Drug Exposure in CINC Cases
What specimen to Test?
What do the results tell us?

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Everyone knows learning must be serious and difficult and you must remain seated at all times. No fun allowed.
Poll Questions
Drug Tests

- **Common Matrices**
  - Blood
  - Urine
  - Oral Fluid
  - Hair

- **Purpose**
  - Compliance
  - Recent
  - Usage
  - Deterrence
  - Intoxication
How Long Do Drugs Stay in your “System”

DEPENDS
Drug Detection Periods

- Blood
- Saliva
- Oral Fluid
- Urine
- Sweat
- Hair

*From E.J. Cone, Addiction Research Center, NIDA*
Detection Times

- Varies number of factors
  - Frequency of use
  - Dose
  - Route of administration
  - Race and age [e.g. 2D6 polymorphisms]

- Hair Detection Time
  - Standard time frame – 90 days
    - Head hair
    - 1 cm/ month
  - Body Hair
    - Grows more slowly
    - Drug do not appear as rapidly
    - 1.5 may represents 0.5–1.0 yr exposure
Collection Site Testing

- Test Cup/Test Strip – Urine only
  - Immunoassay-based
  - No confirmation
- Subjective
  - Looking at color change or “band”
- Non-technical testing personnel
Laboratory Based Testing

**Instrumental – objective**
- Two tier
- Immunoassay
- Mass Spectrometry Confirmation
  - GC/MS
  - LC/MS

**Objective – highly reliable**
- Multiple checks and balances to assure quality

**Reviewable data**

**Technically trained scientists**
Screens for classes of drugs
- Not 100% X-reactivity
- “Cut-off” driven as deterrent

“Amphetamine” Scrn
- Tests for class of drugs
  - Phenethylamines
    - Methamphetamine
    - Amphetamine*
    - Ephedrine
    - Pseudoephedrine
  - ADHD medication
    - Amphetamine
False Positives

Not Really!

Ephedrine  Pseudoephedrine

amphetamine  methamphetamine
False Positives

- Lab based or “test cup”
- May occur with IM screening
  - PCP
    - DXM or Diphenhydramine
  - Reason for confirmation
  - GC/MS or LC/MS
- Misinterpretation
False Negatives

- **Time**
- **Different Assay/Cross-reactivity**
  - Opiates: OxyC/Fentanyl
- **Different “Cut-off” or confirmation target**
  - Opiates: Confirmation for only Codeine/Morphine
- **Different matrix**
  - Urine
  - Hair
  - Oral fluid
  - Blood
Myth #1

- Passive inhalation of marijuana smoke can cause a “positive” drug test result.
- NO - not if standard cutoffs are used
- THC (cannabinoid) assay uses variable cutoffs (20, 50, 100 ng/mL)
- passive inhalation research indicates less than 10 ng/mL in volunteer urines
- Shotgunning and hot box is not passive
- no passive inhalation for “crack”
Second Hand Exposure

- Increased potency of cannabis
  - Resurface of secondhand exposure

John Hopkins Study
- 6 smoker/6 non-smokers
- High potency MJ
- Small room
  - Smoke so intense—goggles/eye irritation
  - 1 non-smoker scrn pos [50 ng/ml]
  - GC/MS confirmed ranged up to 57.5 ng/ml
    - 15 ng/ml std confirmation cut-off
  - Non-smokers pos behavioral effects

Cone et al ThOP #2 TIAFT meeting Firenze Italy 2015
Cone et al JAT 39:1 [2015]
Myth #2

- Advil® (ibuprofen) causes “false-positive” drug tests for marijuana
- NO!
- problem with EMIT® method corrected 15+ years ago
Myth #3

- Consuming poppy seeds causes “false-positive” drug tests for heroin
- NO! - but?
- poppy seeds contain trace amounts of both codeine and morphine
- can causes “positive” drug test results for “opiate” class
- confirm positive opiates
Hair Testing
Hair Testing

- Alternative and/or complementary matrix to blood and urine testing
- Primary advantage(s)
  - Document longer drug use/exposure history
  - Ease of collection – no privacy/gender issues
  - Chemical stability of certain drugs
  - Less susceptible to adulteration
- Accepted by a US court – 1982
  - St of Alaska v Richard Gene Majdic
Hair Testing

