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## European guidelines for workplace drug and alcohol testing in hair

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## European Guidelines for Workplace Drug and Alcohol Testing in Hair

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**Abstract**

These guidelines for Legally Defensible Workplace Drug Testing have been prepared and updated by the European Workplace Drug Testing Society (EWDTs). They are based on the 2010 version published by Pascal Kintz and Ronald Agius (Guidelines for European workplace drug and alcohol testing in hair. *Drug Test Anal.* 2010; 2(8):367-76) and in concordance with the Society of Hair Testing guidelines (Society of Hair Testing guidelines for drug testing in hair, *Forensic Sci. Int.*, 2012; 218:20-24). The European Guidelines are designed to establish best practice procedures whilst allowing individual countries to operate within the requirements of national customs and legislation. The EWDTs recommends that all European laboratories that undertake legally defensible workplace drug testing should use these guidelines as a template for accreditation.

## 1. General

### 1.1 Objectives

- To provide a common framework for European providers of workplace drug testing services in Europe within which Workplace Drug Testing (WDT) in hair should be performed.
- To provide guidance to European laboratories interested in the determination of drugs of abuse in hair, in order to provide reliable results for the purpose of WDT.
- To help promote and harmonise efforts by providing guidelines which are accepted at the European level.
- To ensure that the processes undertaken are capable of legal scrutiny.
- To provide safeguards to protect the specimen donors.
- To define common quality assurance and quality control criteria for laboratories that are capable of being accredited by an external body.

## 2. Definitions

For purposes of these guidelines the following definitions have been adopted:

<b>Adulteration</b>	See Tampering
<b>Aliquot</b>	A fractional part of a sample used for testing. It is taken as a sample representing the whole sample.
<b>Authorising Scientist</b>	A person who reviews all pertinent data and quality control results in order to attest to the validity of the laboratory's test reports.
<b>Calibrator</b>	A solution of known concentration used to calibrate a measurement procedure or to compare the response obtained with the response of a test sample/sample. The concentration of the analyte of interest in the calibrator is known within limits ascertained during its preparation. Calibrators may be used to establish a calibration curve over a concentration range of interest.
<b>Chain of Custody</b>	Procedures to account for each specimen by tracking its handling and storage from point of collection to final disposal. These procedures require that the donor identity is confirmed and that a chain of custody form is used from time of collection to receipt by the laboratory. Within the laboratory appropriate chain of custody records must account for the samples until disposal.
<b>Chain of Custody Form</b>	A form used to document the procedures from time of collection until receipt by the laboratory.
<b>Collecting officer</b>	A person trained to collect specimens from donors.
<b>Collection Site</b>	A place where individuals present themselves for the purpose of providing a specimen for analysis.
<b>Confirmation Test</b>	An analytical procedure to identify and quantify the presence of a specific drug or metabolite which is independent of the initial test and which uses a different technique and chemical principle from that of the screen test in order to ensure reliability and accuracy.

<b>Customer</b>	The organisation requesting the drug testing service.
<b>Cut-off</b>	A concentration level set to determine whether the sample is positive or negative for the presence of a drug.
<b>Donor</b>	The individual from whom a hair specimen is collected.
<b>Laboratory</b>	The facility providing the analytical services to detect drugs of abuse.
<b>Negative result</b>	A result reported by the laboratory that indicates that either no drug is present in the sample or that any drug present is below the cut-off.
<b>Positive result</b>	A result reported by the laboratory as positive means that there is conclusive evidence that a drug is present in the sample tested at a level greater than or equal to the confirmation cut off concentration.
<b>Quality control sample</b>	A sample used to evaluate whether or not an analytical procedure is operating within pre-defined tolerance limits.
<b>Medical Review Officer (MRO)</b>	A medical physician responsible for receiving laboratory results from the drug-testing laboratory who has knowledge of substance abuse and has appropriate training or experience to interpret and evaluate an individual's positive test result, in light of declared information.
<b>Sample</b>	A representative portion of a specimen submitted to a laboratory for testing.
<b>Screening Test</b>	A test to eliminate negative samples from further consideration and to identify the presumptive positive samples that require confirmation testing.
<b>Service Provider</b>	The organisation contracted to provide the drug testing service. This may be a laboratory, or a third party providing other elements of the service, and sub-contracting the tests to another laboratory.
<b>Specimen</b>	The portion of hair that is collected from a donor.
<b>Standard (1)</b>	A reference material of known purity or a solution containing a reference material at a known concentration.
<b>Standard (2)</b>	An agreed protocol or procedure (e.g. EN ISO/IEC 17025 and EN ISO 15189)
<b>Standard Operating Procedure (SOP)</b>	A written document giving the detailed steps to be followed when undertaking a particular task (e.g. the analysis of a given drug in a hair sample).
<b>Tampering</b>	Any process by which an individual knowingly interferes with (or attempts to interfere with) the processes of specimen collection, transport or analysis with the intention of avoiding a legitimate test result.
<b>Toxicologist</b>	A person responsible for interpreting a toxicological analytical result for the customer or the customer's designated Medical Review Officer.

