NEGATIVE

** ALTERNATIVE EXPLANATIONS SHOULD BE CONSIDERED FOR ANY POSITIVE FINDINGS. **

Certified by: TOMAZENKO, JEFF
** FINAL REPORT **
Collected at 5182900140 - MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS FALLS, NY
LABORATORY REPORT

MEDTOX LABORATORIES INC.
402 WEST COUNTY ROAD D
ST PAUL, MN 55112
651-436-1466

Account #: 5184
CENTER FOR OCCUPATIONAL HEALTH
Prof: Dr. Michael Holland
135 NORTH ROAD
WILTON, NY 12895

General Information
Accession #: MH862857
Specimen I.D.: 
Donor Name/ID: 
SSN: 
Age: 
Sex: 
Reason for test: Follow Up

Date
Collect: 01/23/2012 15:15
Received: 01/24/2012
Reported: 01/30/2012 21:14PM

TEST(S) REQUESTED
ALCOHOL BIOMARKERS
ETHYL GLUCONONIDE

RESULTS
+++POSITIVE+++

THIS SPECIMEN WAS SCREENED BY IMMUNOASSAY. ANY POSITIVE RESULT WAS CONFIRMED BY LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY (LC/MS/MS).

THE FOLLOWING THRESHOLD CONCENTRATIONS WERE USED FOR THIS ANALYSIS:

DRUG    SCREENING THRESHOLD    CONFIRMATION THRESHOLD
ETHYL GLUCONONIDE $50 Ng/mL    $250 Ng/mL
ETHYL SULFATE

ALTERNATIVE EXPLANATIONS SHOULD BE EXPLORED FOR ANY POSITIVE FINDING.

PLEASE NOTE THAT INCIDENTAL EXPOSURE TO ALCOHOL MAY RESULT IN DETECTABLE LEVELS OF ETG AND/OR ETX. THE CENTER FOR SUBSTANCE ABUSE PREVENTION (CSAP) ADVISES CAUTION IN INTERPRETATION AND USE OF BIOMARKERS ALONE TO ASSESS ALCOHOL USE. ETG/ETX RESULTS SHOULD BE INTERPRETED IN THE CONTEXT OF ALL AVAILABLE CLINICAL AND BEHAVIORAL INFORMATION.


Certified by: Nicklas, Renee

ALCOHOL BIOMARKERS
ETHYL GLUCONONIDE
ETHYL SULFATE

ng/mL
>10000
41202

**FINAL REPORT**

Collected at 518920146 MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS FALLS, NY
LABORATORY REPORT

Account #: 91687
CENTER FOR OCCUPATIONAL HEALTH
MHO: DR MICHAEL HOLLAND
135 NORTH ROAD
WELLS, NY 12888

General Information

Date Collected: 11/26/2012
Date Received: 11/26/2012
Date Reported: 11/28/2012
Reported: 12/1/2012
12:17PM

TEST(S) REQUESTED

ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE

RESULT(S)

+++POSITIVE+++

THIS SPECIMEN WAS SCREENED BY IMMUNOASSAY. ANY POSITIVE RESULT WAS CONFIRMED BY LIQUID CHROMATOGRAPHY WITH TANDER MASS SPECTROMETRY (LC/MS/MS).

THE FOLLOWING THRESHOLD CONCENTRATIONS WERE USED FOR THIS ANALYSIS:

DRUG SCREENING THRESHOLD CONFIRMATION THRESHOLD
ETHYL GLUCURONIDE 200 mg/dL 500 mg/dL
ETHYL SULFATE 250 mg/dL

ALTERNATIVE EXPLANATIONS SHOULD BE EXPLORED FOR ANY POSITIVE FINDING.

PLEASE NOTE THAT INCIDENTAL DECOURS TO ALCOHOL MAY RESULT IN LETHAL LEVELS OF ETG AND/OR ETX. THE CENTER FOR SUBSTANCE ABUSE PREVENTION (CSAP) ADVISES CAUTION IN INTERPRETATION AND USE OF BIOMARKERS ALONE TO ASSESS ALCOHOL USE. ETG/ETX RESULTS SHOULD BE INTERPRETED IN THE CONTEXT OF ALL AVAILABLE CLINICAL AND BEHAVIORAL INFORMATION.


Certified by: SIMCOX, JELAINE

ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE 4655 mg/dL
ETHYL SULFATE 1469 mg/dL

**FINAL REPORT**

Collected at 5189242140 MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH = GLENS FALLS
GLENS FALLS, NY
<table>
<thead>
<tr>
<th>Alcohol Biomarkers</th>
<th>RESULTS</th>
<th>UNITS THERAPEUTIC RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EICHLER GLUCURONIDE</td>
<td>+++POSITIVE+++</td>
<td></td>
</tr>
</tbody>
</table>

This specimen was screened by immunoassay. Any positive result was confirmed by liquid chromatography with tandem mass spectrometry (LC/MS/MS).

