Breath and Urine Alcohol Testing: Relationship to BAC and Use Patterns

ACMT ASM
Huntington Beach, CA
March 17, 2016

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Senior Medical Toxicologist, Center for Toxicology and Environmental Health; North Little Rock, AR
Disclosures

- No financial relationships to disclose
- I perform medical-legal consulting regarding drug and alcohol impairment
- My practice of Occupational Medicine uses BAT devices and I am a BAT trainer
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 cause elevations
  - Some abusers do not have elevations
Indirect Biomarkers

- Newest: CDT- Carbohydrate-deficient transferrin
  - Elevated after > 2 weeks of heavy EtOH abuse (>5 drinks/day)
  - 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

- Analytes are measured in the blood or urine.
Alcohol Metabolism

Unchanged in breath, urine, sweat

< 5%

Ethyl Glucuronide (EtG)

< 1%

UDP-glucuronosyltransferase

Ethanol in Blood

< 1%

Sulfotransferase

Ethyl Sulfate (EtS)

> 95%

ADH & AIDH

Acetaldehyde and acetic acid
Breath Alcohol Concentration (BrAC)

- BrAC of terminal portion of a prolonged exhalation reflects the arterial BAC
- Expressed as g ethanol per 210 L breath
- Machines designed to ignore initial (2/3) of breath and measure terminal portion only
- Salivary alcohol gone in 15 minutes
  - Mandatory retest in 15 minutes
  - 2nd test must agree with first by ≤ 0.02g/210L
- Eructation, vomiting can contaminate
BrAC testing Devices

- BrAC testing devices must be calibrated
- Air blanks and gas cylinders with known [EtOH]
- QA procedures and device user training logs
- Device manufacturers must submit their equipment to NHTSA to get on CPL
- BrAC frequently subject to challenges by DWI defense counsel
BrAC Testing Devices

- Three types in common use on NHTSA CPL:
  - Infrared
  - Fuel cell
  - Chemical oxidation

- Challenges to accuracy are rampant in DWI defense

- Most attack the human element, not the technology

- Many jurisdictions use BrAC screening, get BAC confirmatory
BrAC Testing Devices:

1) Infrared Spectroscopy

- All chemicals absorb infrared light at specific wavelengths.
- Ethanol absorbs strongly at 3.3-3.5 µ (acetone too), but more specific band at 9.5 µ.
- Amount of infrared energy lost from Br from chamber entry to exit reflects infrared absorption by EtOH, is proportional to [EtOH].
- Acetone potential interferent if 9.5 µ not used.
2) Fuel Cell, aka Electrochemical Oxidation

- Converts fuel and an oxidant into DC
- EtOH converted to acetic acid
  - Gives off two electrons
  - Current produced is proportional to [EtOH]
- Very specific for alcohols (MeOH, IsOH too)
- Can be very small, low power usage: ideal for hand-held devices
- Acetate can build up on fuel cell with many consecutive + tests, prolongs time to return to baseline
Alkosensor IV
Alkosensor IV
3) Chemical Oxidation/Photometry

- First type of BrAC device developed and brought into widespread LE use
- Breath enters chamber of oxidizing mixture
- Reaction of EtOH with the oxidizers causes a decrease in UV light absorbed, measure by photometer, proportional to [EtOH]
- Still in use in a few jurisdictions
- No longer manufactured, Breathalyzer 900A was the only one left
BrAC Testing Devices: Dual Detectors: FC and IR

- Employs both Fuel Cell and Infrared
- Usually one method is used to validate the other
- Any discrepancy invalidates the test
- Can be used stationary or mobile
- Very accurate and hard to challenge due to redundancy
BrAC to BAC ratio

- Assumption is that blood: breath alcohol ratio is 2100:1, and that is basis for all testing in NA
- Actual ratio is closer to 2300:1
- This actually favors the arrestee
  - Proven in side by side roadside tests
  - BrAC always underestimates true BAC
Converting Serum EtOH to BAC

- Serum: WB ratio:
  - Averages 1.15:1 to 1.10:1
  - So a serum EtOH value is divided by 1.1 to 1.15 to convert to “legal” BAC language

- Some DWI arrestees with positive BrAC present to EDs demanding a retest- will usually not be helpful to them
Other 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
Ethylsulfate- EtS

- EtG: Ethyl β-D-6-glucosiduronic acid

- Approx 0.02% of ethanol is metabolized by phase II conjugation with uridine 5'-diphospho-glucuronic acid (UDPGA) via UDP-glucurolyosyltransferase to form EtG

- EtS produced by sulfotransferase
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

- However, cannot prove beverage alcohol was the source
  - Hand sanitizers
  - Mouthwashes
  - Non-alcoholic beer
  - Old fruit juices
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
- \[\text{EtG}\] Varies with hydration, often corrected for creatinine
- ?: should urine be refrigerated or preserved?
  - EtOH produced from glucose fermentation in urine
  - Refrigeration or preservative in specimen prevents
  - However, UDPGA & ST-ase do not exist in urine
  - Our practice does not refrigerate urine, no preservative in our bottles
- Not FDA-approved medical test, not covered by health insurance
EtG and EtS Kinetics

Winkler Int J Legal Med.