### 3. Specimen Collection

#### 3.1 Introduction

The collection of donor specimens involves some of the most difficult and sensitive areas of the WDT process.

To ensure the integrity of the entire process, the collector must be very sensitive to each individual's privacy and respect the dignity of the donor while at the same time ensuring that the sample is accurately collected and has not been tampered with in any way.

To ensure a balance between the privacy of the donor and the need to ensure the proper identification and integrity of the specimen, the following steps must be documented:

- The verification of the identity of the donor e.g. by valid and unexpired ID card.
- The proper identification of the specimen with its donor.
- Ensuring that no adulteration or tampering took place.
- Ensuring that no unauthorised access to the specimen was possible.
- The secure transfer of the specimen to each person handling it.

This documentation process is the first link in what is referred to as the chain-of-custody process. This process follows a data trail that, when reconstructed at a later date, can be used to prove that the final result properly matches the sample to the donor.

The procedures for collection of hair specimens for workplace drug testing are very specific. It is essential for each collection site to have written standard operating procedures and for collectors to comply with those procedures, in order to minimise the possibility of procedural or administrative errors.

A collector is a trained individual who instructs and assists the donor at a collection site, who receives and makes an initial inspection of the specimen provided by the donor, and who initiates and completes appropriate sections of the Chain of Custody Form (CCF).

The specimen collection kit is usually provided by the testing laboratory.

The collector should conduct only one collection at a time, to prevent specimen misidentification and avoid distraction that could compromise specimen security. The collector must guarantee the secure handling and storage of the specimen from the time the specimen is received from the donor until the specimen leaves the collection site for transport to the testing laboratory. Instructions for hair collection are provided in Appendix A.

#### 3.2 Collector Qualifications

Specimens must be collected by suitably trained personnel (Collecting Officers). Although no healthcare professional education is required, documented training, which includes a demonstration of competence, must be undertaken before collections are performed. The training can for example be organized by a laboratory or an independent organisation or company.

1 Collectors can be trained by various methods (video, classroom, internet, etc).

2 Training must include, as a minimum, the following:

- 3
- 4 • The collection process
  - 5 • The chain-of-custody process
  - 6 • The process involved with “problem” collections (e.g. baldness)
  - 7 • The responsibility of the collector for maintaining donor privacy, confidentiality of
  - 8 information, and specimen integrity
  - 9 • Ethical issues, especially regarding the declaration by the donor of past and
  - 10 present use of prescribed medications which may influence the result
  - 11 • Legal, ethical and human rights of the donor
- 12

13

14 It is highly recommended that, upon completion of the training, each collector be tested

15 on all subject matters covered in the training course to verify their understanding of the

16 topics. It is also highly recommended that each training course include mock collections

17 to assess collector competency. On successful completion of training, the trainee

18 collector may begin performing collections. However, there are a few instances in which

19 a collector should not perform a collection. These situations are:

20

- 21 • If the collector is the immediate supervisor of the donor (unless no other
  - 22 collector is available), or if the collector is a co-worker, a relative or a close
  - 23 friend of the donor.
  - 24 • An individual working for a drug testing laboratory may not act as a collector if
  - 25 that individual can link the donor with the specimen drug test result unless
  - 26 he/she is bound by professional confidentiality.
- 27
- 28
- 29
- 30

### 31 3.3 Collection Site

32 A collection site is a facility (permanent or temporary) selected by the employer where

33 donors present themselves for the purpose of providing a specimen.

34

35 Access to the facility must be restricted. Procedures for collection of specimens should

36 allow for individual privacy. Preferably, there should be a sign outside prohibiting entry

37 while a collection is occurring.

38

39 The collection area must be thoroughly cleaned, dedicated to sample collection and

40 cannot be used for storage of any potential source of contamination, such as drugs.

41

### 42 3.4 Chain of Custody Form (CCF)

43 Chain-of-custody is the term used for the process of documenting the handling and

44 storage of the hair specimen from the time the donor gives it to the collector until it is

45 destroyed. A CCF is used to document the collection procedure and the chain-of-

46 custody of the specimen.

47

48 In Europe, there are many different types of CCFs. Almost every laboratory that

49 performs WDT has its own version of this form which may also include a different

50 number of copies for each form.

51

52 The CCF is numbered with a unique specimen identification number and includes a

53 sample label that is printed with the same specimen identification number as the CCF.

