Drug and Alcohol Testing in Hair, Collection and Analysis

Version 1.0, valid from August 2010

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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 harmonize efforts by providing guidelines which are accepted at the European level.
- To ensure that the processes undertaken are capable of legal scrutiny.
- To define for laboratories common quality assurance and quality control criteria that are capable of being accredited by an external body.

2 Specimen Collection
2.1 General Rules

The collection of donor specimens involves some of the most difficult and sensitive areas of the workplace drug testing process. To ensure the integrity of the entire process, the collector must be very sensitive to each employee’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.
- The proper identification of the specimen with its donor.
  - Ensuring that no adulteration or tampering took place.
  - Ensuring that no unauthorized 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 minimize 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 custody and control form (CCF).

2.2 Collector Qualifications

Although no certification or medical education is usually required, a training course is necessary. The training course can for example be organised by a laboratory or an independent organisation or company.

On successful completion of collector training a person may begin performing collections. However, there are a few instances in which a collector may not perform a collection. These situations are:

- If the collector is the immediate supervisor of the donor (unless no other collector is available), or if the collector is a co-worker, a relative or a close friend of the employee,
- An individual working for a drug testing laboratory may not act as a collector if that individual can link the donor with the specimen drug test result unless he/she is bound by professional secrecy.

Collectors can be trained by various methods (video, classroom, internet, etc). However, the training must include, at a minimum, instructions on the following:

- The collection process
- The chain-of-custody process
- The process involved with “problem” collections (e.g. baldness)
- The responsibility of the collector for maintaining donor privacy, confidentiality of information, and specimen integrity
- Ethical issues, especially regarding the declaration by the donor of the present use of prescribed medications which may influence the result.

It is highly recommended that, upon completion of the training, each collector be tested on all subject matters covered in the training course to verify their understanding of the topics. It is also highly recommended that each training course include mock collections to assess collector competency.

2.3 Collection Site

A collection site is a facility (permanent or temporary) selected by the employer where donors present themselves for the purpose of providing a specimen.

Access to the facility must be restricted. Procedures for collection of specimens should allow for individual privacy. Preferably, there should be a sign outside prohibiting entry while a collection is occurring.

The collection area must be dedicated to sample collection and cannot be used for storage of any active material (drug).
All the necessary precautions should be taken in order to minimize contamination.

2.4 Custody and Control Form (CCF)

Chain-of-custody is the term used for the process of documenting the handling and storage of the hair specimen from the time the donor gives it to the collector until it is destroyed. A CCF is used to document the collection procedure and the chain-of-custody of the specimen. In Europe, there are many different types of CCFs. Almost every laboratory that performs workplace drug testing has its own version of this form which may also include different number of copies for each form.

The CCF is numbered with a unique specimen identification number and includes a sample label that is printed with the same specimen identification number as the CCF. The tamper-evident label also serves to seal the specimen collection kits and are applied across the hair collection envelope.

The information on the CCF can include:
- Unique specimen identification number.
- Name, address, e-mail address, and phone number of the testing laboratory.
- Information identifying the donor (e.g., birth date (DDMMYY), name, and home address).
- Information on how to reach the donor during daytime (i.e., telephone number).
- Information on how to reach a representative of the employer (i.e., name and telephone number).
- Medical review officer (MRO)/ occupational physician information (i.e., name, address, telephone, e-mail and fax numbers).
- Collection site information (i.e., collector name, telephone number).
- Date and time of the collection.
- Names and signatures of all individuals who had custody of the specimen during the collection process.
- Declaration by the donor of the present use of prescribed medications, of sample authenticity, correctness of sample labelling and package and permission for sample to be analyzed at the lab.
- Hair length to be analysed, hair colour, cosmetic treatment
- Substances abused, period of abuse, frequency of abuse
- Required analysis

There should be three parts or copies of the CCF to be distributed by the collector as follows:
- One part or copy to the testing laboratory (with the specimen)
- One part or copy to the donor
- One part or copy retained by the collector.
2.5 Specimen Collection

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. A sample protocol for hair collection is provided in Appendix 1.

2.6 Collection Process

The sampling does not need to be performed by a physician, but a responsible authority respecting legal, ethical and human rights of the customer. See section “Collector qualifications” above.

The following describes steps for a hair collection:

1. **Verify donor identity.** When the donor arrives at the collection site, the collector should request photo identification to verify donor identity. If a photo ID is not available, it is acceptable for the donor’s supervisor or other employer representative to identify the donor. If the individual’s identity cannot be established without a doubt, the collector should not proceed with the collection.