- Calling a hair test a **hair follicle test** is a common misnomer.
- Hair follicle is actually the pocket, below the scalp, from which the hair grows.
- Follicle anchors the hair into the skin.
- “Pluck” hair for follicle.
Hair Testing

- Accepted & objective method to determine drug use
  - Similar technology as with UAs
  - Safe guards in place to identify passive exposure

- Drug incorporation
  - Blood
  - Sweat

- Broader window of drug detection
  - 1 cm/mo [0.6–1.4 cm/mo]
  - 90 days w/ 3 cm sample
    - 2.54 cm per inch [1.18 inches]
Hair Sample Collection

- Slide hair clip horizontally under hair at crown of head
- Lift 1-2 inch wide section of hair exposing base of hair underneath
- Use fingers below clip to take the required diameter of hair
  - Pencil
  - Shorter hair (less than 1 ½ inch) requires more strands of hair to have adequate “bulk” for sample
- Lift and Cut the hair
- Cut as close to scalp as possible
Hair Sample Collection

- Align root ends of hair sample so they are even
  - Ends closest to the scalp
- Place the root end of the hair sample against the tab end of the Collection Foil
- Lab measures 3 cm from scalp end for 90 days surveillance window
Poll Questions
<table>
<thead>
<tr>
<th>Drug</th>
<th>DTAB 2004</th>
<th>EWDT 2010</th>
<th>SOHT 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabinoids</td>
<td>THC-COOH: 0.05</td>
<td>THC: 50 THC-COOH: 0.2</td>
<td>THC: 50 THC-COOH: 0.2</td>
</tr>
<tr>
<td>Methadone</td>
<td></td>
<td>Methadone: 200 EDDP: 50</td>
<td></td>
</tr>
<tr>
<td>PCP</td>
<td>PCP: 300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* BE/EME in vitro degradation
CE/NorCoc in street samples
Chemical Treatments

- Multiple products claim to mask or remove drugs
- Dasgupta (2007) look at several shampoos
  - Don’t work
- Yeagles et al (2000) evaluated effectiveness of bleaching hair
  - Some impact on benzos
- Only true way beat
  - Shave head

Dasgupta Clin Chem 128:491 [2007]
### Amount of Use Tables

<table>
<thead>
<tr>
<th>Substance</th>
<th>Confirm cutoff</th>
<th>Low use (recreational)</th>
<th>Medium use (daily/weekends)</th>
<th>High use (constant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines</td>
<td>500pg/mg hair</td>
<td>500–2500pg</td>
<td>2500–7500pg</td>
<td>7500+pg</td>
</tr>
<tr>
<td><em>Amphetamine,</em> Methamphetamine &amp; Ecstasy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>500pg/mg hair</td>
<td>500–2000pg</td>
<td>2000–10000pg</td>
<td>10000+pg</td>
</tr>
<tr>
<td><em>Cocaine</em> &amp; Benzoylecgonine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opiates</td>
<td>300pg/mg hair</td>
<td>500–1000pg</td>
<td>2000–8000pg</td>
<td>9000+pg</td>
</tr>
<tr>
<td><em>Codeine, Morphine</em> &amp; 6-MAM (Heroin metabolite)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phencyclidine (PCP)</td>
<td>300pg/mg hair</td>
<td>300–500pg</td>
<td>500–1000pg</td>
<td>2000+pg</td>
</tr>
<tr>
<td>Marijuana</td>
<td>0.3pg/mg hair</td>
<td>Qualitative – amount does not correlate to usage</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Carboxy-THC</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This data was acquired from various academic studies with admitted drug users and are thus subject to numerous sources of variability (e.g., purity, inadequate self-report, etc.).
Establishing relationship between amt of drug intake and drug/drug metabolite concentration highly controversial

- NOT recommended

At best a positive correlation seems to exist

- Welp et al [2003] found crude correlation between reported drug use and concentration
  - 0.45 to 0.59
Limited studies are retrospective and based upon self-reported use

Overall drug intake unknown
  ◦ Unknown purity
  ◦ Highly variable purity

Drug incorporation depends upon hair type and age

Drug removal depends upon type and frequency of washing and cosmetic treatments
Conclusions