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1 A tamper-evident label must be applied across the hair collection envelope. The  
2 numerical sample label can be used as a tamper-evident label and also serves to seal  
3 the collection kit by applying it across the hair collection envelope containing the  
4 collected hair specimen. A purpose tamper-evidence seal can also be utilised.  
5  
6

7 The information on the CCF should include:

- 8 • Unique specimen identification number.
- 9 • Name, address, e-mail address, and phone number of the testing laboratory.
- 10 • Information identifying the donor (e.g., birth date, name, and home address).  
11 However, the identification could be an identification code purposely used so  
12 the sample remain anonymous but it will be linked to the individual being tested,  
13 known by the employer. The testing laboratory will not know the actual identity  
14 of the individual being tested but the employer will.
- 15 • Date and time of the collection.
- 16 • Names and signatures of all individuals who had custody of the specimen  
17 during the collection process.
- 18 • Declaration by the donor of the use of prescribed and non-prescribed  
19 medications, of sample authenticity, correctness of sample labeling and  
20 package and permission for sample to be analyzed at the laboratory.  
21  
22 • ~~Substances abused, period of abuse, frequency of abuse~~
- 23 • Required analysis including period to be tested and drugs. However, when  
24 testing is already covered by a contract, this information is not required at the  
25 point of specimen collection.
- 26 • Site of hair collection (e.g. head, pubic, chest)
- 27 • The colour and any obvious, observed or declared cosmetic treatments
- 28 • Optionally, the CCF may contain information on how to reach the donor during  
29 daytime (i.e., telephone number); information on how to reach a representative  
30 of the employer (i.e. name and telephone number);
- 31 • Medical Review Officer (MRO)/ occupational physician information (i.e. name,  
32 address, telephone, e-mail and fax numbers); collection site information (i.e.  
33 collector name, telephone number).

34 There should be a minimum of three parts or copies of the CCF to be distributed by the  
35 collector as follows:

- 36 • One part or copy to the testing laboratory (with the specimen)
- 37 • One part or copy to the donor
- 38 • One part or copy retained by the collector

### 3.5 Collection Process

39 The following describes steps for a hair collection (see Appendix A):

- 40 i **Verification of donor's identity.** When the donor arrives at the collection  
41 site, the collector should request photo identification to verify donor identity. If  
42 photo ID is not available, it is acceptable for the donor's supervisor or other  
43 employer representative to identify the donor. If such a third party is used to  
44 confirm the identity of the donor, they should provide their own photo  
45 identification and their confirmation should be recorded in writing. If the  
46 individual's identity cannot be established without a doubt, the collector  
47 should not proceed with the collection. If proof of identity is not available, then  
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2 a photograph can be taken at the time of collection, as verification of the  
3 donor's identity.  
4

- 5  
6 ii **Hair collection.** The specimen should be cut from the posterior vertex region  
7 of the head, as close as possible to the scalp, since this is the region of least  
8 variation in growth rate. A lock of hair, with the thickness of a thin pencil –  
9 **about 0.3 – 0.5 cm in diameter**, is usually collected. The root end must be  
10 clearly indicated. Unless otherwise required by local laws or employer policy,  
11 the collector is permitted to submit a single specimen. **When both A and B**  
12 **specimens are required, two specimens should be collected side by**  
13 **side.** The amount of hair collected will vary according to the needs of each  
14 laboratory. Sufficient hair must be collected to allow initial testing, followed by  
15 confirmatory or re-testing of the sample if necessary. In cases where head  
16 hair is not available, body hair (i.e. chest and pubic) can be collected, if  
17 permitted by the company's WDT policy and the donor. Other source of body  
18 hair (i.e. arm and leg) is a suitable alternative. Axillary hair should never be  
19 collected **because drugs and alcohol biomarkers may be underestimated**. The  
20 different physiology of non-head hair has to be considered during  
21 interpretation (see also Paragraph 8.2). In general, head hair is estimated to  
22 grow at approximately 1.0 cm per month. If segmental analysis is required,  
23 head hair is needed. The length of each segment and number of required  
24 segments should be specified (e.g. 3 x 2 month segments, 2 x 3 month  
25 segments etc). Segmental analysis is only applicable to head hair.  
26  
27  
28  
29 iii **Fill in the required details.** The colour, length, body site and any obvious  
30 cosmetic treatment of the hair should be recorded. The donor should be  
31 asked to declare all cosmetic treatments in addition to the observed  
32 treatments. The root (proximal) end of the hair must be clearly identified.  
33  
34  
35 iv **Put the lock(s) of hair into the collection kits.** When A and B samples are  
36 required, **the collector put the two locks of hair into two different**  
37 **collection kit envelopes.** Each envelope will contain the minimum quantity  
38 needed for the analysis. Both specimen A and B should be stored in a dry  
39 environment, at room temperature and protected from daylight (see also  
40 Paragraph 5.1)  
41  
42  
43 v **Seal the kit.** In front of the donor, the collector places the tamper evident  
44 label/seal over both collection kit envelopes. **The label/seal should be**  
45 **arranged in a way that any manipulation can be detected**  
46  
47  
48 vi **Instruct the donor to annotate kit seals.** The collector instructs the donor to  
49 record her/his initials and date on each of the specimen kit seal.  
50  
51  
52 vii **Annotate the CCF.** The collector completes the appropriate sections of the  
53 CCF with donor information (e.g. date of birth, telephone numbers), collection  
54 information (e.g. date and time of the collection), and chain-of-custody  
55 entries, and instructs the donor to sign the CCF.  
56  
57  
58 viii **Check the CCF.** The collector checks all copies of the CCF for legibility and  
59 completeness. If all copies are legible and complete, the collector then  
60 provides the donor a copy of the CCF and permits the donor to leave.