2. **Hair collection.** 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. Sufficient hair must be collected 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 is recommended. A sample protocol for hair collection is provided in Appendix 1.

3. **Fill in the required details.** The colour, length, body site and any obvious cosmetic treatment of the hair should be recorded. The donor should be asked to declare all cosmetic treatments in addition to the observed treatments. 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. chest, arm, thigh, pubic, axillary hair) can be collected if head hair is unavailable, if permitted by the company’s workplace drug testing policy and the donor.

4. **Put the locks of hair into the collection kits.** The collector splits the lock of hair into two collection kit envelopes.
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5. **Seal the kit.** The collector places the tamper-evident label/seal over both collection kit envelopes.

6. **Instruct the donor to annotate kit seals.** The collector instructs the donor to record her/his initials and date on each of the specimen kit seal.

7. **Annotate the CCF.** The collector completes appropriate sections of the CCF with donor information (e.g. date of birth, telephone numbers), collection information (e.g. date and time of the collection), and chain-of-custody entries, and instructs the donor to sign the CCF.

8. **Check the CCF.** The collector checks all copies of the CCF for legibility and completeness. If all copies are legible and complete, the collector then provides the donor a copy of the CCF and permits the donor to leave.

9. **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. Samples must be stored and shipped at room temperature, away from direct sunlight or humidity.

3 **Sample Preparation, Analysis and Storage**

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. 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 analyte, or contamination from other sources. Dry hair should be stored in the dark at room temperature.

In general, a decontamination strategy to remove gross environmental contamination must include an initial organic solvent, to remove lipophilic contaminations such as hair wax followed by aqueous washes.

The washings should be stored at 4°C for later analysis, if necessary.

After washing, the required hair segment is dried, and pulverized or cut into small pieces. A fixed amount of hair, for instance 25 mg is weighed.

Several extraction procedures have been published. These include:

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

Immunoassay screening (ELISA) can be applied. Presumptive positive samples need to be confirmed with a second different method, usually GC-MS or LC-MS. In order to improve the signal-to-noise ratio, a clean-up procedure, such as liquid-liquid extraction or solid-phase extraction followed by derivatization (if GC-MS is used) is recommended.

Receipt of specimen should be indicated by handwritten or electronic signature (or initials) of individuals receiving the specimens; at a minimum the date of receipt should also be included.

Any transfer of specimens must be documented as part of the permanent laboratory record.

Specimens must be stored for the time period agreed with the customer in a secure manner.

4 Cut-offs - Criteria for a Positive Drug Test

The positive result of a hair analysis may be used to confirm if a person has used or was exposed to a drug.

The Society of Hair Testing (SOHT) recommends the following cut-offs for hair testing in forensic cases¹ ²:

4.1 Alcohol

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.

From the different fatty acid ethyl esters, ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate can be chosen. For interpretation, the sum of the concentrations of these four esters can 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.

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

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 proximal segment.

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.

An EtG concentration equal to or greater than 7 pg/mg scalp hair indicates alcohol consumption and provides evidence to refute a claim of abstinence ³.

### 4.2 Opiates

**Immunochemical test:**
A morphine or 6-acetylmorphine level of 0.2 ng/mg must produce a positive result.

**Chromatographic 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.

### 4.3 Cocaine

**Immunochemical test:**
A cocaine level of 0.5 ng/mg must produce a positive result.

**Chromatographic 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 one of the following: benzoylecgonine, cocaethylene, norcocaine or eckgonine methyl ester.

### 4.4 Amphetamines

**Immunochemical test:**
A concentration of 0.2 ng/mg of each substance must separately produce a positive result: amphetamine, methamphetamine, MDMA, MDEA or MDA

**Chromatographic test:**

³ These cut-offs are not part of the SOHT recommendations; they are however adopted since 1st July 2009 by the German driving licence re-granting guidelines: Schubert W and Mattern R. (2009) Beurteilungskriterien: Urteilsbildung in der Medizinisch-Psychologischen Fahreignungsdiagnostik, ISBN 978-3-7812-1678-5, Kirschbaum Verlag Bonn, p.178
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Recommended cut-off: 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.

4.5 Cannabinoids

Immunochemical test:
A THC concentration of 0.05 ng/mg \(^4\) must produce a positive result.

Chromatographic test:
Recommended cut-off:
THC: \(\leq 0.05 \text{ ng/mg}^4\)
THC-COOH: \(\leq 0.2 \text{ pg/mg}\)

Confirmation of THC-COOH is required to definitively prove the use of cannabinoids.

