#### 2021 Soht Consensus on Drugs of Abuse (DOA) testing in Hair

Consensus revision – presented in Santiago September, 17<sup>th</sup> 2021 after expert meeting in Sevilla Feb 7-8<sup>th</sup>, 2020, and on-line meeting in June 29<sup>th</sup>, 2020,

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The present consensus deals with DoA only, whereas general aspects of hair testing covering collection and storage, decontamination, segmentation, extraction, analytical techniques, general interpretation, quality assurance and quality controls, will be revised and presented in the future.

The consensus was finalized and agreed during the SoHT board meeting on the 16<sup>th</sup> November, 2021

### 1. Sample preparation

1.1 The preparation of hair samples involves a number of steps including washing/decontamination, segmentation (optional) and obtaining a representative sample from the material available.

#### 2. Pre-analytical steps

- 2.1 Before extraction, hair samples must be homogenized by pulverization or digestion, or cut in small pieces.
- 2.2 A decontamination step is necessary.
- 2.3 Organic solvents and/or aqueous solutions can be used. Any wash protocol will require validation by the laboratory to assess its efficacy.
- 2.4 For drug or metabolites that are present at low levels, pulverization is recommended

#### 3. Extraction

- 3.1 Hair analysis involves an initial pre-treatment step to release the drugs from within the hair matrix. The resulting extract can be analyzed directly or will require further clean-up
- 3.2 Method efficiency is compromised significantly through use of unsuitable extraction procedures not targeted to specific drugs.
- 3.3 It is important to consider the potential deleterious effect on the drugs in question when choosing the extraction solutions. In vitro hydrolysis of labile compounds such as cocaine and heroin has been reported under acidic or alkaline conditions. Incubation procedures with methanol do not suffer from the same issues but recoveries may be lower.
- 3.4 Acetonitrile solely should be used with caution due to possible low extraction efficiency
- 3.5 Extraction recovery must be evaluated with incurred hair

# 4. Screening techniques

If screening techniques are used the following points should be considered:

- 4.1 Screening assays should prove sufficient sensitivity to detect drug levels in hair.
- 4.2 All presumptively positive screening tests must be confirmed using mass spectrometry applying internationally accepted identification criteria.

# 5. Confirmation techniques

- 5.1 Identification and quantification of the analytes of interest are achieved using chromatographic methods hyphenated with mass spectrometry
- 5.2 Laboratories must ensure that confirmation techniques have sufficient sensitivity and specificity for low levels of the analytes of interest found in hair.
- 5.3 Confirmation methods should include the determination of the parent drug and metabolite(s), where applicable

## 6. Analytes

- 6.1 Minimum required detection levels are different between drug classes.
- 6.2 Different decision levels may be required depending on the purpose of the test.

#### 7. Cut-off

- 7.1 Cut-off is the value that enables identification of drug users
- 7.2 When hair analysis is utilized in drug facilitated crimes (DFCs) or single intake/exposure cases, the cut-off is not to be considered but lower limit of quantification are required
- 7.3 For hair analysis in children the cut-off is not to be considered but lower limit of quantification are required
- 7.4 The cut-off is applied to head hair
- 7.5 The cut-off is applied to hair segments no longer than 3 cm; when applied to longer segments, dilution must be considered
- 7.6 At the time of preparing this document, the scientific literature is insufficient to establish cutoff values for any other drugs not listed in Table 1

Table 1: Analytes and cut-offs

<b>Drug group</b> Analyte	Cut-off pg/mg	Comments
Opiates group	P8/ 1118	Comments
Morphine, Codeine, Dihydrocodeine	200	Heroin consumption must be differentiated from codeine or morphine use by the presence of 6-acetylmorphine and/or heroin
6-Monoacetylmorphine, Heroin	200	
Cocaine group		The presence of benzoylecgonine, norcocaine ,
Cocaine	500	cocaethylene, hydroxyl-cocaines or hydroxy- benzoylecgonine must be considered to confirm use. For crack cocaine use, anhydroecgoninemethylester must be considered
Amphetamine group		
MDMA, MDEA, MDA, methamphetamine, amphetamine	200	
Canabinoids		
THC	50	Detection of THC-COOH strongly supports THC use/intake (*)
CBD	50	
Opioids group		Confirmation of desmethyltramadol definitively proves the use of tramadol
Tramadol	200	
Oxycodone	100	
Methadone		Confirmation of EDDP definitively proves the use of methadone
Methadone	200	
Buprenorphine		Confirmation of norbuprenorphine definitively proves the use of buprenorphine
Buprenorphine	10	
Ketamine		Confirmation of norketamine definitively proves the use of Ketamine
Ketamine	200	

For metabolites, additional cut-offs can be established according to scientific knowledge

<sup>\*</sup> For the confirmation of THC-COOH the minimum required LOQ is 0.2 pg/mg

# 8. Interpretation

- 8.1 The incorporation of drugs into hair may be influenced by natural hair colour, depending on physical/chemical properties of the drug molecule
- 8.2 The concentration of drugs in hair can be influenced by chemical and/or physical hair treatments (e.g. bleaching, dyeing, perming, straightening, UV exposure, etc);
- 8.3 The type of hair treatments should be documented at the time of sample collection and considered during interpretation
- 8.4 To identify a potential external contamination of the hair, the analysis of the washings can be useful
- 8.5 The identification of certain metabolites (see table above) is required to identify drug users
- 8.6 The results of hair analysis should be interpreted considering all relevant factors surrounding the case
- 8.7 The concentration of drug in hair cannot be used to determine the quantity of drug consumed
- 8.8 Segmental analysis can provide information about the individual pattern of drug consumption over time
- 8.9 If hair from other body sites is used, the quantitative results should be interpreted with caution and with consideration of the different growth cycles representing different time periods.