- ix **Prepare specimen for shipment.** The collector places the specimen kits along with the laboratory copy (original) of the CCF in an envelope. It is important that the collector ensures each specimen collected is shipped (or picked up by the laboratory's courier) in accordance with the company policy. Specimens must be stored and shipped at room temperature, away from direct sunlight and humidity.

#### 4. Laboratory Organisation

Laboratories performing hair analysis should ideally be accredited to ISO/IEC 17025 standards and the guidelines of the SoHT (Society of Hair Testing) should be considered.

The complex nature of hair analysis and the concomitant analytical challenges necessitates well-trained scientists (see Paragraph 7.2) and sufficiently equipped laboratories.

The laboratory should use hyphenated techniques such as chromatography-mass spectrometry (GC-MS, LC-MS and tandem MS technologies – see Paragraph 6.2), well-documented experience in method validation, data handling and reporting of results.

Staff should have the ability to interact with investigating officers and with employment tribunals and the courts of law.

It is recommended that analyses are directed to designated laboratories, which have the analytical capacity to achieve the performances required by hair analysis.

Adequate sampling, evidence collection and storage should still be performed at a local level to ensure that the results produced by the regional laboratories are scientifically accurate and valid.

#### 5. Laboratory analyses and procedures

##### 5.1 Sample receipt

Receipt of specimens in the laboratory should be indicated by a handwritten or electronic signature (or initials). Any transfer of specimens must be documented as part of the permanent laboratory record.

Once the analyses are completed, specimens must be stored for the time period agreed with the customer in a secure manner. However, a minimum 1 year storage period is recommended.

Areas of possible contamination must be considered before and during the analysis and when interpreting the results. These may include external drug exposure and laboratory contamination.

The sample and any aliquots or extracts must be handled and stored in a manner so as to minimize degradation, loss of analytes, or contamination from other sources. Dry hair should be protected from UV light sources and at room temperature whilst in storage.

## 5.2 Sample decontamination and extraction

When hair analysis is being used to identify drug use, potential external contamination of the hair needs to be minimised, as if not removed it can wrongly result in an incorrect finding of actual drug use.

In general, a decontamination strategy to remove gross environmental contamination must include a wash protocol before analysis of the sample. The wash protocol could be a combination of aqueous and/or organic solvents which has been validated by the laboratory. The wash residues can be stored for later analysis if necessary.

After washing, the required hair segment is submitted to a validated extraction protocol that may involve drying, pulverising, cutting into small pieces or chemical disintegration of the hair sample.

The minimum amount of hair used in the analysis varies between different laboratories and each laboratory must establish their requirement during method validation.

Several extraction procedures have been published. These include:

- Methanolic incubation
- Acidic incubation
- Alkaline incubation
- Buffer incubation
- Enzymatic incubation

Extraction procedures vary between different laboratories and each laboratory must validate their method of choice prior to use.

## 5.3 Sample Analysis

Immunoassay screening can be applied to eliminate all negative samples and identify presumptive positive samples. All presumptive positive samples need to be confirmed with a second different method according to international guidelines. Usually mass spectrometry (MS)-based techniques, e.g. GC-MS or LC-MS, are used.

In order to improve the signal-to-noise ratio, a clean-up procedure, such as liquid-liquid extraction or solid-phase extraction followed by derivatisation (especially if GC-MS is used) is usually performed. Testing by GC-MS or LC-MS alone is also acceptable.

Clean-up procedures vary between different laboratories and each laboratory must validate their chosen method prior to use.

## 6 Analytical methods validation criteria

All methods must be validated and their suitability for intended purpose must be evaluated in accordance with EN ISO/IEC 17025 requirements. The following parameters have to be determined at least for quantitative confirmation analyses and whenever possible, for screening analyses: precision, cut-off verification, selectivity, limit of detection, limit of quantification, sensitivity, specificity, stability, measurement uncertainty and matrix effects.

## 6.1 Screening Tests

Since the majority of the samples tested for WDT are negative and hence will only be screened, high quality screening tests are **critical** for WDT. The precision of the assay around the reported cut-off must be demonstrated. Screening tests must be validated for the hair matrix.

The following validation criteria are suggested:

- intra-assay imprecision (< 30%)
- inter-assay imprecision (< 30%)
- sensitivity (>90%) calculated using at least 100 authentic hair samples which can be pooled together or a maximal false negativity rate of 10 % using at least 50 authentic hair samples with target drug concentrations at the required cut-off.

However, some analytes may be more difficult to analyse and a validation criteria outside the limits above may be used. This will be reflected in the uncertainty of the measurement and must be given to the clients in the service level agreement.

## 6.2 Confirmation Tests

National or European guidelines for mass spectrometric identification and quantification of drugs must be taken into account. MS criteria for identification by either scanning (e.g. Full Scan, Product Ion Scan) or non-scanning (e.g. Selected Ion Monitoring, Selected Reaction Monitoring) techniques are based on the presence and relative abundance of a number of ions which are defined by the Laboratory as diagnostic for the analyte. Any data processing (e.g. integration, subtraction, averaging, etc.) shall be performed consistently across the analytical batch [1]. The use of deuterated internal standards is recommended.