4.6 Benzodiazepines \(^5\)

Immunochemical test:
A concentration of 0.05 ng/mg of Bromazepam, Nordiazepam, Oxazepam, Lorazepam, Alprazolam, Diazepam or Flunitrazepam must produce a positive result.

Chromatographic test:
Recommended cut-off: 0.05 ng/mg for each compound.

5 Quality Assurance

The quality standards set by ISO/IEC 17025 or ISO/IEC15189 must be fulfilled, meaning that the testing laboratory must be accredited by a recognised regulatory body. Specific areas relevant to hair analysis are listed below.

5.1 Personnel
The Laboratory must be staffed by suitably qualified personnel.

The Laboratory must keep 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.

\(^4\) The SOHT recommends 0.1 ng/mg hair for THC; The German guidelines for driving licence re-granting recommend 0.02 ng/mg hair. The cut-off of 0.05 ng/mg hair for THC is chosen as a compromise.

\(^5\) These cut-offs are not part of the SOHT recommendations; they are however adopted since 1\(^{st}\) July 2009 by the German driving licence re-granting guidelines: Schubert W and Mattern R., (2009) Beurteilungskriterien: Urteilsbildung in der Medizinisch-Psychologischen Fahreignungsdiagnostik, ISBN 978-3-7812-1678-5, Kirschbaum Verlag Bonn, p.178
All laboratory personnel must have received training in laboratory safety to ensure compliance with relevant legislation.

5.1.1 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).

Qualifications:
At least a University degree or degree equivalent in the chemical or biological sciences or medical technology. Training, experience and a thorough understanding of chain of custody procedures, quality control practices, and the theory and practice of all analytical methods and procedures used in the laboratory.

Responsibilities:

• Ensure that there are sufficient personnel with adequate training and experience to supervise and conduct the work of the drug-testing laboratory.
• Assure the continued competency of laboratory personnel by documenting their in-service training, reviewing their work performance, and verifying their skills.
• Ensure that the laboratory has a manual of Standard Operating Procedures (SOPs), which is complete, up-to-date, and available for personnel performing tests, and followed by those personnel.
• Maintain a quality control program to assure the proper performance and reporting of all test results in compliance with SOPs.
• Maintain acceptable analytical performance for all controls and standards for maintaining quality control testing.
• Assure and document the validity, reliability, accuracy, precision, and performance characteristics of each test and test system.
• Ensure that all remedial actions necessary to maintain satisfactory operation and performance of the laboratory are taken (eg in response to quality control systems not being within performance specifications, errors in result reporting or in analysis of external QA results), and that sample results are not reported until all appropriate corrective actions have been taken.
• Ensure that the results provided are accurate and reliable.

5.1.2 Authorising Scientist

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

- At least a degree or degree equivalent in, for example, the chemical or biological sciences or medical technology.
- Training and experience in the theory and practice of all methods and procedures used in the laboratory, including a thorough understanding of chain of custody procedures, quality control practices, and analytical procedures relevant to the results that the individual certifies.

Responsibilities:

- Ensure that the results provided are accurate and reliable.

5.1.3 Laboratory Analyst

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

Qualifications:

- Appropriate training and experience in the theory and practice of the procedures used in the laboratory.

Responsibilities:

- Maintenance of chain of custody.
- Day-to-day analytical procedures following SOPs.
- Remedial actions to be taken in response to test systems being out of control limits or detecting non-conforming test or quality control results.

5.1.4 Toxicologist

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

Qualifications:

- At least a degree or degree equivalent in, for example, the chemical or biological sciences/medical technology and pharmacology/toxicology.
- Training and experience in the theory and practice of all methods and procedures used in the laboratory, including a thorough understanding of chain of custody procedures, quality control practices, and analytical procedures relevant to the results that the individual interprets. Thorough understanding of pharmacology and/or toxicology.

Responsibilities:

- The interpretation of drug test results to the customer or the customer’s designated medical representative.
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5.1.5 Expert Witness

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

The qualifications and experience of this individual must be acceptable to the court or enquiry.

5.1.6 Other personnel

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

5.1.7 Quality Manager

The person who assumes responsibility for quality assurance within the laboratory.

Qualifications:
- Training and experience in auditing within an ISO or other relevant regulatory environment.

Responsibilities:
- Monitoring the laboratory's quality control programme.
- Auditing the laboratory operations in accordance with these guidelines.
- Verify that all remedial actions necessary to maintain satisfactory operation and performance of the laboratory are taken.