- Drug use
- Hair and Urine
  - No gauge of intoxication
- Urine
  - Broad est of last time of use [hours to a few days]
  - No conclusion on amt/dose
- Hair
  - Very broad time of use [week – 90 day* window]
  - No conclusion on amt/dose
    - Ethyl Alcohol exception

* Sample size/area dependent
Test Results
“Conflicting” Test Results

- **Urine Test Window:** generally 2-4 days
  - MJ possible exception
- **Hair Test Window:** generally 90 days
  - First few days/week missed, esp if hair cut v pulled
  - Head hair
  - Single dose exposures for many drugs
- **Consensus of SOHT**
  
  *A negative hair test result cannot exclude the administration of a drug in question and cannot overrule a positive urine drug test*

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Poll Questions
Alcohol Use
Non-oxidative Pathway

FAEE ← +fatty acids
PEth ← +phosphatidylcholine
EtG ← +glucuronic acid
EtS ← +sulfate

Non-oxidative pathway

Oxidative Pathway

Alcohol → Acetaldehyde
ADH → Acetate → Circulation

Excess alcohol

Acetaldehyde

Lipid accumulation
Inflammation
Fibrosis

ROS

Catalase

CYP2E1

Oxidative pathway
EtOH v EtG

Ethyl alcohol

Ethyl glucuronide

Time (days)
# Non-invasive biomarkers for excessive alcohol use

## Acute alcohol use biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Description</th>
<th>Timeframe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Most accurate determination of a patient’s alcohol level</td>
<td>1-12 hrs in blood or urine</td>
</tr>
<tr>
<td>Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS)</td>
<td>Direct minor metabolites of ethanol and are considered good markers of acute, short-term</td>
<td>Up to 36 hours in the blood, up to 5 days in urine) alcohol ingestion. Results do not accurately correlate with the amount or frequency of ethanol use</td>
</tr>
</tbody>
</table>

## Chronic alcohol use biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Description</th>
<th>Timeframe</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST/ALT</td>
<td>Low sensitivity and specificity</td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td>Inexpensive and lack of specificity</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>Low sensitivity and specificity</td>
<td>Not useful for monitoring abstinence</td>
</tr>
</tbody>
</table>
| Carbohydrate deficient transferrin | An indirect metabolite of ethanol  
A marker of long-term, heavy alcohol use (≥40 g/day for up to 2 weeks) or relapse | 2-3 wks in serum/plasma          |
| Phosphatidylethanol (PEth)    | Formed directly after alcohol intake via the enzyme phospholipase D from phosphatidylcholine (PC) in the presence of alcohol | 1-2 wks or longer                |
**Urine EtG**

**Alcohol Detection Timeline***

- **LARGE**
  - 10+ Drinks

- **MEDIUM**
  - 5–9 Drinks

- **SMALL**
  - 2–4 Drinks

**HOURS AFTER DRINKING EVENT**

- 12
- 24
- 36
- 48
- 60
- 72
- 80

**Alcohol Detected**  **Alcohol Not Detected**

* Based on a positive cut-off of 100 ng/mL. A cutoff of 500 ng/mL is recommended in criminal justice settings.

**Cutoff level for EtG confirmation may be 100 ng/mL, 250 ng/mL, 500 ng/mL or 1000 ng/mL. EtS confirmation cut-off level is 75-100 ng/mL.**
What Cut-Off to Use?

“High” positive (e.g., >1,000 ng/mL) may indicate: – Heavy drinking on the same day or previously (e.g., previous day or two). – Light drinking the same day.

“Low” positive (e.g., 500–1,000 ng/mL) may indicate: – Previous heavy drinking (previous 1–3 days). – Recent light drinking (e.g., past 24 hours). – Recent intense “extraneous” exposure (within 24 hours or less).

“Very low” positive (100–500 ng/mL) may indicate: – Previous heavy drinking (1–3 days). – Previous light drinking (12–36 hours). – Recent “extraneous” exposure.
Incidental exposure to alcohol-containing products such as mouthwash and hand sanitizers, etc.

