Hair Analysis for Drugs:
Cut-off Concentrations
Analytes
Stability

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Drug Testing Advisory Board
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Overview

• Proposed guidelines
  ◦ Cut-off concentrations
  ◦ Analytes
  ◦ Drug stability

• Further considerations for program
Rates of ED visits per 100,000 population involving illicit drugs, 2011

- Cocaine: 162
- Marijuana: 146
- Heroin: 83
- Amphetamines/methamphetamines: 51
- PCP: 24
## Proposed guidelines: Immunoassay / Screening

<table>
<thead>
<tr>
<th>Drug</th>
<th>Recommended cut-off (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DTAB 2004</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>300</td>
</tr>
<tr>
<td>Opiates</td>
<td>200</td>
</tr>
<tr>
<td>Cocaine</td>
<td>500</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>500</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>1</td>
</tr>
<tr>
<td>Methadone</td>
<td></td>
</tr>
<tr>
<td>Buprenorphine</td>
<td></td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td></td>
</tr>
</tbody>
</table>
Proposed screening cut-off concentrations

- Opiates
- Cocaine
- Amphetamines
- Cannabinoids

**pg/mg**

- DTAB 2004
- EWDTS 2010
- SOHT 2012

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Considerations for immunoassay: Cocaine

![Bar chart showing the percentage of cocaine, BZE, CE, and norcocaine in urine and hair samples.]

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Considerations for immunoassay: Heroin
Targeted immunoassay screens

• Basic drugs:
  ◦ Incorporate well into hair
  ◦ Parent compound (e.g. cocaine) incorporated to greater extent than metabolites (e.g. BZE)
  ◦ So immunoassay must target cocaine, OR, if urine immunoassay used, degree of conversion of cocaine to BZE in method must be measured

  ◦ 6-AM in higher concentration than morphine
  ◦ Immunoassay should target 6-AM, OR, degree of conversion to morphine must be measured
Considerations for immunoassay: Cannabinoids

![Bar chart showing cannabinoid levels in urine and hair](image-url)
Targeted immunoassay screens

- **Acidic drugs:**
  - *Do not incorporate well* (e.g., marijuana)
  - *THC in higher concentration in hair than metabolite*
  - *Why not use an immunoassay targeted to THC?*
  - *Confirmatory procedure would identify metabolite THC-COOH*
<table>
<thead>
<tr>
<th>Drug</th>
<th>Recommended cut-off (pg/mg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DTAB 2004</td>
</tr>
<tr>
<td></td>
<td>Federal Register</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>PCP: 300</td>
</tr>
<tr>
<td>Opiates</td>
<td>MOR, COD, 6-AM: 200</td>
</tr>
<tr>
<td></td>
<td>MOR, COD, 6-AM: 200</td>
</tr>
<tr>
<td></td>
<td>MOR, COD, 6-AM: 200</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Cocaine: 500</td>
</tr>
<tr>
<td></td>
<td>BZE, Norcocaine, CE: 50</td>
</tr>
<tr>
<td></td>
<td>Cocaine: 500</td>
</tr>
<tr>
<td></td>
<td>BZE, Norcocaine, CE, EME: 50</td>
</tr>
<tr>
<td></td>
<td>Cocaine: 500</td>
</tr>
<tr>
<td></td>
<td>BZE, Norcocaine, CE, EME: 50</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>AMP, METH, MDMA, MDA, MDEA: 300</td>
</tr>
<tr>
<td></td>
<td>AMP, METH, MDMA, MDA, MDEA: 200</td>
</tr>
<tr>
<td></td>
<td>AMP, METH, MDMA, MDA, MDEA: 200</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>THC-COOH: 0.05</td>
</tr>
<tr>
<td></td>
<td>THC: 50</td>
</tr>
<tr>
<td></td>
<td>THC-COOH: 0.2</td>
</tr>
<tr>
<td>Methadone</td>
<td>Methadone: 200</td>
</tr>
<tr>
<td></td>
<td>EDDP: 50</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Buprenorphine: 10</td>
</tr>
<tr>
<td></td>
<td>Nor-BUP: 10</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Bromazepam, Nordiazepam, Oxazepam, Lorazepam, Alprazolam, Diazepam, Flunitrazepam: 50</td>
</tr>
</tbody>
</table>
Proposed confirmatory cut-off concentrations

- MOR
- COD
- 6-AM
- COC
- BZE
- NC
- CE
- EME
- AMP
- METH
- MDMA
- MDA
- MDEA
- THC
- THC-COOH

[Chart showing proposed confirmatory cut-off concentrations for various substances, with bars for DTAB 2004, EWDTS 2010, and SOHT 2012.]
Are proposed cut-offs appropriate?
Phencyclidine

- Only North America suggests inclusion
- Hair analysis for drugs of abuse. XVII. Simultaneous detection of PCP, PCHP, and PCP-diol in human hair for confirmation of PCP use.

