Appendix C
of the GTFCh Guidelines for Quality Control in Forensic-Toxicological Analyses

Quality requirements for the analysis of hair samples

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Preface

Toxicological analyses of hair samples for the detection and determination of illicit drugs, medicinal drugs or other xenobiotic substances are carried out particularly in the context of jurisdiction, but may also be performed for clinical problems. In the analysis of hair samples and the interpretation of results, many characteristics must be considered compared to the analysis of other biological matrices, due to the nature (solid matter) and heterogeneity of the matrix, the stability of analytes and possible influences during sample preparation.

In general, the “GTFCh guidelines for quality control in forensic-toxicological analyses“ must be observed. General requirements on the competence of testing and calibration laboratories are to be found as the basis for accreditation in the respectively valid version of DIN-Norm EN ISO/IEC 17025.

In this appendix, certain requirements regarding forensic-toxicological analyses of hair samples are substantiated.

The chapter numeration refers to the “GTFCh guideline for quality control in forensic-toxicological analyses“. The specifications are to be regarded as a supplement to the respective sections of the guidelines.

1 General measures for quality control and quality assurance

Pure substances and hair samples should be analysed in different laboratory rooms in order to exclude contamination. The samples should be stored at room temperature. The available equipment should provide an unambiguous identification of single substances and the determination of their concentrations.

2 Requirements on samples and their handling

2.1 Requirements on sample collection and sample transport

Samples to be analysed can either be collected by the laboratory or by other qualified institutes. Already when collecting a sample, contamination by substances (e.g. powders) must be prevented by using separate rooms Since the collection of a hair sample is non-invasive, the presence of a physician is not a necessity. The identity of the person tested must be authenticated (identity card, photo comparison, signature, finger print if necessary). The sample must be labelled unambiguously and completely.

The application form should be clear and should also contain the date of sample collection, sample origin and the required analysis, including problem definition and case history.

A sufficiently large hair sample should be collected from the posterior vertex region of the of the head, as close as possible to the scalp, generally in the form of several strands of hair. In that way, reference sample is reserved. The length of the remaining hair must be documented. The strand of hair to be collected should be fixed with a thread before cutting, in order to avoid shifting of segments. The hair end closest to the scalp (proximal) is to be marked. In special, critical cases some hair may be taken by pulling out, in order to facilitate future classification of the sample by DNA-analysis.

Alternatively, body hair such as axillary, pubic, chest or beard hair may be collected. Upon collection of body hair it is recommended to either rule out or to record previous hair shaving or cutting, via light microscopic examination. If there is no scalp hair, body hair may be used to check drug use, e.g. prior to medical psychological examinations (MPU) and to verify an alleged abstinence of several months. Body hair is not suitable for an estimation of the time of consumption by taking into account the growth cycle, as with scalp hair (see above). The sampling site must be clearly stated. For the rest the procedure is as above (as far the hair length allows).

A suitable form must be chosen for transport and storage (e.g. aluminium foil as primary packaging material in an envelope).

2.2 Sample receipt

Prior to analysis, the sample must be characterised (length, weight, condition, colour, evidence of cosmetic treatment, etc.).

At the beginning of analysis, the person in charge determines the strategy of the examination. Depending on the question, in particular the segmentation of the hair strand is decided and documented. An unsegmented reference sample should be retained.

2.3 Sample storage

Primary, secondary and reference hair samples must be appropriately transported and stored at room temperature under dry conditions and protected from light. before and after analysis. Refrigeration or freezing should be avoided in order to protect the material from moisture. Moisture and crystallisation could lead to decomposition of analytes and matrix.

3 Requirements on immunoassay-determinations

In preliminary immunochemical testing care should be taken that sample preparation does not lead to denaturation of antibodies to be used. Calibrators and controls should be prepared with blank hair samples. In some substance classes, the sensitivity to hydrolysis of the analyte can be used to obtain the required sensitivity in the preliminary test, by incorporating a hydrolysis step in the sample pretreatment. This implies that so-called “equivalents“ are measured that are related to the hydrolysis product.

When using immunochemical methods for a preliminary result, an analyte concentration in the region of the required detection limit of the chromatographic method (see appendix A) should always give a positive immunochemical result for the relevant substance class. This should be documented appropriately (see appendix B).

4 Evidential, identifying and confirmatory methods

The determination of xenobiotic substances in hair is divided into different methodical steps: decontamination, digestion/extraction, clean-up , and analysis

Generally, for hair analyses as for other analyses, only a GC or HPLC-method coupled with a mass specific detector or tandem-MS-system should be considered for evidential analysis.

4.1 Sample preparation

Hair samples should be examined for contamination prior to the actual analysis or sample preparation, or at least the possibility should be created that contamination can be

excluded or confirmed later. Presently there is no consensus regarding an optimal decontamination method. When performing several washing steps, a balance should be found between adequate decontamination and incipient extraction of the analytes from the hair sample. This can be studied by analysing the washing solutions. Since decontamination by treatment with solvents may also influence the hair matrix and thus the subsequent extraction efficiency, this must generally be considered in the interpretation of test results, especially when quantitative results have to be compared.
A possible segmentation must be performed according to the problem. For a routine examination, the analysis of 3 cm-segments is recommended.

