

FACTA Recommended Practice Guidelines for Drug Testing in Hair

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Foreword

This document was prepared by the FACTA Hair Drug Testing Working Group* and provides recommendations for best practice in the collection, analysis and interpretation of hair drug tests in Australasia. These guidelines are not mandatory but represent a consensus of the working group and FACTA membership in this area.

**HDTWG members:*

Amanda Thompson (chair)
Dimitri Gerostamoulos
Sue Nolan
David Batty
George Marshall
Sarah Russell
Christine Sellings
Michael Robertson

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1 Scope

The testing of drugs in hair has become commonplace and can be undertaken by accredited laboratories for the purpose of workplace testing, forensic cases, family law matters and other settings where this type of analysis is required. This document provides evidence-based guidelines for the collection and analysis of hair specimens as well as the interpretation of the testing results. These recommended practice guidelines incorporate current scientific knowledge of the advantages and limitations of drug testing in hair.

2 Specimen Collection

2.1 Human hair growth rates

It is generally accepted that hair from the vertex region of the head grows at an average rate of 1 cm per month, however this may vary between individuals [1,2]. Hair does not grow at a consistent rate, but transitions through various stages of growth and rest before falling out. Consequently, a segment of hair may contain drugs from a period outside of the expected or predicted timeframe.

Following drug exposure, it takes approximately 1-2 weeks for the hair containing incorporated drug to grow beyond the scalp, and possibly longer for it to be in hair which is long enough to cut. In the case of a single exposure to a drug (e.g. drug facilitated crime), hair should not be collected for several weeks following the incident [3]. (See 2.6 for information on non-head hair).

2.2 Collector

A collector is an appropriately trained / competent individual who completes a chain-of-custody (COC) procedure using a collection kit to collect an appropriate specimen of hair from a donor for analysis. A step-by-step procedure for specimen collection should be provided with the collection kit (see Appendix 1). This procedure should be formally documented by the laboratory and/or collection facility with appropriate reviews and updates, as required.

2.3 Collection of the hair specimen

Prior to specimen collection, the collector should explain to the donor the sampling procedure and the purpose of the testing. The donor must be identified by photo identification, or by a reliable witness (e.g. caseworker).

Collection should be from one donor at a time, and the hair specimen sealed in the kit prior to collecting from another donor. The collector must change gloves between donors to

avoid potential contamination, and fresh or decontaminated scissors/equipment should be used for each collection.

Ideally, the hair specimen should be taken from the posterior vertex (crown) region of the head as this region shows the least variation in growth rates between individuals. The hair should be cut as close to the scalp as possible, and the root end clearly identified for the testing laboratory. It is recommended to collect 2 hair specimens from each donor, stored in separate sealed specimen containers (envelopes), so that one may be kept as a reference specimen.

If insufficient head hair is available for sampling, hair from other regions of the body may be collected, however this will limit the interpretive value of the test results.

The amount of hair that needs to be collected will depend on the requirements of the testing laboratory. Sufficient hair must be collected to allow initial testing followed by re-testing of the specimen if necessary or confirmatory testing by a second laboratory. If there is insufficient weight of hair from one sampling site, or if the collection of a large area of hair would cause unnecessary trauma to the donor, multiple samplings may be combined. A larger weight of hair may be required if:

- the hair is very fine
- shorter segments are required to be analysed (e.g. 1cm)
- multiple tests are required (e.g. drugs, alcohol, steroids)

2.4 Chain-of-custody

Chain-of-custody (COC) is a series of procedures followed to account for the integrity of each hair specimen by tracking its handling and storage from the point of specimen collection to final disposal of the specimen. A COC form, either hard copy or electronic, is used from the time of collection of the specimen to its receipt at the laboratory as well as dispatch between laboratories (if relevant). Thereafter, appropriate documentation accounts for the hair specimen or aliquots within the laboratory.