- 8 PCP users
- Positive: 330 – 14,000 pg/mg
- Minor metabolites also detected in lower concentration

- Suggested cut-off appears appropriate
Cocaine

- Good agreement between professional societies
- Detection of metabolites mandated
- However, benzoylecgonine (& EME) not indicative of ingestion due to “in vitro” cocaine degradation
- Cocaethylene and norcocaine were initially thought to be indicative of use, but are apparently present in street cocaine
- Some discussion of m-OH and p-OH BZE metabolites
Cocaine users

- Determination of opiates and cocaine in hair using automated enzyme immunoassay screening methodologies followed by GC-MS confirmation

- Authentic samples from cocaine users (n=103)
- GC/MS confirmation results:
  - Cocaine: 100 – 21,370 pg/mg  \textbf{Mean: 2,610}
  - BZE: 30 – 10,510 pg/mg  \textbf{Mean: 1,110}
  - CE: 50 – 1260 pg/mg  \textbf{Mean: 270}

- Users have high cocaine concentrations in hair
**Self report of cocaine use**

- Hair testing and self-report of cocaine use

*Proposed cut-offs will identify cocaine users*
Amphetamines

- Some agreement between professional societies
  Incorporation of methamphetamine and amphetamine in human hair following controlled oral methamphetamine administration

- 7 volunteers: METH administration at low and high doses
  **Maximum detected concentrations:**
  - METH: 600 – 3500 pg/mg; AMP 100 – 300 pg/mg (low dose)
  - METH 1200 – 5300 pg mg; AMP 200 – 500 pg mg (high dose)
  - AMP/MAMP ratio: Mean: 0.15; Median: 0.13

  - **Proposed cut-off: 300pg/mg; At least 50pg/mg of AMP if 300pg/mg METH (0.16)**

  - **Suggested cut-off appears reasonable for METH with AMP metabolite**
MDMA in Hair

- Simultaneous analysis of six amphetamines and analogues in hair, blood and urine by LC-ESI-MS/MS. Application to the determination of MDMA after low ecstasy intake.

- Detection of MDMA in a forensic case
- Single administration of ecstasy to a 16-year-old female without her knowledge during a party
- Hair collected 60 days after incident
- Hair positive for MDMA (22 pg/mg); no MDA
Amphetamines

- DTAB proposals higher than other societies
  - Discussion of lower threshold values
- METH with AMP as a metabolite appears appropriate
- If MDMA/MDA and MDEA are included in test profile, consider a lower cut-off
- Consider including ratio requirement for MDMA/MDA
THC

- Limited agreement between professional societies
- Parent THC in higher concentration than metabolite (similar to oral fluid analysis)
- Screening for parent THC proposed in other organizations
- Consensus that THC-COOH minimizes claim of passive exposure
- Consider allowing THC screen with THC-COOH confirmation
Opiates

- Good agreement between professional societies
- Detection of 6-AM identifies heroin usage
- Distinct advantage over urine testing
  - Opiate concentrations in hair from subjects in a controlled heroin-maintenance program and from opiate-associated fatalities
  - 46 individuals tested – all different hair colors
    - 100% positive for 6-AM; 89% positive for morphine

Suggested cut-off appears appropriate
What about other analytes?

Currently, additional drugs being considered for other matrices, so potential addition of:

- Hydrocodone
- Hydromorphone
- Oxycodone
- Oxymorphone

What data on these drugs in hair is available?
Drug concentrations in hair following self-reported heroin use

Moore et al. JAT 2006 30; 353-359
Drug concentrations in hair following self reported codeine use

- **Codeine**
- **HYC**

Subject took both drugs daily

<table>
<thead>
<tr>
<th>pg/mg</th>
<th>Daily codeine dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
</tr>
<tr>
<td>3</td>
<td>2000</td>
</tr>
<tr>
<td>4</td>
<td>2500</td>
</tr>
<tr>
<td>5</td>
<td>3000</td>
</tr>
</tbody>
</table>

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Heroin / Codeine

Heroin:
- 9 self-reported heroin users: 8 provided hair specimens positive for MOR and 6-AM (89%) (9th subject once a month use)
- 6 samples also contained COD, 5 of those had HYC and 2 HYM

Codeine:
- 5 subjects reported daily codeine intake
- MOR not detected in any hair specimens, but both COD and HYC were present

Summary:
- Presence of MOR suggests heroin or morphine intake, not codeine
- Apparent linear relationship between reported frequency of heroin use and morphine and 6-AM concentrations in hair
Oxycodone / Oxymorphone

- Notable paucity of information and literature

- Oxycodone in hair PubMed: 8 citations
  - None discuss concentrations from users

- Oxymorphone in hair PubMed: 4 citations
  - 2 to do with cats/dogs (surgery)
  - 1 was general analytical screen
  - One discussed real patients, but focused on other pain medications (tramadol, fentanyl)
2007 Wayne County, MI: Post-mortem hair specimens

