Recommendations for Hair Testing in Forensic Cases

Society of Hair Testing

Introduction
On Tuesday October 7th 2003, representatives from 15 countries gathered in Heraklion, Crete, to discuss some issues involved in the analysis of hair for drugs of abuse. All representatives were either currently engaged in the analysis of hair samples in their own laboratories, or were conducting hair analysis through a third party laboratory facility.

The countries represented were as follows (in alphabetical order):
Canada, Chile, France, Germany, Greece, Italy, Luxembourg, Norway, Poland, Portugal, Spain, Sweden, Switzerland, United Kingdom, and the USA.

While the Consensus was discussed, there was no final result. The Board of the Society met, and agreed upon the wording of the Consensus in Sevilla, Spain, on January 24th 2004. They included the sections upon which the Assembly had agreed.

Areas for discussion

1. **Sampling, Shipping, Storage**
2. **Decontamination**
3. **Hair disintegration and extraction**
4. **Screening test**
5. **Criteria for mass spectrometric analysis**
6. **Specific Drug Classes**
   - **6.1. Opiates**
   - **6.2. Cocaine**
   - **6.3. Amphetamines**
   - **6.4. Cannabinoids**
7. **Internal Quality Control**
8. **External Quality Control**

1. *Sampling, Shipping, Storage*

These areas were addressed in a previous Consensus from the Society of Hair Testing
Forensic Science International 84 (1997) 3-6

- The sample should be cut from the posterior vertex region of the head, as close as possible to the scalp, since this is the region of least variation in growth rate. If not, the source of the sampling should be described. In general, head hair is estimated to grow at approximately 1.0 cm per month.
- The sampling does not need to be performed by a physician
- The colour, length, body site and any obvious cosmetic treatment of the hair should be recorded
- Root (proximal) and tip (distal) sections of the hair should be clearly defined
- If segmental analysis is required, a lock of hair must be fixed before cutting
- Head hair is the preferred specimen. Alternative hair (e.g. pubic, axillary) can be collected if head hair is unavailable
- The sample and any aliquots or extracts must be handled and stored in a manner so as to minimize degradation, loss of analyte, or contamination from other sources. Dry hair should be stored in the dark at room temperature.
- There must be adequate sample to allow initial testing, followed by confirmatory or re-testing of the sample if necessary. A lock of hair, with the thickness of a pencil, or several locks with the thickness of a straw is recommended.
- In post-mortem cases, hair should be collected at the beginning of an autopsy

2. Decontamination

When hair analysis is being used to identify drug use, the major limitation is external contamination, which if not removed, can confuse exposure with actual drug use. The issue of external contamination must be addressed through multiple methodologies and cannot be solved through the simple application of any single approach.

- Areas of possible contamination must be considered before and during the analysis. These may include, but are not limited to, external drug exposure and laboratory contamination
- A simple use of cut-off levels is insufficient because external contamination can be at any level.
- In general, a decontamination strategy must include an initial organic solvent, to remove oils, followed by aqueous washes.
- The washings should be stored for later analysis, if necessary
- In autopsy or exhumation cases, additional pre-treatment of the hair in the laboratory may be necessary, depending on the condition of the sample

3. Hair disintegration and extraction

- Different analytical procedures can produce different quantitative results. Each laboratory has the choice of disintegrating the hair matrix before extraction, or of extracting the drug directly from the solid hair after suitable preparation
- Degradation compounds may be produced during the assay. In order to assess the degree of conversion, the laboratory must include adequate controls

4. Screening Test

- If a screening test is used, an appropriate method validation including calibrators and controls in a hair matrix must be performed
- Analytes of interest must be identified to minimize false negatives

5. *Criteria for mass spectrometric analysis*

- The method must be validated according to good laboratory practice.
- The possible influence of the internal standard at low concentrations must be assessed and documented
- For information on valid criteria for mass spectrometric analysis, refer to recommended rules from scientific organizations or national guidelines

6. Specific Drug Classes

Drugs and metabolites in hair should be analyzed using valid methods. Detection levels will be different between drug classes. Some examples for specific drugs are presented below.

6.1 Opiates

- *Immunochemical test*:
  - A morphine or 6-acetylmorphine level of 0.2 ng/mg must produce a positive result
- *Chromatographic test*:
  - Recommended Limit of Quantification (LOQ): ≤ 0.2 ng/mg for each compound
  - Heroin consumption must be differentiated from codeine or morphine use by the presence of 6-acetylmorphine

6.2 Cocaine

- *Immunochemical test*:
  - A cocaine level of 0.5 ng/mg must produce a positive result
- *Chromatographic test*:
  - Recommended Limit of Quantification (LOQ): ≤0.5 ng/mg for cocaine, ≤0.05 ng/mg for other compounds
  - The chromatographic analysis should include cocaine, and at least one of the following: benzoylecgonine, cocaethylene, norcocaine or ecgonine methyl ester

6.3 Amphetamines

- *Immunochemical test*:
  - A concentration of 0.2 ng/mg of each substance must separately produce a positive result: MDMA, methamphetamine, amphetamine, MDEA or MDA
- *Chromatographic test*:
  - Recommended Limit of Quantification (LOQ): ≤0.2 ng/mg for each compound

Note: Laboratories should be aware of the possible ingestion of legal drugs producing positive results for methamphetamine and amphetamine

6.4 Cannabinoids

- *Immunochemical test*:
  - A THC concentration of 0.1 ng/mg must produce a positive result
- *Chromatographic test*:
  - Recommended Limit of Quantification (LOQ):
  - THC:≤ 0.1 ng/mg
  - THC-COOH:≤ 0.2 pg/mg
  - Confirmation of THC-COOH is required to definitively prove the use of cannabinoids

7. Internal Quality Control

Internal Quality Control for hair is more difficult than for other homogenous body fluids, since spiked control samples cannot substitute for the actual hair of a drug user. However, spiked controls may be substituted for hair from drug users if properly prepared.

- One technique is to expose drug-free hair to aqueous solutions of drugs at high concentrations, for several days and then thoroughly wash the hair before drying and analysis. When suitably homogenized, these spiked samples can be used for precision studies, routine QCs, and as internal degradation controls. Various hair types should be employed.
- Although controls may be homogenized pools, the substitution of properly prepared spiked controls is acceptable
- For endogenous drugs, controls may be prepared using an alternative medium, for example, synthetic melanin

8. External Quality Control

- For external quality control, the laboratory should enrol in a proficiency testing program, where **authentic standard** hair specimens are sent for testing
- The laboratory must analyze proficiency specimens in the same way as routine samples
- If a laboratory does not consistently perform adequately in external proficiency programs, corrective actions must be taken
- In the case of the Society of Hair Testing, reference laboratories will be named, and their results will be used as a reference method and result source
- The results of their analysis are collated and compared to those from selected reference laboratories