There should be multiple copies of each set of forms. The number of copies will depend on the purpose of the hair testing, e.g. for workplace drug testing, copies should be available for the laboratory, donor, collector, requesting authority, and Medical Review Officer (MRO). Each set of documents should have the unique specimen identification number.

COC forms shall be completed by the collector and donor. Handling and transportation of hair specimens from one individual or place to another shall always be accomplished through chain-of-custody procedures. The number of persons handling specimens shall be kept to a minimum.

The COC documentation should have as a minimum the following information:

- Unique specimen identification number or barcode
- Testing laboratory details (name, address, email, phone number)
- Documented informed consent or a notation that a separate informed consent form has been completed
- A notation about the means used to unequivocally verify the donor's identity (e.g. driver's licence, passport, company ID or other appropriate measure)
- Two identifiers unique to the donor (e.g. name and date of birth)
- Date and time of specimen collection
- Confirmation by the donor that the specimen was their own. (This may require the donor's signature or initials on both the chain-of-custody form and the sealed specimen to be dispatched to the laboratory)
- Identification and signature of collector
- Declaration by the collector that the collection was performed in accordance with documented procedures
- Requesting authority details
- Required laboratory testing including period to be tested and drugs
- Site of hair collection (e.g. head, pubic, chest)
- Segments and length of specimen to be tested
- The hair colour and any obvious, observed or declared cosmetic treatments.

The COC documentation **may** also include on some copies:

- **Voluntary** declaration by the donor of the use of prescribed or over-the-counter medication
- **Voluntary** declaration by the donor of illicit or recreational substances used (do not include if there is a risk of self-incrimination)

2.5 Transport of the specimen

The hair specimen should be delivered directly to the testing laboratory, along with the relevant forms, via fully tracked postage or courier. Hair specimens should be stored at room temperature in a dry environment rather than refrigerated/frozen [4].

2.6 Non-head hair

Analysis of hair other than head hair (e.g. pubic, facial or axillary hair) is possible [4]. However, the analysis and interpretation of results has some particular limitations and drug concentrations cannot be directly correlated with head hair. Where non-head hair samples are collected the specimens should be limited to a single site e.g. facial hair or pubic hair. Some of the limitations in analysing non-head hair include:

- Obtaining enough specimen weight for analysis
- Different growth rates compared with head hair make it difficult to establish timelines of drug use
- The hair may be more susceptible to environmental contamination (e.g. pubic hair can be contaminated by sweat and/or urine).

2.7 Post-mortem collection

Postmortem hair specimens should be collected prior to autopsy to minimise contamination from body fluids. It is essential to note the condition of the hair (for example covered in blood, vomit or other bodily fluids) as these may lead to extensive contamination of the hair. As described above, ideally two separate specimens should be collected, and the locks be of a suitable size such that there is a straight cut edge. The cut edge must be clearly identified.

3 Laboratory and Staff Requirements

3.1 Laboratory accreditation

Although there is no International Standard that specifically relates to hair analysis, laboratories should be accredited to ISO/IEC 17025 or an equivalent standard.

3.2 Staff qualifications

The analysis of drugs in hair should be performed by a competent scientist according to the accreditation criteria of the laboratory.

Hair drug test results should be interpreted by an expert: that is, an individual with formal qualifications, training and experience with the application of hair drug test results and familiarity with the variables and nuances of the testing process. Examples include but are not limited to: toxicologist (clinical or forensic), physician, forensic medical officer (FMO), senior analytical chemist, medical review officer (MRO).

A laboratory may include interpretive comments on a hair drug test report if the laboratory has access to an expert.

3.3 Proficiency testing

The testing laboratory must be enrolled in an ongoing proficiency testing program. It should be noted that hair proficiency samples often display a wide range of drug concentrations between participating laboratories due to the inhomogeneity of the sample and the different extraction techniques used.

