

Hair Analysis for Drugs:
Cut-off Concentrations
Analytes
Stability

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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

Drug	Rate of ED visits per 100,000 population
Cocaine	162
Marijuana	146
Heroin	83
Amphetamines/methamphetamines	51
PCP	24

Proposed Guidelines: Immunoassay / Screening

- Recommended cut-off (pg/mg)

Drug	DTAB 2004	EWDTS 2010	SOHT 2012
Phencyclidine	300	--	--
Opiates	200	200	200
Cocaine	500	500	500
Amphetamines	500	200	200
Cannabinoids	1	50	50
Methadone	--	--	200
Buprenorphine	--	--	10
Benzodiazepines	--	50	--

Proposed screening cut-off concentrations

Drug	DTAB 2004, pg/mg	EWDTS 2010, pg/mg	SOHT 2012, pg/mg
Opiates	200	200	200
Cocaine	500	500	500
Amphetamines	500	200	200
Cannabinoids	1	50	50

Considerations for immunoassay: Cocaine

Drug	Urine, %	Hair,%
Cocaine	5	60
BZE	90	25
CE	0	10
Norcocaine	0	5

Considerations for immunoassay: Heroin

Drug	Urine, %	Hair, %
Heroin	0	10
6-AM	5	50
Morphine	10	35
M-3-g, M-6-g	85	2

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

Drug	Urine, %	Hair, %
THC	2	75
11-OH-THC	7	5
THC-COOH	91	20

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*

Mass Spectrometry /Confirmation

- Recommended cut-offs (pg/mg)

Drug	DTAB 2004 Federal Register	EWDTs 2010 DTA 2(8): 367-376	SOHT 2012 FSI 218: 20 - 24
Phencyclidine	PCP: 300	--	--
Opiates	MOR, COD, 6-AM: 200	MOR, COD, 6-AM: 200	MOR, COD, 6-AM: 200
Cocaine	Cocaine: 500 BZE, Norcocaine, CE: 50	Cocaine: 500 BZE, Norcocaine, CE, EME: 50	Cocaine: 500 BZE, Norcocaine, CE, EME: 50
Amphetamines	AMP, METH, MDMA, MDA, MDEA: 300	AMP, METH, MDMA, MDA, MDEA: 200	AMP, METH, MDMA, MDA: 200
Cannabinoids	THC-COOH: 0.05	THC: 50 THC-COOH: 0.2	THC : 50 THC-COOH: 0.2
Methadone	--	--	Methadone: 200 EDDP: 50
Buprenorphine	--	--	Buprenorphine: 10 Nor-BUP: 10
Benzodiazepines	--	Bromazepam, Nordiazepam, Oxazepam, Lorazepam, Alprazolam, Diazepam, Flunitrazepam: 50	--

Proposed confirmatory cut-off concentrations

Drug	DTAB 2004, pg/mg	EWDTS 2010, pg/mg	SOHT 2012, pg/mg
MOR	200	200	200
COD	200	200	200
6-AM	200	200	200
COC	500	500	500
BZE	50	50	50
NC	50	50	50
CE	50	50	50
EME	--	50	50
AMP	300	200	200
METH	300	200	200
MDMA	300	200	200
MDA	300	200	200
MDEA	300	200	200
THC	--	50	50
THC-COOH	0.5	20	20

***Are proposed cut-offs
appropriate ?***

Phencyclidine

- *Only North America suggests inclusion*
- *Nakahara et al. 1997 J Anal Toxicol. 21(5):356-62.*
- *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

- *Lachenmeier et al. 2006 Forensic Sci. Int. 159(2-3):189-99*
- *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 **Mean: 2,610***
- *BZE: 30 – 10,510 pg/mg **Mean: 1,110***
- *CE: 50 – 1260 pg/mg **Mean: 270***
- *Users have high cocaine concentrations in hair*

Self report of cocaine use

- *Vignali et al. 2012 Forensic Sci. Int. 215: 77-80.*
- *Hair testing and self-report of cocaine use*
- *Proposed cut-offs will identify cocaine users*

Use	African heritage, mg/pg	Caucasian heritage, mg/pg
Daily use COC	60740	39820
BZE	6010	5690
2-4x/week COC	33420	18960
BZE	4050	2610
1-2x/month COC	14980	3990
BZE	1830	560

Amphetamines

- *Some agreement between professional societies*
- *Polettini et al. 2012 Anal Chim Acta. 726:35-43*
- *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

- *Cheze et al. 2007 Forensic Sci Int. 170(2-3):100-4.*
- *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*
 - *Musshoff et al. 2005 J Anal Toxicol 29(5): 345-52*
 - *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*

Drug	Mean, pg/mg	Median, pg/mg
6-AM	1240	1460
Morphine	930	1020

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 are available?*

Drug concentrations in hair following self reported heroin use

- *Moore et al. JAT 2006 30; 353-359*

Frequency per week	COD	MOR	6-AM	HYC	HYM
0.25	--	--		155	--
1	339	1379	1758	764	--
1	2197	2501	932	--	--
2	285	734	667	135	--
3	--	570	831	--	--
4	841	2006	1622	130	--
4	--	399	684	--	--
7	1958	9160	9925	202	268
14	5743	15206	7623	161	504

Drug concentrations in hair following self reported codeine use

Dose (mg/day)	Codeine, pg/mg	HYC, pg/mg
500	6516	592
500	19489	15933
1000	20543	15852
2000	851	4019
3000	575	3150

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

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

Drug	Heart Blood (mg/L)	Hair (pg/mg)
Cocaine	<0.025	>10,000
BZE	2.9	>10,000
CE	ND	1041
Norcocaine	NA	532
Oxycodone	0.066	2079
Hydrocodone	ND	2231
Alprazolam	ND	261

Case 3

- *Other PM Findings: None*
- *Cause of Death: Oxycodone Intoxication*
- *Manner of Death: Accident*

<i>Drug</i>	<i>Liver (mg/kg)</i>	<i>Hair (pg/mg)</i>
<i>Morphine</i>	<i>6.4</i>	<i>541</i>
<i>Hydrocodone</i>	<i>0.67</i>	<i>ND</i>
<i>Oxycodone</i>	<i>279</i>	<i>>10,000</i>
<i>Oxymorphone</i>	<i>NA</i>	<i>1236</i>
<i>Propoxyphene</i>	<i>ND</i>	<i>375</i>

Case 12

- *Other PM Findings: None*
- *Cause of Death: Opiate Use*
- *Manner of Death: Accident*

Drug	Spleen (mg/kg)	Hair (pg/mg)
Cocaine	ND	977
BZE	ND	229
Morphine	0.78	288
Codeine	0.084	86
Oxycodone	ND	82
Hydrocodone	ND	704
6-AM / 6-AC	ND	865 / 76

Summary of Results – 14 Cases

Drug Class	Traditional PM Samples	Hair
<i>Cocaine / BE / CE</i>	3 / 4 / 0	12 / 12 / 4
<i>Morphine / 6-AM</i>	8 / 5	10 / 11
Codeine / HYC	7 / 4	10 / 8
Oxycodone	2	6
<i>Benzodiazepines</i>	5	6
<i>Methadone</i>	1	3
<i>Fentanyl</i>	4	4
<i>Tramadol / PPX / Carisoprodol</i>	1 / 0 / 0	1 / 1 / 1

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*

Drug	1 hour, % recovery	2 hours, % recovery	3 hours, % recovery
AMP	64.5	86.6	89
METH	70	83.4	86
PCP	50	77	85
COC	47	75	83
BZE	61	88	94

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*
 - *Oxymorphone*
 - *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.....