The following validation criteria are suggested:

- selectivity – to ensure no interference: measurement of 6 blank samples and 2 zero samples (blanks with internal standard)
- linearity: at least five replicate measurement at 5 different, approximately equidistant spiked concentrations; linearity shall be verified with an adequate statistical test, such as Grubbs-Test in order to eliminate outliers.
- bias (<20%): calculated at least at two different controls along the linearity range
- intra-assay imprecision (<20%): calculated at least at two different controls along the linearity range
- inter-assay imprecision (<20%): calculated at least at two different controls along the linearity range
- sensitivity limits and lower limit of quantification (LLOQ) must be lower than the given cut-off

Additionally, the uncertainty of measurement should be assessed.

However, some analytes, including drug and metabolites may be more difficult to analyse and a validation criteria outside the limits above may be used. This is reflected in the uncertainty of the measurement and must be given to the clients in the service level agreement.

## 7. Quality assurance and quality control

### 7.1 Introduction

The quality standards set by EN ISO/IEC 17025 or EN ISO15189 are recommended. The testing laboratory must be accredited by a recognised regulatory body.

### 7.2 Personnel

The Laboratory must be staffed by suitably qualified personnel.

The Laboratory must keep training records that establish the individual's competency for the position(s) held. The individual's file must include a CV showing qualifications and previous employment experience, and training records for the current tasks performed. All laboratory personnel must have received training in laboratory safety to ensure compliance with relevant legislation.

#### 7.2.1 Head of Laboratory

There must be one person who has overall responsibility for the professional, organisational, educational and administrative activities of the drug testing facility. This person is responsible for the day-to-day management of the drug testing laboratory. Some of the functions may be delegated to other appropriately qualified personnel but the overall responsibility for any delegated functions will remain with the designated Laboratory Head (typically the Laboratory Manager).

#### 7.2.2 Authorising Scientist

A person responsible for the review and certification of pertinent data and quality control results, prior to release of analytical results.

#### 7.2.3 Laboratory Analyst

A person responsible for undertaking the day-to-day analytical procedures.

#### 7.2.4 Toxicologist

A person responsible for interpreting a toxicological analytical result for the customer or the customer's designated Medical Review Officer.

#### 7.2.5 Expert Witness

A person to present evidence to administrative or disciplinary proceedings that are based on analytical results reported by the laboratory.

#### 7.2.6 Other personnel

Other technical or non-technical staff must have the necessary training and skills for the tasks assigned.



## 7.2.7 Quality Manager

The person responsible for quality assurance within the laboratory organisation.

## 7.3 Accommodation and Environmental Conditions

The laboratory must be accessible only to authorised visitors.

Visitors should be always be accompanied and are required to sign a log-book upon entry and departure from the laboratory, recording date and purpose of the visit and times of arrival and departure.

Due to their high surface area to volume ratio, hair samples are highly susceptible to external contamination. As a measure to avoid contamination, the analysis and the storage of hair samples in laboratories where seized drugs are analysed must be carried out in separate rooms. As an additional precaution, laboratory coats must be changed if the laboratory analyst is handling seized drugs and also carrying out the analysis of hair samples.

## 7.4 Quality Control

### 7.4.1 Internal Quality Control

- It is recommended that low and high level controls must be measured at least at the start and end of each batch of specimens.
- It is suggested that a batch of specimens includes at least 5% controls.
- The low level control concentration should be around the confirmation cut-off concentration (not greater than twice the confirmation cut-off concentration).
- For spiked controls, the laboratory must define the upper and lower limits according to acceptable statistical criteria.

### 7.4.2 External Quality Control

Participation in proficiency testing schemes should be performed on at least two occasions each year where available.

## 8. Interpretation of results

### 8.1 Introduction

Interpretation of results must be achieved by an experienced scientist.

The following aspects need to be taken into account when interpreting a hair analysis result.

### 8.2 Theoretical Background

Hair is a unique matrix because no active metabolism and excretion is present to remove drugs once deposited.

Drug concentration in normally treated hair depends mainly on:

- dosage of abused drug

- metabolism
- distance from the root – significant decrease in drug concentration can be observed after several months due to washing and U.V. radiation
- position along the hair and polarity of drug
- hair colour
- percentage of hair in the anagen and telogen phase

This is why it is often not straightforward to correlate the concentration of drugs found in hair with consumption pattern.

The growth rate of head hair could range from 0.7 cm - 1.4 cm/month when about 80%-95% of the follicles remain in the anagen phase (active growing phase). Growth rate could be influenced by therapeutic drugs, age, sex, race and depends even on seasonal fluctuations. The calculation of the period covered by head hair uses the average of 1 cm/month to give an approximate period of the detection covered by the hair sample or segment analysed.

Body hair has a slightly slower growth rate (0.5 - 1.1 cm/month) but a different percentage in the three stages of growth cycle in comparison to scalp hair. The calculation of the period covered by body hair uses the average of 1 cm/month to give an approximate period of the detection covered by the length of body hair, but because 40-80% remains in the resting phase, the period covered may be extended by 3-4 months. Body hair is not suitable for segmental analysis.