4 Analysis

4.1 Initial examination

An initial examination of the hair should record observations about the colour, length, alignment (or misalignment) of the root ends, and any obvious hair treatments such as bleaching or dyeing. Any relevant notes on the accompanying collection form should be considered along with these observations. Visible evidence of external contamination should be noted, particularly if the hair has been collected post-mortem.

4.2 Segmentation

The hair should be cut into appropriately sized segments following the directions of the request form. Analysis of segments longer than 3cm is not recommended due to the possibility of diluting any drug present in the hair. Multiple segments may be analysed if a longer timeframe of drug use is being investigated (e.g. 3 x 3cm).

4.3 Decontamination

A decontamination (washing) step is required prior to the extraction of drugs from the hair. In theory, this step is intended to remove drugs adhered to the outside of the hair, although this approach does have limitations. It should not be assumed that the post-wash hair is free of external drug contamination, nor that drugs found in the wash solvent are a result of external contamination.

The decontamination procedure may consist of one or more washing steps and may use aqueous and/or organic solvents. Decontamination should be performed after segmentation and prior to further cutting/pulverisation of the sample. A more thorough decontamination procedure may be warranted in specimens which are grossly contaminated (e.g. from post-mortem).

The effectiveness of the decontamination procedure should be assessed during method validation; however complete validation of this step is difficult due to the unavailability of control samples with known internal and external drug concentrations. It is recommended that hair specimens taken from drug users should be used to assess the efficacy of this step, rather than drug-fortified blank hair.

The analysis of the wash solvent may aid in the assessment of external contamination in some instances, and drug concentrations in the wash solvent may be reported with the hair results. The use of wash/hair drug ratios to assess the amount of external contamination in a specimen should be done with caution.

4.4 Cutting/Pulverisation

Cutting or pulverisation of the hair prior to extraction are both suitable techniques. The effectiveness of either method must be judged by sequential extractions of a hair specimen which has incorporated drugs following use (rather than spiked with drugs in the laboratory). For laboratories undertaking analysis of alcohol markers the use of powdered hair is recommended over cut hair [5]. Please refer to Appendix 2 for more information on alcohol markers.

4.5 Extraction

The hair should be incubated/extracted using solvent prior to instrumental analysis (unless using a direct analytical technique such as Matrix-Assisted Laser Desorption Ionisation, which is not covered in these guidelines). The use of a solvent such as methanol is recommended for broad drug screening. Alkaline or acidic solvent conditions may be required for more targeted analysis, but some drugs (or metabolites) may degrade during extraction under these conditions.

4.6 Identification/Quantification

Identification and quantification of drugs should be performed using a technique which couples chromatography and mass spectrometry. Validation of the method should be based on relevant guidelines for analytical methods. Identification criteria should take into account any selectivity issues observed during method validation. The detection of metabolites or specific biomarkers in addition to the parent drug assists in the interpretation of a result.

Due to the inherent heterogeneity of hair samples, duplicate analysis may produce wider variations in concentration than with other sample types. Where the drug concentration is greater than the validated range, it may be more appropriate to dilute the extract than to re-extract with a smaller amount of hair, providing that such a dilution has been validated. Alternatively, a laboratory may report a very high concentration as “greater than curve” (or a similar phrase).

4.7 Quality controls

The testing laboratory should incorporate the use of matrix matched quality controls into every batch and have defined acceptance criteria for these. Ideally, these controls should cover both low and high concentrations and may be commercial or prepared in-house (or a combination of these).

4.8 Cut-off concentrations

Hair drug testing is often used to determine whether a person has been regularly using a drug, so it is desirable to establish reporting cut-off concentrations for drugs which indicate repeated use. There are, however, a number of limitations of this approach, such as:

- reported drug concentrations are highly dependent on the extraction method used
- drug concentrations above the cut-off may persist in hair for some time after ceasing drug use
- high drug concentrations may result from external contamination rather than use.

Therefore, the use of reporting cut-off concentrations is dependent on the purpose of the testing.

