2022 SoHT CONSENSUS ON GENERAL RECOMMENDATIONS FOR HAIR TESTING

Consensus revision – presented in Verona June 10th 2022 following the expert meeting in Malaga March 4-5, 2022.

Participants in the discussion: Brice Appenzeller, Carmen Jurado, Donata Favretto, Donna Muldoon, Elena Lendoiro, Frank Musshoff, Frank Sporkert, Gail Cooper, James Nutt, Jochen Beyer, Kieran Spellman, Lolita Tsanaclis, Luca Morini, Maria del Mar Ramirez, Mario Barroso, Maristela Andraus, Markus Baumgartner, Michel Yegles, Richard Poulton, Robert Kronstrand, Ronald Agius, Sabina Strano Rossi, Sian Beven, Tina Binz and Vincent Cirimele.

The present consensus deals with general aspects of hair testing covering sampling, sample continuity, storage, segmentation, washing, pre-analytical steps and analytical techniques as well as quality control and considerations for interpretation.

The consensus was finalized and agreed during the SoHT board meeting on the 27th September, 2022.

1. Sampling, sample continuity, and storage

Hair analysis can be performed using hair collected from different body sites. Sample collection should be performed following these recommendations.

- Head (scalp) hair is generally the preferred specimen however, hair from other body sites can be collected if deemed more suitable for testing. Hair samples should be collected by cutting as close to the skin as possible. Alignment of the hair strands should be maintained until analysis. The sample collection site (e.g. head, chest, leg, etc) should be documented.
- Head hair should be cut from the posterior vertex region, since this is the region of least variation in growth rate.
- Colour and length of the hair, and any topical, chemical or physical hair treatments that have been used should be documented.
- The proximal (root) end of the hair should be clearly identified.
- Hair should be stored dry in the dark at room temperature. The sample and any aliquots must be handled and stored in a manner that prevents contamination and minimizes degradation and loss of analyte.
- The amount of hair needed for analysis should be in line with the laboratory requirements

2. Segmentation

Segmentation may be performed to provide additional information on patterns of use or exposure over time taking into consideration the hair growth rate. An approximate head hair growth rate of 1.0 cm per month is generally accepted, however each individual´s hair growth rate varies.

- Hair cut from the head and aligned with the root end identified, can be subjected to segmental analysis**.**
- Segmentation of body hair is generally not recommended.

3. Washing of the hair samples

Washing of hair samples prior to analysis has two main purposes. First, to remove hair care products, sweat, sebum or surface material (e.g. skin cells, head lice, body fluids, etc.) that may interfere with the analysis or that may reduce extraction recovery. Second, to remove potential external contamination of xenobiotics from the environment.

- Organic solvents and/or aqueous solutions can be used. Any wash protocol will require validation by the laboratory to assess its efficacy.
- The washings may be stored for additional analyses.

4. Pre-analytical steps

Hair analysis involves procedures to facilitate the release of analytes from within the hair matrix.

It is recommended that hair samples are homogenized by techniques such as pulverization, digestion or cut into small pieces.

Extraction

- Extraction procedures should be suitable for the target analytes.
- It is important to consider the potential deleterious effect on the analytes when choosing the extraction procedure.
- It is recommended that extraction efficiency is evaluated with authentic hair containing the analytes.

5. Analytical techniques

Identification and quantification of the analytes of interest should be performed using chromatographic methods combined with mass spectrometry and applying internationally accepted identification criteria.

Laboratories must ensure that their techniques have sufficient sensitivity and specificity for the concentrations of the analytes of interest expected in hair.

Screening methods are useful when the expected proportion of negative samples is high and are used to identify presumptive positive samples that require further confirmatory analysis. Screening methods can be immunoassays specifically developed for hair analysis or other analytical techniques such as mass spectrometry. Immunoassays are not recommended for single/seldom intake/exposure cases.

- Screening assays should have sufficient sensitivity to detect expected analyte levels in hair.
- All presumptive positive screening tests must be confirmed using mass spectrometry.

6. Quality control

As hair is a non-homogenous solid matrix, the quality control is more challenging than for homogenous body fluids such as urine and blood.

Spiked internal control samples (prepared from analyte free authentic hair) can be used to monitor the analytical performance but not the extraction efficiency.

For external quality control, the laboratory must participate in a proficiency testing program or inter-laboratory comparison including authentic hair.

Proficiency test or inter-laboratory comparison samples should be treated, as far as possible, in the same way as routine samples in the laboratory.

7. Considerations for interpretation

The incorporation and retention of compounds into hair may be influenced by natural hair colour and type, depending on their physical and chemical properties.

The concentration of compounds in hair can be affected by topical, chemical or physical hair treatments (including but not limited to bleaching, dyeing, perming, straightening, UV exposure).

The concentrations of compounds in hair can be affected by external contamination.

The growth rates and growth cycles have to be considered to establish the time frame represented by the analyzed scalp hair or body hair.

If body hair is analyzed, the higher proportion of dormant (telogen) hair should be considered.

The results of hair analysis should be interpreted considering all relevant factors surrounding the case (for example, other analytical testing, declarations and statements).