### 8.3 Cut-offs - Criteria for a Positive Drug Test

The positive result of a hair analysis may be used to confirm if a person has frequently used or was exposed to a drug. The Society of Hair Testing (SOHT) has recommended specific cut-offs for hair testing in forensic cases<sup>[2-4]</sup>. Recommended confirmation cut-off concentrations for workplace drug testing are given in Appendix B.

#### 8.3.1 Alcohol

Currently, according to the World Health Organization and a literature survey, chronic excessive alcohol drinking corresponds to consumption of higher than 60 g of pure ethanol per day for several months.

The direct determination of ethanol itself in hair is not possible due to its volatility and its potential absorption from external sources. Instead, the minor ethanol metabolites ethyl glucuronide (EtG) and/or fatty acid ethyl esters (FAEE) should be measured in hair as a direct alcohol consumption marker.

Either gas or liquid chromatography coupled to (tandem) mass spectrometry ~~with deuterated EtG as internal standard~~ should be used to test for EtG in hair.

For FAEE analysis, four different fatty acid ethyl esters should be tested: ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate.

For interpretation, the sum of the concentrations of these four esters should be used. Headspace solid phase microextraction in combination with gas chromatography-mass spectrometry ~~and use of d5-FAEE's as internal standards~~ is a suitable technique for determination of FAEE in hair.

The cut-off for EtG in hair to strongly suggest chronic excessive alcohol consumption is proposed at 30 pg/mg scalp hair measured in the 0-3 cm up to 0-6 cm proximal



segment. The same cut-off can be used for hair sampled from other body sites, with the exception of axillary and pubic regions.

The cut-off for the sum of the four esters in hair to strongly suggest chronic excessive alcohol consumption is proposed at 0.5 ng/mg scalp hair measured in the 0-3 cm proximal segment. If the proximal 0-6 cm segment is used the proposed cut-off concentration is 1.0 ng/mg scalp hair.

EtG should be the first choice in abstinence assessment. An EtG concentration equal to or greater than 7 pg/mg strongly suggests repeated alcohol consumption. A lower concentration does not contradict self-reported abstinence of a person during the corresponding time period before sampling<sup>[4]</sup>. The possibilities of a higher sensitivity of pubic hair should be considered in interpretation.

The analysis of FAEEs alone is not recommended to determine abstinence from ethanol, but may be used in cases of suspected false negative EtG results, utilising a FAEEs cut-off of 0.2 ng/mg for a 0-3 cm proximal scalp hair segment<sup>[4]</sup>.

### 8.3.2 Opiates/opioids

#### *Screening test:*

A morphine or 6-acetylmorphine concentration of 0.2 ng/mg must produce a positive result.

#### *Confirmation test:*

Recommended cut-off: 0.2 ng/mg for each compound.

Heroin consumption must be differentiated from codeine or morphine use by the presence of 6-acetylmorphine.

Other opioids (e.g. oxycodone, tramadol, fentanyl, tilidine): in case of positive results, interpretation must be completed by an experienced scientist.

### 8.3.3 Cocaine

#### *Screening test:*

A cocaine concentration of 0.5 ng/mg must produce a positive result.

#### *Confirmation test:*

Recommended cut-off: 0.5 ng/mg for cocaine, 0.05 ng/mg for other compounds. The chromatographic analysis should include cocaine, and at least two of the following: benzoylecgonine, aryl hydroxycocaines, cocaethylene, norcocaine or ecgonine methyl ester. The detection of two aryl hydroxycocaines (*ortho*, *meta* or *para*) can be reported as positive for cocaine use.

### 8.3.4 Amphetamines

#### *Screening test:*

A concentration of 0.2 ng/mg of each substance must separately produce a positive result: amphetamine; methamphetamine; 3,4-methylenedioxy-amphetamine (MDA); 3,4-methylenedioxymethamphetamine (MDMA); 3,4-methylenedioxyethylamphetamine (MDEA)

#### *Confirmation test:*

1  
2 Recommended cut-off: 0.2 ng/mg for each compound.

### 3 8.3.5 Cannabinoids

#### 4 *Screening test:*

5  
6 A  $\Delta^9$ -tetrahydrocannabinol (THC) concentration of 0.1 ng/mg must produce a positive  
7 result.  
8

#### 9 *Confirmation test:*

10 Recommended cut-off:

11 THC: 0.05 ng/mg  
12  
13 11-nor-9-Carboxy-THC (THC-COOH): 0.0002 ng/mg  
14

15  
16 THC may be present due to passive exposure to cannabis smoke, therefore  
17 confirmation of THC-COOH is required to definitively prove the active use of  
18 cannabinoids.  
19

### 20 8.3.6 Methadone

#### 21 *Screening test:*

22  
23 A methadone concentration of 0.2 ng/mg must produce a positive result.  
24

#### 25 *Confirmation test:*

26 Recommended cut-off:

27 Methadone: 0.2 ng/mg  
28  
29 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP): 0.05 ng/mg  
30  
31

### 32 8.3.7 Buprenorphine

#### 33 *Screening test:*

34  
35 A buprenorphine or a norbuprenorphine concentration of 0.01 ng/mg must produce a  
36 positive result.  
37