Table 1 contains recommended cut-off concentrations in head-hair to establish repeated use. Laboratories reporting to these cut-offs may consider reporting metabolites at lower concentrations if it is likely to assist in interpretation and providing all criteria for identification are met. For cases where any detection of a drug is relevant (e.g. drug-facilitated sexual assault, zero-tolerance workplace settings, children), it may be more appropriate to report any drugs detected above the laboratory's limit of reporting.

Drug	Cut-off concentration (pg/mg)	relevant metabolites
AMPHETAMINES		
MDMA	200	MDA
MDA	200	
methylamphetamine	200	amphetamine
amphetamine	200	
OPIOIDS		
6-monoacetylmorphine	200	morphine
codeine	200	morphine, hydrocodone
morphine	200	
buprenorphine	10	norbuprenorphine
dihydrocodeine	200	
methadone	200	EDDP
oxycodone	200	
tramadol	200	O-desmethyl tramadol
COCAINE		
cocaine	500	benzoylecgonine, norcocaine, hydroxycocaine(s), cocaethylene
benzoylecgonine	50	
CANNABINOIDS		
delta-9-THC	50	THC-COOH
THC-COOH	0.2	
OTHER DRUGS		
ketamine	200	norketamine

Table 1. Cut-off concentrations to establish repeated use (for segments no longer than 3cm). These are based on the cut-off concentrations recommended by the Society of Hair Testing [3].

For many other drugs, including novel psychoactive substances, not enough data exists to establish cut-off concentrations. Drugs not included in this list should be reported at the discretion of the laboratory.

5 Reporting/Interpretation

It is important for laboratories to clearly communicate with their clients the appropriate uses and limitations of their testing methods. It is not essential to include a full interpretation of the results in the primary report; however, it is recommended to include at least a brief statement highlighting the complexity of interpreting drug concentrations in hair, and the necessity of consulting with an expert regarding any interpretation. This will reduce the risk of misunderstandings by those reading and using the report.

Where an interpretation of results is provided, the following should be taken into account:

- The uptake of drugs into hair following drug use may be influenced by hair colour
- The concentration of drugs in hair may be influenced by treatments such as bleaching or dyeing
- The possibility of external contamination rather than (or in addition to) use cannot always be discounted. The detection of metabolites greatly aids in identifying drug use
- The results of proficiency tests from the laboratory are a good indication of the extraction efficiency of their method relative to other laboratories
- The concentration of a drug in the hair cannot be used to estimate the dose consumed
- Precise dates and times of drug exposure cannot be determined from hair analysis
- Segmental analysis is useful to determine patterns of drug use over a longer time period, however limitations of segmental analysis (such as overlap of time ranges between segments, and movement of drug along the hair shaft) should be considered
- Single use versus repeated use
- A negative result does not necessarily exclude drug use/exposure
- All relevant factors in the case should be considered.

References

1. M.A. LeBeau, M.A. Montgomery, and J.D. Brewer, *The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair*. Forensic Sci Int, 2011. **210**(1-3): p. 110-6.
2. F. Pragst and M.A. Balikova, *State of the art in hair analysis for detection of drug and alcohol abuse*. Clinica chimica acta, 2006. **370**(1-2): p. 17-49.
3. G.A. Cooper, R. Kronstrand, and P. Kintz, *Society of Hair Testing guidelines for drug testing in hair*. Forensic Sci Int, 2012. **218**(1-3): p. 20-4.

4. *Guidelines for Testing Drugs under International Control in Hair Sweat and Oral Fluid*. 2014, UNODC, <https://www.unodc.org/unodc/en/scientists/guidelines-for-testing-drugs-under-international-control-in-hair-sweat-and-oral-fluid.html>.
5. P. Kintz, *2014 Consensus for the use of alcohol markers in hair for assessment of both abstinence and chronic excessive alcohol consumption*. Forensic Science International, 2015. **249**: p. A1-A2.
6. *2019 Consensus for the use of alcohol markers in hair for supporting the assessment of abstinence and chronic alcohol consumption*. Society Of Hair Testing <https://www.soht.org/consensus>.