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

Winkler *Int J Legal Med.*

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


- 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
  - The sweet spot is probably EtG> 500, with EtS >250 (this is what our program uses)
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.
MEDTOX LABORATORIES INC.
402 WEST COUNTY ROAD D
ST PAUL, MN 55112
651-636-7466

LABORATORY REPORT

Account #: 91687
CENTER FOR OCCUPATIONAL HEALTH
MRO: DR MICHAEL HOLLAND
135 NORTH ROAD
WILTON, NY 12831

Accession #: J2207105
Specimen I.D.: 223533774
Donor Name/I.D.: [Redacted]
SSN:
Age: Sex: Follow Up

Date Collected: 12/28/2012
Date Received: 12/29/2012
Date Reported: 12/29/2012
10:25
4:26PM

TEST(S) REQUESTED
-------------------------------------
ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE

RESULTS
NEGATIVE

UNITS THERAPEUTIC RANGE

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:

<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>
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<tr>
<td>ETHYL SULFATE</td>
<td>250 NG/ML</td>
<td></td>
</tr>
</tbody>
</table>

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 ALONE TO ASSESS ALCOHOL USE. ETG/ETS RESULTS SHOULD BE INTERPRETED IN THE CONTEXT OF ALL AVAILABLE CLINICAL AND BEHAVIORAL INFORMATION.


Certified by: TOMASZEWSKI, JEFF

** FINAL REPORT **

Collected at 5189262140  MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS FALLS, NY
LABORATORY REPORT

Account #: 91687
CENTER FOR OCCUPATIONAL HEALTH
MSO: DR. MICHAEL HOLLAND
135 NORTH ROAD
WILTON, NY 12831

Accession #: N8862087
Specimen I.D.: 21033427
Donor Name/ID:
SSN:
Age: Sex:
Reason for test: Follow Up

Date Collected: 01/23/2012
15:15
Date Received: 01/24/2012
Date Reported: 01/27/2012
2:14PM

TEST(S) REQUESTED

ALCOHOL BIOMARKERS
ETHYL GLUCORONIDE

RESULTS

+++POSITIVE+++  

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

THE FOLLOWING THRESHOLD CONCENTRATIONS WERE USED FOR THIS ANALYSIS:

DRUG SCREENING THRESHOLD CONFIRMATION THRESHOLD
ETHYL GLUCORONIDE 500 NG/ML 500 NG/ML
ETHYL SULFATE 250 NG/ML

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Certified by: WICKLUND, RENEE
ALCOHOL BIOMARKERS
ETHYL GLUCORONIDE  >10000  ng/ml
ETHYL SULFATE  41202  ng/ml

** FINAL REPORT **

Collected at 5189262140  MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS FALLS, NY
LABORATORY REPORT

Account #: 51687
CENTER FOR OCCUPATIONAL HEALTH
MNO: DR MICHAEL HOLLAND
135 NORTH ROAD
WILTON, NY 12831

Accession #: J1922947
Specimen I.D.: 880000443
Donor Name/ID: 
SN: 
Age: Sex: 
Reason for test: Other

Date
Collected: 11/26/2012
Received: 11/28/2012
Reported: 12/01/2012
Time: 16:17

TEST(S) REQUESTED
---------------------
ALCOHOL BIOMARKERS

TEST RESULTS

ETHYL GLUCURONIDE
+++POSITIVE+++ 

UNITs THERAPEUTIC RANGE

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

THE FOLLOWING THRESHOLD CONCENTRATIONS WERE USED FOR THIS ANALYSIS:

DRUG SCREENING THRESHOLD CONFIRMATION THRESHOLD
ETHYL GLUCURONIDE 500 NG/ML 500 NG/ML
ETHYL SULFATE 250 NG/ML

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Certified by: SIMCOX, JELALNE
ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE 4635 ng/ml
ETHYL SULFATE 1669 ng/ml

** FINAL REPORT **

Collected at 5189262140 MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS FALLS, NY
LABORATORY REPORT

Account #: 91687
CENTER FOR OCCUPATIONAL HEALTH
MRG: DR MICHAEL HOLLAND
135 NORTH ROAD
WILTON, NY 12831

Laboratory Report

Accession #: J1706045
Specimen I.D.: ???????
Donor Name/ID: 
SSN: 
Age: Sex: 
Reason for test: Not indicated

General Information

Date Collected: 11/01/2012
Date Received: 11/02/2012
Date Reported: 11/04/2012
Time: 5:24PM

TEST(S) REQUESTED
ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE

RESULTS
+++POSITIVE+++

UNITS THERAPEUTIC RANGE

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:

DRUG SCREENING THRESHOLD CONFIRMATION THRESHOLD
ETHYL GLUCURONIDE 500 NG/ML 500 NG/ML
ETHYL SULFATE 250 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 ALONE TO ASSESS ALCOHOL USE. ETG/ETS RESULTS SHOULD
BE INTERPRETED IN THE CONTEXT OF ALL AVAILABLE CLINICAL AND
BEHAVIORAL INFORMATION.