5.2 Accommodation and Environmental Conditions

The physical layout of the forensic toxicology laboratory must be such that unauthorized visitors cannot enter without detection.

Unauthorized visitors should be escorted and may be required to sign a log-book upon entry and departure from the laboratory, recording the time, date and purpose of the visit.

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 storage of hair samples in laboratories where seized drugs are analysed shall 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.
5.3 Method Validation

5.3.1 Screening Tests

Since the majority of the samples tested for WDT are negative and hence will only be screened, screening tests of high quality are a must 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 (>80%) calculated using at least 100 real hair samples,
- the number of false negative samples shall not exceed 2%.

5.3.2 Confirmation Tests

National or European guidelines for mass spectrometric identification and quantification of drugs must be taken into account.

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%);
- At least two different controls along the linearity range shall be
  - intra-assay imprecision (<20%),
  - inter-assay imprecision (<20%),
  - sensitivity limits

The lower limit of quantification (LLOQ) shall be calculated according to DIN 32645 and must be lower than the given cut-off.
- stability
- recovery

Additionally, the uncertainty of measurement should be calculated.

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5.4 Quality Control

5.4.1 Internal Quality Control

- A low and high level control 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 must be around the confirmation cut-off concentration (not greater than twice the confirmation cut-off concentration).
- For self-made spiked controls, the laboratory must define the upper and lower limits according to acceptable statistical criteria.

5.4.2 External Quality Control

Participation in proficiency testing schemes should be performed a minimum of two occasions each year.

6 Interpretation of Results

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.

6.1 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 – the higher the concentration, the longer the detection time;
- 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 e.g. the highest concentration of 9-carboxy-THC will rather be in the proximal than in the distal segment;
- 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 0.6 cm - 1.5 cm/month about 80-95% of the follicles remain in anagen stage (active growing phase). Growth rate could be influenced by therapeutic drugs age, sex, race and depends even on seasonal fluctuations.
European Workplace Drug Testing Society

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Body hair has a slower growth rate (0.5 - 1.1 cm/month) but a different growth cycle in comparison to scalp hair. In beard, axillary and pubic hair 40-60% remains in the resting phase. Therefore, body hair is not suitable for segmental analysis.

6.2 Passive Contamination

The following criteria are recommended for the exclusion of passive contamination:

a. the identification of metabolites; the following metabolites are recommended:
   i. Benzoylecgonine and cocaethylene for the confirmation of cocaine consumption;
   ii. 6-acetylmorphine and morphine for the confirmation of heroin consumption;
   iii. Carboxy-THC for the confirmation of cannabis consumption;
   iv. MDA for the confirmation of Ecstasy (MDMA) consumption;

b. the use of metabolite-to-parent drug ratios; the following may be used:
   i. Cocaine: benzoylecgonine/cocaine >0.05 (since benzoylecgonine is not always present, hydrolysis controls should be used)
   ii. Heroin: 6-acetylmorphine/morphine >1.3 (corrected for hydrolysis)

6.3 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, blonding 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 (e.g. a higher porosity) will be altered.

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 L.O.D. and hence cause false negative results.

7 Reporting the Results

Before reporting the results to the Medical review officer (MRO) or occupational physician, it must be ensured that the quality criteria for both the screening and confirmation tests are fulfilled. This must be checked by 2 qualified personnel, (for instance the laboratory analyst and the authorising scientist) and documented in the laboratory information system.

The minimum quality criteria are:
(i) the daily internal Q.C. (for both the screening and confirmation tests) must fit the acceptance criteria and
(ii) the fulfilment of the chromatographical identity criteria.
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The test report shall contain as a minimum the following information:

- Coded identification of the donor
- Date of sample collection
- Date of receipt of the hair sample in the laboratory
- Address and/or fax of occupational physician/MRO ordering the test
- Characterization of the hair sample:
  - type of hair (scalp, pubic, axillary, beard, chest, arm or leg hair)
  - original length of the hair segment
  - length of the analyzed hair segment
  - hair colour
  - 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 used
- Interpretation of the result:
  - Decision about negative or positive outcome
  - Statement about the minimal and maximal 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.
- Appendix 1 – Collection Instructions For Hair

A lock of hair about the width of a thin pencil, or several locks of hair are cut from the back of the head, twisted and fastened with a string.

The hair is to be cut just above the skin, as close to the scalp as possible. The length of remaining segment on the head should be recorded on the request form.

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. Both hair samples shall be sent to the laboratory in an envelope by post or by courier.