General Approach to Hair Analysis

1 Introduction

Drugs and poisons in the bloodstream can be incorporated into the keratin matrix of hair through the root. This can make hair a suitable forensic toxicological specimen for determining a history of drug or poison exposure. Because of the nature of hair as a forensic toxicological specimen, the window of detection after exposure may be wider than that of blood or urine. Since hair grows at an average rate of one centimeter per month, segmental hair analysis can be performed to approximate when the exposure occurred.

2 Scope

This document addresses sample preparation and analysis of hair specimens for the presence of drugs and poisons. Specific procedures for hair analysis can be found in the Toxicology Subunit Manual.

3 Principle

Samples are weighed, pulverized and extracted with methanol, or another appropriate solvent. The initial extracts are screened for the presence of a poison, drug or drug class. Positive specimens are subjected to a second sampling and extraction, when sample size permits. Wash steps may be incorporated to address the concern of exterior contamination. Segmentation and/or quantitation may be performed, when appropriate.

4 Specimens

4.1 Specimen Collection

Note: Although the examiner will not have complete control over the collection of hair samples for analysis, certain suggestions can and should be made to those individuals prior to collecting the specimens to be sent into the Laboratory, such as:

- Hair collectors should wait at least four weeks after an alleged exposure to a drug/poison to collect hair samples for toxicological testing.
- Hair samples collected for analysis should be cut as close to the scalp as possible, from the posterior vertex of the head, just above the neck.
- The diameter of the sample collected should be about the width of a jumbo pencil, or approximately 1 cm. A rubberband or twist tie should be placed securely around the cut end of the hair sample prior to cutting, to mark the proximal end of the bundle.
- A properly labeled, sealed white paper envelope is suggested for packaging the hair sample.
- Two samples should be collected and packaged separately to allow one specimen for testing by the FBI Laboratory, while a second is left untouched.

4.2 Specimen Requirements

Typically, head hair is the specimen of choice for screening. If unavailable, body hair may be analyzed.

By dividing the hair into discrete segments that represent growth over a particular time period (i.e., one centimeter lengths represent one month time periods), the time of an exposure of an individual to a drug may be estimated. This process is called segmental analysis. Of course, the longer the hair, the more historical information may be available. Segmental analysis is only appropriate when the cut end of the hair is known and will require larger amounts of hair to be collected.

5 Equipment/Materials/Reagents

Guidance for the preparation of reagents may be found in the *Preparation of Chemical Reagents* standard operating procedure (Tox 103).

a. Scissors

b. Ruler

c. Analytical Balance

d. Mini Beadbeater-8 Cell Disruptor, or equivalent (Biospec Products, Bartlesville, OK)

e. 2-mL Screw Cap Microtubes

f. Micro Stir Bars

g. 2.5-mm Glass Beads

h. Methanol (HPLC grade, or better)

i. Vortex Mixer

j. Heating/Stirring Block

k. Microcentrifuge

l. Disposable Syringe Filters (Anotop 25 Plus, 0.2 μm, or equivalent)

m. Disposable Syringes (2.5 cc, or equivalent)

n. 22-gauge hypodermic needles

o. Liquid nitrogen

p. 12 x 75 mm test tubes, disposable pipettes, and other common laboratory glassware

q. Deionized water

r. Evaporator with nitrogen

s. SPEX 6870 freezer/mill (cryogrinder), or equivalent (SPEX Sample Prep, Metuchen, NJ)

t. Cryogrinder tubes, or equivalent (SPEX Sample Prep, Metuchen, NJ)

6 Standards and Controls

a. Negative Control Hair:
Obtain in house. Store at room temperature. Stable indefinitely. A Negative Control sample will be extracted and analyzed with every analysis.

b. Positive Control Hair (any of the following appropriate controls may be used):

1. Prepared in house following the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101). Typically methanolic solutions are spiked into weighed portions of Negative Control Hair. Prepare fresh.

2. Standard Reference Material® 2379 (purchased from the National Institute of Standards & Technology Gaithersburg, MD) is drug-free human hair material that has been fortified with drugs and drug metabolites. It contains the following analytes at the listed concentrations: amphetamine (6.00 ng/mg), benzoylecgonine (4.01 ng/mg), cocaethylene (2.67 ng/mg), cocaine (7.45 ng/mg), methamphetamine (5.20 ng/mg), and phencyclidine (6.24 ng/mg). It can be used as a positive control for the listed analytes. Storage conditions and stability are determined by the manufacturer.

3. Standard Reference Material® 2380 (purchased from the National Institute of Standards & Technology Gaithersburg, MD) is drug-free human hair material that has been fortified with drugs and drug metabolites. It contains the following analytes at the listed concentrations: codeine (9.82 ng/mg), morphine (10.54 ng/mg), 6-monoacetylmorphine (2.71 ng/mg), and tetrahydrocannabinol (0.99 ng/mg). It can be used as a positive control for the listed analytes. Storage conditions and stability are determined by the manufacturer.

7 Calibration

Not applicable.

8 Sampling

Not applicable.

9 Procedure

a. Make note of approximate length of questioned hair sample, color, curvature, condition, and any other pertinent observations.

b. Thoroughly clean scissors with methanol. Cut a portion of the hair sample into fine snippets. (If segmental analysis is to be performed, cut the hair into 1-2 cm segments and analyze them individually.)

c. If washing of the hair is required, perform wash using an appropriate organic or aqueous solvent. A series of washes may be desirable. Typically, the last wash will be saved for possible analysis.

d. Weigh approximately 25 mg of the hair snippets into a microtube. Add 5 glass beads. If sample size permits, two separate aliquots of the hair may be weighed and analyzed. Process control samples in a similar manner.

   Alternatively, add a sample of cut hair into a cryogrinding tube. Process control samples in a similar manner.

e. Pulverize hair in the Beadbeater or cryogrinder.

f. For cryoground hair, weigh pulverized hair into a microtube.

g. Add stir bars to microtubes and incubate overnight in methanol or another appropriate solvent while stirring. Apply heat during this process, if appropriate.

h. Centrifuge microtubes. Remove methanol to a test tube. A hypodermic needle may be used for this process. Methanol extracts can be filtered at this point, if necessary.

i. Concentrate methanol extracts to dryness. Reconstitute extracts and further purify via liquid or solid phase extraction. (See *Toxicology Subunit Manual* for guidance.)

j. Analyze final extracts using appropriate instrumentation.

10 Instrumental Conditions

Not applicable.

11 Decision Criteria

Not applicable.

12 Calculations

Not applicable.

13 Uncertainty of Measurement

Not applicable.

14 Limitations

Not applicable.

15 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

16 References

Guidelines for Toxicological Quantiations (Tox 101); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

Preparation of Chemical Reagents (Tox 103); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

Society of Hair Testing. *For. Sci. Int.*, 2004, *145*, 83-84.

Mandatory Guidelines for Federal Workplace Drug Testing Programs, proposed. Substance Abuse and Mental Health Services Administration, 2006.

Welch, M.J.; Sniegoski, L.T.; Tai., S. *Anal. Bioanal. Chem.*, 2003, *376***,** 1205-1211.

FBI Laboratory Division Safety Manual.

FBI Laboratory Toxicology Manual.

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0	01/25/13	New document that replaces a previous document titled "*Extraction and Analysis of Drugs in Hair*".

Approval

Redacted - Signatures on File