

Practical Aspects of Drug Testing in Human Hair: Laboratory Perspective

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Overview of Collection Process

Hair Collection

- Site of Collection - Occipital Crown / Posterior Vertex
- Cut with Scissors in 2-3 locations close to the scalp
- Hair aligned in collection container root and distal ends

Hair Collection Challenges

- No head hair
- Body Hair
- Weaves
- Short Head Hair
- Contamination Claims
- Variability cutting from the scalp

Preparing Hair Collection Kit

- Alcohol prep used to clean scissors
- Hair collected from at least 3 regions occipital crown area
- Root ends aligned in the foil
- Foil placed inside the cardboard envelope
- Cardboard envelope initialed, dated and sealed
- Test request form/ chain of custody filled out
- Envelope placed into plastic bag
- Test request form placed in outer pocket of plastic bag
- Plastic bag sealed
- Specimen shipped to the laboratory

Overview of Accessioning Process

Overview

- Sample arrived at the lab
- Sorting process
 - Hair
 - Urine/Oral fluid
- Accessioning process
 - Hair – cut/weighed

Hair Accessioning Requirements

- Ideal to have Separate Area Isolated
- Each Station:
 - Scissors
 - Cleaning Solution for Scissors
 - Scale
 - Sharpie
 - Disposable Pads
- Accurate Scales Needed to Weigh hair
- Humidity Control (Reduce static electricity)

Aliquotting of Hair

- Hair cut at 3.9 cm from root end
- 3.9 cm segment of hairs cut into 2-5 mm segments
- Cut hair mixed for homogeneity
- 20 mg weighed
 - 1st aliquot: ELISA screen
 - 2nd aliquot: GC/MS and/or GC/MS/MS confirm

Pre-Analytical Process

Pre-Analytical Process

- Not a liquid Matrix
- Pre-Analytical Process Needed Prior to:
 - Screening by Immunoassay
 - Extraction for GC/MS, GC/MS/MS or LC/MS/MS
- Pre-Analytical Process should include Wash Procedure(s) to remove sources of external contamination
- Two main approaches
 - Digestion of the hair matrix to a liquid
 - Pre-Extraction of the Drug dissolved in a liquid
- Challenges
 - Drugs and metabolites must remain intact
 - Extraction or Digestion Efficiency
 - Time – Balance between Efficiency and TAT

Hair Confirmation: Extractions

- Cocaine and Amphetamines
 - 4 washes
 - Dilute Acid + Heat Extraction
- Opiates
 - 4 washes
 - Methanol/Buffer/KOH Digestion
- PCP
 - 4 washes
 - Methanol + Heat Extraction
- THC/THCA
 - 4 washes
 - NaOH + Heat Digestion
- All Drugs: SPE

Extraction

Extraction

- After Pre-analytical Process Extraction is similar to other matrices
- Solid Phase Extraction
- Liquid-Liquid
- Derivatization
- Deuterated Internal Standards
- Calibration Curve
- Quality Controls

Confirmation Technology

- GC/MS
- GC/GC/MS
- GC/MS/MS
- LC/MS
- LC/MS/MS

Drugs/ Metabolites Tested & Cut-Off Values in Hair

Quest Diagnostics

DRUG CLASS	INITIAL TEST LEVEL	CONFIRMATORY LEVEL
AMPHETAMINES	300 pg/mg	--
Amphetamine	--	300 pg/mg
Methamphetamine	--	300 pg/mg
MDA	--	300 pg/mg
MDMA	--	300 pg/mg
COCAINE/METABOLITES	300 pg/mg	
Benzoyllecgonine	--	300 pg/mg
Cocaine	--	300 pg/mg
Cocaethylene	--	300 pg/mg
Norcocaine	--	300 pg/mg
MARIJUANA	1.0 pg/mg	--
THC Metabolite	--	0.1 pg/mg
OPIATES	500 pg/mg	--
Morphine	--	500 pg/mg
Codeine	--	500 pg/mg
6-Acetylmorphine (6-AM)	--	500 pg/mg
Hydrocodone*	--	500 pg/mg
Hydromorphone*	--	500 pg/mg
Oxycodone*	--	500 pg/mg
Oxymorphone*	--	500 pg/mg

Omega Laboratories

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Benzoyllecgonine	--	500 pg/mg
Cocaine	--	500 pg/mg
Cocaethylene	--	500 pg/mg
Norcocaine	--	500 pg/mg
MARIJUANA	1.0 pg/mg	--
THC Metabolite	--	0.3 pg/mg
OPIATES	300 pg/mg	--
Morphine	--	300 pg/mg
Codeine	--	300 pg/mg
6-Acetylmorphine (6-AM)	--	300 pg/mg
Hydrocodone*	--	300 pg/mg
Hydromorphone*	--	300 pg/mg
Oxycodone*	--	300 pg/mg
Oxymorphone*	--	300 pg/mg

Drug Test Results in Hair vs. Urine

Combined Hair + Urine Profile

- Hair and Urine collected
- Urine 7-panel
 - THC, Cocaine, Amps, Opiates and PCP
 - Barbs and Ethanol
- Hair 5-panel
 - THC, Cocaine, Amps*, Opiates and PCP
 - *MDMA and MDA
- N = ~ 7,000 samples

Reason For Test

Reason	Positive, #	Positive, %
NM	932	12.9
NR	38	0.5
OT	557	7.7
PA	44	0.6
PE	5066	70.3
RA	471	6.5
RC	54	0.7