#### 38 *Confirmation test:*

39 Recommended cut-off:

40 Buprenorphine: 0.01 ng/mg  
41  
42 Norbuprenorphine: 0.01 ng/mg  
43  
44

### 45 8.3.8 Benzodiazepines and analogues

#### 46 *Screening test:*

47  
48 A concentration of 0.05 ng/mg of benzodiazepines must produce a positive result.  
49

#### 50 *Confirmation test:*

51  
52 Recommended cut-off: 0.05 ng/mg for each compound. These cut-offs are not part of  
53 the SOHT recommendations while they are based on the German driving licence re-  
54 granting guidelines<sup>[5]</sup>.  
55

56  
57 The chromatographic analysis should include (but not limited to) the following drugs or  
58 their metabolites: alprazolam, bromazepam, clonazepam, diazepam, flunitrazepam,  
59  
60

1  
2 flurazepam, lorazepam, lormetazepam, midazolam, nitrazepam, nordiazepam,  
3 oxazepam, phenazepam, temazepam, z- drugs (zolpidem, zopiclone and zaleplon).  
4

### 5 8.3.9 Ketamine 6

#### 7 *Screening test:*

8 A ketamine concentration of 0.5 ng/mg must produce a positive result.  
9

#### 10 *Confirmation test*<sup>[6]</sup>:

11 Recommended cut-off:

12 Ketamine: 0.5 ng/mg

13 Norketamine: 0.1 ng/mg  
14  
15

### 16 8.3.10 New psychoactive substances 17

18 Serious challenges are posed to laboratories to detect new psychoactive substances  
19 (NPS), so nowadays most routine analyses do not include screening procedures for  
20 these compounds. Nevertheless, WDT protocols should consider this investigation  
21 when the laboratory is offering screening and confirmation for NPS.  
22  
23

24 Considering the wide range of compounds forming this class, it would be difficult to  
25 speculate about possible cut-off levels. The expected concentrations in hair for synthetic  
26 cathinones are comparable to ones of amphetamines/methamphetamines. Since the  
27 pharmacological potency *in vitro* of synthetic cannabinoids is extremely high, it is likely  
28 that also *in vivo* these compounds are active at relatively low doses, reducing the  
29 detectable levels in hair. Therefore, some laboratories have set the limit of detection as  
30 the minimum criterion to establish the use of NPS. These positive samples, at very low  
31 levels, should be interpreted with caution by an experienced scientist.  
32  
33  
34  
35

## 36 8.4 Reporting the Results 37

38 Before reporting the results to the Medical Review Officer (MRO) or occupational  
39 physician, it must be ensured that the quality criteria for both the screening and  
40 confirmation tests are fulfilled. This must be checked by 2 qualified personnel (for  
41 instance the laboratory analyst and the authorizing scientist) and documented in the  
42 laboratory information system. The minimum quality criteria are:  
43  
44

- 45 (i) the daily internal Q.C. (for both the screening and confirmation tests) must meet  
46 the acceptance criteria and
- 47 (ii) the fulfillment of the chromatographic and mass spectral identification criteria.  
48

49 The test report should contain as a minimum the following information:

- 50 • Coded identification of the donor
  - 51 • Date of sample collection
  - 52 • Date of receipt of the hair sample in the laboratory
  - 53 • Address and/or fax of occupational physician/MRO ordering the test
  - 54 • Characterisation of the hair sample:
    - 55 a. type of hair (e.g. head, pubic, chest, arm or leg hair)
    - 56 b. original length of the hair specimen
    - 57 c. length of the analysed hair segment and the corresponding time frame
    - 58 d. hair colour
- 59  
60

- e. cosmetic treatment
- Type of analysis performed (screening and/or confirmation)
- Analytical method applied
- List of drugs, metabolites or alcohol markers analyzed and their detected concentrations
- Cut-offs values used
- Interpretation of the result:
  - a. Decision about negative or positive outcome
  - b. Statement about the minimum and maximum time period represented by the investigated hair length or segment length.
- Name of the person who is authorised to declare the final result of the analysis

## 9. Challenges to drug test results

In situations where there is a challenge to the results of a confirmed positive drug test result, the following guidelines must be used.

The residue of the original specimen, or the B specimen when available, are re-tested. The analyses can be performed in the same Laboratory or by a different drug testing laboratory accredited by a recognised external accrediting body and working according to these guidelines.

The donor shall be informed of his/her right to attend the new analyses or be represented.

The final report must say either that there was no drug found, or a named drug was found at a level that is either consistent or inconsistent with the level in the corresponding first analysis. .