The following concentration thresholds were used for this analysis:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Screening Threshold</th>
<th>Confirmation Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETHYL GLUCURONIDE</td>
<td>500 ng/mL</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>VINYL SULFATE</td>
<td>250 ng/mL</td>
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</tbody>
</table>

Alternative explanations should be explored for any positive findings.

Please note that incidental exposure to alcohol may result in detectable levels of EICHLER GLUCURONIDE. The Center for Substance Abuse Prevention (CSAP) advises caution in interpretation and use of biomarkers along with other assessment tools to assess alcohol use. Interpretation should be interpreted in the context of all available clinical and behavioral information.


Certified by: MARETTI, SUBULA

** Final Report **

Collected at 5188262140 MEDTOX collection site #2072 CENTER FOR OCCUPATIONAL HEALTH - GLEN FALLS GLEN FALLS, NY
LABORATORY REPORT

Test(s) Requested:
ALCOHOL BIOMARKERS
ETHYL GLUCOCHONIDE

Results:
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Units Therapeutic Range

This specimen was screened by Toxscreen. Any positive result was confirmed by liquid chromatography with tandem mass spectrometry (LC/MS/MS). The following threshold concentrations were used for this analysis:

SCREENING THRESHOLD
ETHYL GLUCOCHONIDE 500 mg/mL
ETHYL SULFATE 250 mg/mL

CONFIRMATION THRESHOLD
ETHYL GLUCOCHONIDE 500 NG/mL
ETHYL SULFATE 500 NG/mL

Alternative explanations should be explored for any positive finding.

Please note that incidental exposure to alcohol may result in detectable levels of EtG and/or EtS. The Center for Substance Abuse Prevention (CSAP) advises caution in interpretation and use of biomarkers along with access alcohol use. EtG/EtS results should be interpreted in the context of all available clinical and behavioral information.


Certified by: WIECKLAND,cMNS

ALCOHOL BIOMARKERS

<table>
<thead>
<tr>
<th>Substance</th>
<th>Result</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETHYL GLUCOCHONIDE</td>
<td>1326</td>
<td>mg/mL</td>
</tr>
<tr>
<td>ETHYL SULFATE</td>
<td>564</td>
<td>ng/mL</td>
</tr>
</tbody>
</table>

**Final Report**

Collected at 5180920140 MEDTOK collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - MEDTOK
GLOVER FALLS, NY
MEDTOX LABORATORIES INC.
428 WEST COUNTY ROAD D
ST PAUL, MN 55112
651-634-7498

JENNIFER A. COLLINS, Ph.D.

LABORATORY REPORT

Accession #: 21803647
Specimen: Urine
Donor Name: John Doe
Age: 35
Sex: Male
Reason for test: Random

Date Collected: 11/15/2012
Date Received: 11/16/2012
Date Reported: 11/16/2012
Time: 5:09 PM

TEST(S) REQUESTED
ALCOHOL BIOMARKERS
ETYL GLUCURONIDE
++POSITIVE++

RESULTS
ETYL GLUCURONIDE: 539 ng/mL
ETHYL SULFATE: 219 ng/mL


Certified by: James R. Miller, Ph.D.

** FINAL REPORT **

Collected at 519962140 - MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS FALLS, NY
LABORATORY REPORT

Account #: 91427
CENTERS FOR OCCUPATIONAL HEALTH
BROOKLYN MIDDLE HOLLAND
135 NORTH ROAD
WILTON, CT 06897

Date
Collect 4/23/2012

Reason for test: Follow-Up

Donor Name: RH

Age: 53

Alcohol Biomarkers

Ethyl glucuronide
+++POSITIVE++++

This specimen was screened by Immunoassay. Any positive result was confirmed by liquid chromatography with tandem mass spectrometry (LC/MS/MS).

The following threshold concentrations were used for this analysis:

Ethanol Screening Threshold: 50 mg/dL
Ethanol Confirmation Threshold: 200 mg/dL
Ethyl glucuronide

509 mg/dL

Ethyl sulfate

250 mg/dL

Alternative explanations should be explored for any positive finding.

Please note that incidental exposure to alcohol may result in detectable levels of ETOH and/or ETG. The centers for substance abuse prevention (CSAP) advise caution in interpretation and use of biomarkers alone to assess alcohol use. ETOH/ETG results should be interpreted in the context of all available clinical and behavioral information.


Certified by: MICHELINO, ROSE

Alcohol Biomarkers

Ethyl glucuronide

500 ng/mL

Ethyl sulfate

NEGATIVE

**FINAL REPORT**

Collected at 5192462140 M GENERAL collection site #107

CENTER FOR OCCUPATIONAL HEALTH - GLACERN FALLS

GLACERN FALLS, NY
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 >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
Window of Assessment for Various Alcohol Biomarkers

BAC = Blood alcohol concentration
# 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>
<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>
</tbody>
</table>
Bibliography


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

4) Rosano TG. Lin J. Ethyl glucuronide excretion in humans following oral administration of and dermal exposure to ethanol. Journal of Analytical Toxicology. 32(8):594-600, 2008