Marijuana

Specimen type	Positive specimens, #	Positive specimens, %
Hair + Urine	202	2.8
Hair	164	2.3
Urine	84	1.2
Hair, total	366	5.1
Urine, total	286	4.0

Amphetamines

Specimen type	Positive specimens, #	Positive specimens, %
Hair + Urine	173	2.4
Hair	335	4.6
Urine	23	0.3
Hair, total	508	6.1
Urine, total	196	2.7

Cocaine

Specimen type	Positive specimens, #	Positive specimens, %
Hair + Urine	87	1.2
Hair	227	3.8
Urine	3	0.04
Hair, total	364	5.0
Urine, total	90	1.3

Total : All Drug Categories (Combined Hair + Urine Profile)

Specimen type	Positive specimens, #	Positive specimens, %
Hair + Urine	471	6.5
Hair	763	10.6
Urine	219	3.1
Hair, total	1234	17.1
Urine, total	690	9.6

Conclusions

- Drug Positive Rates: Higher in Hair v. Urine
- Most Dramatic Differences : Cocaine and Methamphetamine
 - Pharmacokinetics
 - Drug Use Patterns
- Combination of Both Matrices: Highest Detection Rate

Interpretation Issues

Two Major Roadblocks: Interpretation????

- EXTERNAL CONTAMINATION
 - Use or Exposure?
 - Many claims of exposure
 - Marijuana
 - Cocaine
 - Hair Positives in Children
 - Published studies showing Contamination
 - Approaches to Combat
 - Wash Procedures
 - Metabolite Ratios
- HAIR COLOR BIAS
 - Melanin Content
 - Positives in Gray Hair
 - Published studies showing Bias
 - Approaches to combat claims
 - Statistics
 - Procedural

Methamphetamine Exposure Results

- 80 specimens: Methamphetamine only (no metabolite detected)
- 3 specimens: Amphetamine only
- 14 specimens:
 - Methamphetamine Detected
 - Amp < 50 pg/mg
- 20 specimens:
 - Methamphetamine > 300 pg/mg and
 - Amphetamine > 50 pg/mg

20 “Exposure” Specimens: Methamphetamine “Reportable”

- 2 specimens
 - 5 month and 7 month old
 - Prenatal Exposure?
- 15 specimens
 - Ages 1-5 (8- 2 year olds)
 - Passive Ingestion?
- 1 specimen
 - 7 year old
- 2 specimens
 - 11 and 12 year old
 - Drug Use?

Methamphetamine Adult Use Results

- 110 specimens met reporting criteria
- 6 specimens: amphetamine < 50 pg/mg
- 1 specimen: no amphetamine detected

Methamphetamine parent/metabolite ratios: Adults vs. Children (Exposure)

Group data	Methamphetamine	Amphetamine
Children	--	--
Mean (pg/mg)	979	27
Range	19 – 10,598	0 - 398
n	114	114
Ratio	36:1	--
Adults	--	--
Mean (pg/mg)	6944	663
Range	204 – 62,170	0 – 8,083
n	117	117
Ratio	10:1	--

Methamphetamine in Hair: Adults vs. Children

Group	METH, pg/mg	AMP, pg/mg
Adults	6944	663
Children	979	27

Marijuana (THC) Exposure

- 126 requests for directed analysis for THC in children's hair (01/03-06/03)
- 104 samples positive for THC
- Mean age = 4.15 years
- Age range (3 months to 16 years)

Marijuana (THC) Exposure Results

- 94 specimens: THC only (no metabolite detected)
- 1 specimen:
 - THC < 5.0 pg/mg
 - THCA > 0.1pg/mg
- 4 specimens:
 - THC Detected
 - THCA < 0.10 pg/mg
- 5 specimens:
 - THC > 5.0 pg/mg and
 - THCA > 0.1 pg/mg

5 “Exposure” Specimens: Marijuana “Reportable”

- 2 Specimens
 - 14 year old male and 16 year old female
 - Drug Use?
- 3 Specimens
 - Ages 1-3
 - High Parent THC concentrations
 - Passive Ingestion?
 - 424.9/0.36; 188.5/0.10; 143.6/0.26

Marijuana (THC) Adult Use Results

- 88 specimens met reporting criteria
- 15 specimens: no THC metabolite detected
- 1 specimen: THC metabolite below reporting cut-off

Interpretation ??????

- How many times did the person use in the last 90 days?
- Is the use decreasing, increasing or remaining the same?
- The hair test was negative but the person admits to taking X?
- The hair test was positive but the person says “no way...I don’t do drugs”?
- Could “Y” cause a positive result for “X”?
- The person collecting my hair contaminated it with drug...?
- How long does “X” stay in body hair? What’s the timeline?

Marijuana parent/metabolite ratios: Adults vs. Children (Exposure)

Group data	THC	THCA
Children	--	--
Mean (pg/mg)	33.6	0.04
Range	0.5 – 464.9	0 – 2.5
n	104	104
Ratio	840:1	--
Adults	--	--
Mean (pg/mg)	98.8	1.6
Range	0 – 643.2	0 – 17.0
n	104	104
Ratio	62:1	--

Marijuana in Hair: Adults vs. Children

Group	THC, pg/mg	THCA, pg/mg
Adults	98.8	1.64
Children	33.6	0.04

Thank you. Questions?