4.1.2 Extraction and derivatisation

Hair digestion or extraction and clean-up can be chosen by the laboratory. In the interpretation it must be observed that different results are to be expected from different methods. Direct comparison of quantitative results obtained by different methods is therefore problematic. During the hair digestion process or the extraction of analytes from the matrix, the specific substance characteristics must be considered. The hydrolysis of relevant analytes as well as the internal standards used must be excluded as far as possible.

5 Aspects of quality controle and quality assurance in quantitative determinations

The nature (solid matter) and heterogeneity of hair samples require special recommendations for quality control and quality assurance in forensic-toxicological hair analyses.

Validation of methods for hair analysis

GTFCh standards regarding detection limits to be obtained for drugs of abuse and their metabolites in hair according to forensic requirements, determined with evidential chromatographic methods, are to be found in appendix A of the “GTFCh guideline for quality control in forensic-toxicological analyses“.

Spiked hair samples may be used to perform a basic validation:

- Linearity of calibration

In principle, appendix B (validation) of the GTFCh guideline for forensic-toxicological analyses applies. The number of calibrators, however, can be reduced to 4, and the number of repetitions (repeatability) per calibrator can be reduced to 5.

- Repeatability and laboratory precision

In principle, appendix B (validation) of the GTFCh guideline for forensic-toxicological analyses applies. The number of days, however, can be reduced to 5.

- Stability

Data on stability and storage conditions are necessary. This applies to the stability of reagents, standards, and samples. Due to the sensitivity to hydrolysis of some compounds that should be tested in hair analysis, it is imperative to check the stability of analytes or internal standards during sample preparation.

5.2 Control charts for QC-samples

Laboratory quality control by using internal control samples is difficult in hair analysis. Integration of substances into the heterogeneous hair matrix cannot be modelled adequately by spiking a blank hair sample. Conversely, setting extraction methods and decontamination procedures is only an arbitrary convention, which cannot claim to lead to the “true value”. For the purpose of internal quality control, it is therefore sufficient that in a sequence with spiked samples no false positive results are obtained in a blank hair sample and that the substances with concentrations near the determination limit are detected in the correct retention time window, and that the mass fragments and their signal intensities match within the specified limits, despite co-extracted matrix components,. To include the extraction method in the controls, it is recommended that in each measurement series also authentic samples are tested which contain the relevant analytes (e.g. remains of positive hair samples). As in other analysis methods, the results must be documented on control charts.

5.4 External quality control (collaborative testing/ interlaboratory tests)

The external quality control of hair samples should also include authentic hair samples. Due to the above mentioned peculiarities in hair analyses, apart from collaborative testing, regular inter-laboratory comparisons seem to be most effective to monitor a single laboratory. With these, the repeatability can be objectified, and the deviation of one laboratory from the mean value of all laboratories or reference laboratories can be determined.

6. Result report/Expert report

Taking hair length into account in interpretation

In forensic-toxicological analyses of hair samples, the majority of cases focus on proof or exclusion of the consumption of illegal drugs. For this purpose, standardised methods to interpret the results are required. In view of other uncertainties it is legitimate to assume an average scalp hair growth of 1 cm per month (ca. 0.5 to 1.5 cm per month). With this assumption, an appropriate segmentation can be performed, depending on the case., The intradermal growth section, comprising a time period of 9 to 14 days, is of minor importance. Principally, an interpretation should be made with caution. One should especially consider that

- ca. 1.3% of the scalp hair is in the catagen phase (transition period from growth phase to resting phase), and ca. 10 to 15% in the telogen phase (no growth for up to six months),
- migration and inclusion from (deep) body compartments may occur up to a few months after the last consumption,

- the inter, and intra-individual growth rate may show considerable variation.

The contribution of catagenic and telogenic hair in shorter hair samples may depend much on the kind of hair cut prior to sampling (e.g. total shaving, so that all hair originating from that moment is in the anagen phase, or regular trimming to the same length). Therefore, the following would be valid for the interpretation after analysis of, for instance, a 6 cm long, proximal hair segment:

In case of a positive result, contact or consumption within the last six months (up to a maximum of 12 months) can be assumed.

In case of a negative result there is no evidence of (illicit) drug consumption in the last six months, but a single or very rare consumption cannot be excluded.

For some illicit drugs such as, for example, 6-monoacetylmorphine or cocaine as well as for benzodiazepines and methadone, higher concentrations were found in axillary and pubic hair than in scalp hair. This was ascribed to the absorption via sweat and sebum, and to different phases of growth compared to scalp hair. In pubic hair, uptake via drug-containing urine can also be assumed. For axillary hair, literature indicates an average growth of 0.28-0.44 mm/day; for the anagen phase, a time span of 44-72 weeks, and for the telogen phase of 48-68 weeks were determined. The growth of pubic hair is quoted as 0.20-0.39/day, the period of the anagen phase as 47-77 weeks, and that of the telogen phase 51-73 weeks. After that, about 40-60% of axillary and pubic hair are in the growth phase, in scalp hair the proportion is about 85-90%. Whiskers grow about 0.25-0.29 mm per day, the active growth phase is indicated as 2-11 weeks, the ratio of anagen to telogen hair as 55:45 or 66:34. A body hair sample represents the period of the total growth cycle, provided that it shows the natural hair points.

Literature

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Legal validity

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