6 Appendix 1 - Example Collection Instructions

The below instructions have been kindly provided by ToxLogic Pty Ltd. Please note that these are an example of an information sheet that may be provided to collectors/clients. It is an example only and may need to be modified depending on the requirements of collection agencies and/or laboratories.

- To complete a chain-of-custody (COC) hair specimen collection, you will need a collection kit*, a pair of small sharp scissors (hairdressing scissors are ideal) and powder-free disposable gloves. If the donor has long hair, a comb and hair clips may be useful.

*A collection kit generally has: COC paperwork, foils and envelopes, isopropanol wipe (or non-alcohol based equivalent), unique identifier number/barcode, plastic bag for collected specimens, security seal.

- Ensure the donor has photo identification or that the witness/collector can confirm the donor's identity.
- Complete the details as required on COC paperwork
- Indicate on the form what type of analysis is required (length/segments of hair, drug tests)
- Take out the foils from the glassine envelopes (2-4 will be required, as per test requested), Security Seal, specimen barcodes and isopropanol wipe.
- Clean the scissors with the isopropanol wipe. Ensure the scissors are dry before you cut the hair.



- Put on disposable gloves.
- The crown of the head is the preferred site for specimen collection. Isolate a hair lock for cutting (note that the amount of hair needed for testing will depend on the type and amount of tests).
- Cut this hair as close as possible to the scalp and parallel with the scalp.



- **Important** - Place the hair with the cut end (root end) on the foil at the foil marker (a coloured dot appears on both sides of foil) lengthwise. Put the entire length of hair in the envelopes – even if the donor has very long hair, please do not cut any length off the hair; the laboratory must receive the entire length.

NOTE – If body hair has been collected (chest, beard, axilla, etc.), the hair can be placed in the centre of the foil without aligning the hairs near the dot or overlapping the edge of the foil.



- Fold the foil around the hair to secure it within the foil. Take care not to bend the hair during this process.
- Place the foil containing the hair specimen into the glassine envelope.
- Seal the envelope by placing a barcode across the flap of the envelope. Repeat the process for any additional hair specimens from the same donor.
- Put all glassine envelopes containing locks of hair into the small plastic bag attached to the kit.
- Remove the sealing strip from the bag and seal the bag. Another barcode should be placed across the bag seal and initialled by both the collector and donor.



- Check all details on the request form have been completed, and that it has been signed by both collector and donor.
- On completion of the paperwork, the donor may wish to take a photo/copy of the paperwork.
- The kit can then be folded and the Security Seal placed across the seal or fold.

7 Appendix 2 - Alcohol biomarkers

Metabolites of ethanol include ethyl glucuronide (EtG) and ethyl palmitate (EtPa). EtG and EtPa can be measured in hair to assist in determining average drinking patterns of ethanol across the time period represented by the hair sample.

Natural hair colour does not influence EtG or EtPa concentrations however, like other drugs in hair, these may be affected by hair treatments.

Analysis: Pulverised or powdered hair is preferred for the extraction of EtG and/or EtPa.

Interpretation: When pulverised, the following cut-off concentrations for EtG and EtPa are recommended when analysing 3 cm of head hair. These should be considered approximate concentrations and where possible used in combination with other available evidence of use. When non-head hair is collected or the hair is not pulverised, interpretation should be performed cautiously.

Alcohol consumption	EtG	EtPa
Minimal use or abstinence	≤ 5 pg/mg	≤ 120 pg/mg
Repeated use	> 6 pg/mg	> 120 pg/mg
Excessive use (average of 60 g per day or greater)	≥30 pg/mg	≥ 350 pg/mg

These are based on the cut-off concentrations recommended by the Society of Hair Testing [6]