Published data suggest 500 ng/mL would adequately exclude most instances of extraneous exposure, although, as noted, anecdotal reports suggest higher cut-off's may be needed.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Exposure</th>
<th>EtG ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouthwash</td>
<td>15 min</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Hand Sanitizer</td>
<td>15 min workday</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Hand Sanitizer–Healthcare</td>
<td></td>
<td>Max 770</td>
</tr>
<tr>
<td>Nyquil</td>
<td>1 0z QD 3days</td>
<td>Max 246</td>
</tr>
<tr>
<td>Non–EtOH Beer</td>
<td>Two 12 oz</td>
<td>Max 93</td>
</tr>
</tbody>
</table>

Urine EtG Concentrations Following “Incidental” Alcohol Exposure:
- 0.5 teaspoon of communion wine: 75 ng/mL @ 9 hours
- Purell hand sanitizer: > 40 ng/mL @ 3 hours
Ethyl glucuronide [EtG]
- Minor metabolite of EtOH
- Incorporated into hair

**Interpretation**
- **EtG ≥ 30 pg/mg**: Chronic Excessive consumption
  - 60 g or more per day over several months [4–5 drinks/day]
- **EtG ≤ 7 pg/mg**: Abstinence threshold

2014 SOHT Consensus Statement *FSI* [2014]
R Kronstrand et al *FSI* [2012]
Beer Shampoo
<table>
<thead>
<tr>
<th>Product</th>
<th>EtOH (vol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haarwasser mit echter Birke (Florena)</td>
<td>41.0</td>
</tr>
<tr>
<td>Petrol Hahn: Lotion Toxic Force: White Hair (Eugene Perma)</td>
<td>29.9</td>
</tr>
<tr>
<td>Petrol Hahn: Lotion Force: Normal Hair (Eugene Perma)</td>
<td>28.0</td>
</tr>
<tr>
<td>Lotion capillaire (Auchan)</td>
<td>29.8</td>
</tr>
<tr>
<td>Alkmene (Brennessel)</td>
<td>39.9</td>
</tr>
<tr>
<td>Seborin (Schwarzkopf)</td>
<td>44.0</td>
</tr>
<tr>
<td>Birkin (Garnier)</td>
<td>31.2</td>
</tr>
<tr>
<td>Birkin Haarwasser (Schlecker)</td>
<td>3.5 *</td>
</tr>
<tr>
<td>Petrol Hahn: Hydratation (Eugene Perma)</td>
<td>31.2</td>
</tr>
</tbody>
</table>

* Mostly IPA

Ferreira et al For Sci Int 218:123 [2012]
Q: EtOH containing hair products impacting alcohol markers; EtG

- Gareri et al [2011] looked at 9 patients
  - EtOH concentrations in Product: 10 – 90 vol%
  - 1.9 – 23.5 pg/mg
  - Opined using ≥ 30 pg/mg cut-off no impact on conclusion

- Ferreira et al [2012] studied 7 volunteers
  - Rare, Social or heavy drinkers
  - Use Seborin product: 44 vol% EtOH
  - Tx right side of scalp; Collected from both sides
  - No indication of increase in EtG on Tx side of scalp hair
Poll Questions
“Legal” Drugs
Novel Psychoactive Substances

- Spice/K2: Synthetic Cannabinoids
- Bath Salts: Syn Cathinones
- “Fake LSD”: NBOMEs
- Std urine or hair tests will not detect
  - Limited # Labs
  - Limited #Analytes
Analytical Challenges

- **Synthetic Cannabinoids [aka] K2, Spice etc**
  - Developmental stage for detection in blood
    - Blood concentrations in the < 1 ng/ml range
      - Lower than THC
      - LC/MS\(^x\) technology needed for these concentrations
    - Urine assay not being developed – too many common metabolites for specific drug identification and full kinetics still to be worked out
  - Well over 450 + compounds have been made
    - RFSC: +25 analogs of JWH, AM, RCS, CP, UR 144, XLR–11, FUBINACAs, CHMINACAs and HU compounds identified in Illicit Drug Lab
Analytical Challenges

- New and more potent drugs: eg NBOMEs
  - Potent = lower concentrations
    - Enhanced sensitivity of instrumentation
      - GC/MS – Dean Switch
      - LC/MS
  - Availability of Drug Standards
    - Identification and quantitation

- Substituted Cathinones [aka “Bath Salts”]
  - Directed analyses–urine/blood
    - MDPV
    - Methylone
THANK YOU FOR YOUR ATTENTION

FINALLY OVER!