### 9.1 External contamination

The following identification of metabolites are recommended for the exclusion of external contamination:

- i. At least two of the following: benzoylecgonine and/or cocaethylene and/or hydroxycocaines for the confirmation of cocaine consumption;
- ii. 6-acetylmorphine and morphine for the confirmation of heroin consumption;
- iii. Amphetamine for the confirmation of methamphetamine consumption;
- iv. **THC-COOH** for the confirmation of cannabis consumption;
- v. MDA for the confirmation of Ecstasy (MDMA) consumption;
- vi. Norketamine for ketamine consumption.
- vii. **EDDP for methadone consumption**

### 9.2 Effects of Cosmetic Treatment

Every strong chemical, physical and mechanical influence could have harmful effects on the cuticle: perming, straightening, dyeing, bleaching, excessive washing, intensive illumination with ultraviolet radiation, excessive exposure to sunlight. Bleaching, highlights or lightening involve the irreversible destruction of melanin by oxidation, a partial or even complete degradation of melanin is possible. When strong bleach is used the physical properties of hair will be altered (e.g. a higher porosity).

Hence cosmetic treatment like bleaching, dying and perming must be considered and accounted for pre- and post-analysis since it can reduce the drug concentration below the limit of detection and hence cause false negative results.

Nonetheless, dyed or bleached hair is not automatically useless for drug and EtG detection; conversely, hair analysis is a powerful tool, often the only means to detect retrospective drugs and/or alcohol consumption even in dyed or bleached hair. In case of cosmetical treated head hair, a body hair specimen might be investigated at the same time.

For Peer Review

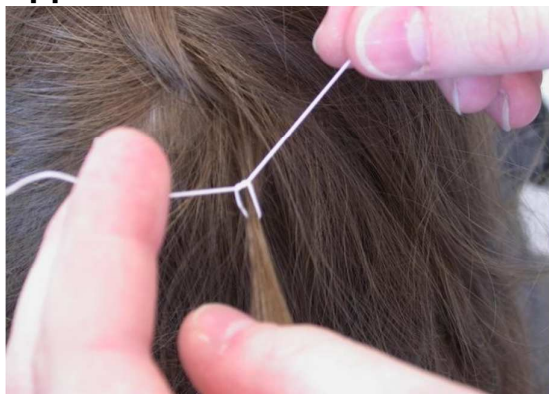
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## REFERENCES

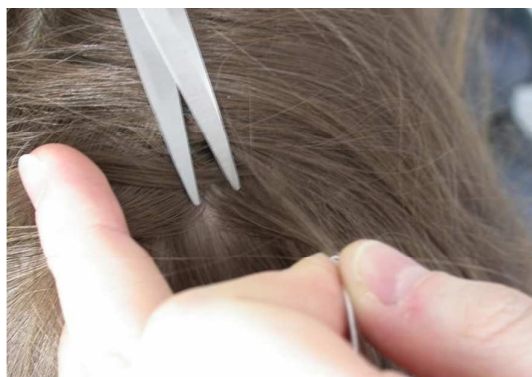
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### Appendix A –Instructions for Hair Collection



A lock of hair (or more, if necessary) about the width of a thin pencil are cut from the back of the head,



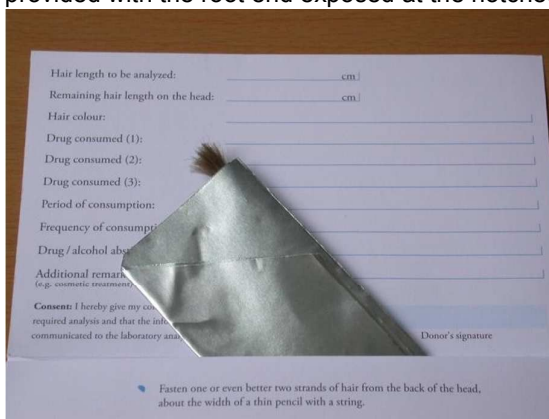
The hair is to be cut just above the skin, as close to the scalp as possible.



The hair lock(s) are put in separate aluminium foils provided with the root end exposed at the notched end.



Each aluminium foil is to be folded once as shown above.



Insert the aluminium foil into the envelope provided. Seal the envelope.



Fill in the donor's particulars and required test. The donor should sign the declaration. Hair samples shall be sent to the laboratory in an envelope by post or by courier.

**Table 1** Recommended cut-offs for screening and confirmation analysis in hair

	<i>Screening ng/mg</i>	<i>Confirmation ng/mg</i>
<b>Alcohol (segment 0-3)</b>	-	EtG: 0.03 FAEE: 0.5
<b>Amphetamines</b> Amphetamine Methamphetamine MDMA MDEA MDA	0.2	0.2 0.2 0.2 0.2 0.2
<b>Cocaine</b> Benzoylecgonine Cocaethylene Norcocaine	0.5 - - -	0.5 0.05 0.05 0.05
<b>Cannabinoids</b> THC THC-COOH	0.1 -	0.05 0.0002
<b>Opiates</b> Morphine Codeine 6-MAM	0.2	0.2 0.2 0.2
<b>Methadone</b> EDDP	0.2	0.2 0.05
<b>Buprenorphine</b> Norbuprenorphine	0.01	0.01 0.01
<b>Ketamine</b> Norketamine	0.5	0.5 0.1
<b>Benzodiazepines/z- drugs</b>	0.05	0.05