REFERENCE: CENTER FOR SUBSTANCE ABUSE PREVENTION, "THE ROLE OF
BIOMARKERS IN THE TREATMENT OF ALCOHOL USE DISORDERS". SUBSTANCE
ABUSE TREATMENT ADVISORY, VOLUME 5, ISSUE 4, SEPTEMBER 2006.

Certified by: MARZITELLI, SUSULA

ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE 3916 ng/ml
ETHYL SULFATE 1876 ng/ml

** FINAL REPORT **

Collected at 5189262140 MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS FALLS, NY
LABORATORY REPORT

Account #: 91687
CENTER FOR OCCUPATIONAL HEALTH
MNO: DR MICHAEL HOLLAND
135 NORTH ROAD
WILTON, NY 12831

Accession #: N615326
Specimen I.D.
Donor Name/I.D.
SSN:
Age:
Sex:
Reason for test: Follow Up

Date Collected: 04/05/2012
Date Received: 04/07/2012
Date Reported: 04/09/2012
15:50
8:18AM

TEST(S) REQUESTED

ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE
+++POSITIVE+++ | RESULTS | UNITS THERAPEUTIC RANGE

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:

DRUG | SCREENING THRESHOLD | CONFIRMATION THRESHOLD
ETHYL GLUCURONIDE | 500 NG/ML | 500 NG/ML
ETHYL SULFATE | 250 NG/ML

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Certified by: WICKLUND, RENEE
ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE 1326 ng/ml
ETHYL SULFATE 564 ng/ml

** FINAL REPORT **

Collected at 5189262140 MEDTOX collection site #2077 CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS GLENS FALLS, NY
LABORATORY REPORT

Account #: 91687
CENTER FOR OCCUPATIONAL HEALTH
MRO: DR MICHAEL HOLLAND
135 NORTH ROAD
WILTON, NY 12831

Accession #: J1837647
Specimen I.D.: 223237167
Donor Name/ID
SSN:
Age: Sex:
Reason for test: Other

Date Collected 11/15/2012
Date Received 11/16/2012
Date Reported 11/19/2012
13:50 5:00PM

TEST(S) REQUESTED

ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE

RESULTS

+++POSITIVE+++ UNITs THERAPEUTIC RANGE

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ETHYL GLUCURONIDE 500 NG/ML 500 NG/ML
ETHYL SULFATE 250 NG/ML

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Certified by: MARETTELI, SUSULA

ALCOHOL BIOMARKERS
ETHYL GLUCURONIDE 1598 ng/ml
ETHYL SULFATE 319 ng/ml

** FINAL REPORT **

Collected at 5189262140 MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENs FALLS
GLENs FALLS, NY
MEDTOX LABORATORIES INC.
402 WEST COUNTY ROAD D
ST PAUL, MN 55112
651-636-7466

LABORATORY REPORT

Account #: 91687
CENTER FOR OCCUPATIONAL HEALTH
MB: DR MICHAEL HOLLAND
135 NORTH ROAD
WELTON, NY 12831

Accession #: N9776558
Specimen ID #: 9716976

General Information

Donor Name: [Redacted]
SSN: [Redacted]

Date:
Date Collected: 04/23/2012
Date Received: 04/24/2012
Date Reported: 04/29/2012
Age: 61

Reason for test: Follow Up

TEST(S) REQUESTED

ALCOHOL BIOMARKERS

TEST(S) REQUESTED

ETHYL GLUCURONIDE

RESULTS

+++POSITIVE+++

UNITS THERAPEUTIC RANGE

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<tr>
<td>ETHYL GLUCURONIDE</td>
<td>500 NG/ML</td>
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<tr>
<td>ETHYL SULFATE</td>
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<td>250 NG/ML</td>
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Certified by: WICKLUND, RENEE

ALCOHOL BIOMARKERS

<table>
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<tr>
<th>DRUG</th>
<th>RESULTS</th>
<th>UNITS</th>
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<tbody>
<tr>
<td>ETHYL GLUCURONIDE</td>
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<td>ETHYL SULFATE</td>
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<td>ng/ml</td>
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** FINAL REPORT **

Collected at 5189262140  MEDTOX collection site #2077
CENTER FOR OCCUPATIONAL HEALTH - GLENS FALLS
GLENS 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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<td>CDT</td>
<td>√</td>
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<td>2- 3 weeks</td>
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<td>1- 3 days</td>
<td>√</td>
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<tr>
<td>GGT</td>
<td>√</td>
<td></td>
<td>2- 4 weeks</td>
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<tr>
<td>MCV</td>
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