- All cases had narcotic paraphernalia at the scene, or
- History of previous drug overdose, or
- Witnessed drug use

Target cases:
- **Acute** drug intoxication suspected
- Hair samples were collected from root end
## Case 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Heart Blood (mg/L)</th>
<th>Hair (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>&lt;0.025</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>BZE</td>
<td>2.9</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>CE</td>
<td>ND</td>
<td>1041</td>
</tr>
<tr>
<td>Norcocaine</td>
<td>NA</td>
<td>532</td>
</tr>
<tr>
<td><strong>Oxycodone</strong></td>
<td><strong>0.066</strong></td>
<td><strong>2079</strong></td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>ND</td>
<td>2231</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>ND</td>
<td>261</td>
</tr>
</tbody>
</table>

**Other PM Findings:**
- Urine: EME, COC, Diltiazem, Levamisole, Clonidine, Oxycodone
- **Cause of Death:** Cocaine use
- **Manner of Death:** Accident
### Case 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>Liver (mg/kg)</th>
<th>Hair (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>6.4</td>
<td>541</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>0.67</td>
<td>ND</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>279</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>NA</td>
<td>1236</td>
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<tr>
<td>Propoxyphene</td>
<td>ND</td>
<td>375</td>
</tr>
</tbody>
</table>

Other PM Findings: None
Cause of Death: Oxycodone Intoxication
Manner of Death: Accident
## Case 12

<table>
<thead>
<tr>
<th>Drug</th>
<th>Spleen (mg/kg)</th>
<th>Hair (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>ND</td>
<td>977</td>
</tr>
<tr>
<td>BZE</td>
<td></td>
<td>229</td>
</tr>
<tr>
<td>Morphine</td>
<td>0.78</td>
<td>288</td>
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<tr>
<td>Codeine</td>
<td>0.084</td>
<td>86</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>ND</td>
<td>82</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>ND</td>
<td>704</td>
</tr>
<tr>
<td>6-AM / 6-AC</td>
<td>ND</td>
<td>865 / 76</td>
</tr>
</tbody>
</table>

Other PM Findings: None  
Cause of Death: Opiate Use  
Manner of Death: Accident
## Summary of Results – 14 Cases

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Traditional PM Samples</th>
<th>Hair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine / BE / CE</td>
<td>3 / 4 / 0</td>
<td>12 / 12 / 4</td>
</tr>
<tr>
<td>Morphine / 6-AM</td>
<td>8 / 5</td>
<td>10 / 11</td>
</tr>
<tr>
<td><strong>Codeine / HYC</strong></td>
<td><strong>7 / 4</strong></td>
<td><strong>10 / 8</strong></td>
</tr>
<tr>
<td>Oxycodone</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Methadone</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tramadol / PPX / Carisoprodol</td>
<td>1 / 0 / 0</td>
<td>1 / 1 / 1</td>
</tr>
</tbody>
</table>
Additional drugs

- Summary of post-mortem cases shows OXYC, OXYM, HYC, HYM detected in hair, often when more traditional specimens were negative

- Should be relatively straight-forward to include in workplace drug testing program
Drug stability in hair

- Several papers state that hair can be “stored for years”
- No supportive data
- Some conditions listed:
  - Cool
  - Dark
  - No plastic bags
  - No refrigeration
- Surprising lack of literature
Other areas for DTAB discussion

1. Extraction efficiency from authentic hair specimens (solid matrix, different issues)
2. Extent of drug conversion during extraction procedure (BZE, 6-AM)
3. Drug stability in storage and during routine transportation
4. Addition of other analytes
1. Extraction efficiency

- Many different published procedures
- Drug recovery from authentic hair
- Point of diminishing returns

![Graph showing % recovery over time for AMP, METH, PCP, COC, BZE](Image)

1 hour, 2 hours, 3 hours
2. Drug conversion

- Cocaine is converted to BZE in many methods (sometimes deliberately to use a BZE immunoassay targeted screen)
- 6-AM may convert to morphine
- Degree of conversion obviously critical if interpretative results are based on drug-metabolite ratio / concentration
3. Drug stability

- Demonstrate drug stability in various storage conditions
- Demonstrate drug stability during transportation

- Re-analysis of collected specimens
  - Brings segmental analysis into the discussion
  - Is re-analysis of “same hair” or hair extract?
  - Is a different “segment” analyzed?
4. Addition of other analytes

As discussed earlier, to harmonize with other matrices:

- MDMA
- MDA
- MDEA
- Oxycodone
- Oxymorphine
- Hydrocodone
- Hydromorphone
Summary

- Proposed cut-offs seem appropriate for PCP, opiates and cocaine
- Amphetamines and cannabinoids may require further discussion
- Expand analyte list to be in harmony with other matrices
- Drug stability in hair needs far more research
- Good alternative or addition to urine/oral fluid
- Provides different information and should be used depending on circumstances for testing
- Method validation may have different requirements to urine and oral fluid
Thank you......