UNITED NATIONS INTERNATIONAL DRUG CONTROL PROGRAMME
Vienna

GUIDELINES FOR TESTING DRUGS UNDER INTERNATIONAL CONTROL IN HAIR, SWEAT AND SALIVA

FOR USE BY
NATIONAL LABORATORIES

Scientific Section

UNITED NATIONS
New York, 2001

ST/NAR/30/Rev.2

UNITED NATIONS PUBLICATION
Sales No. E.01.XI.12
ISBN: 92-1-148141-4

Acknowledgements

UNDCP's Scientific Section wishes to express its thanks to the experts who participated in the Consultative Meeting on "Recommended Methods for the Detection and Assay of LSD, PCP, Psilocybin and Methaqualone in Biological Specimens and on Guidelines for Testing Drugs Under International Control in Hair, Sweat and Saliva", Barcelona, Spain, 24 to 28 November 1997, for their contribution to the contents of this manual. The meeting was organized by UNDCP in collaboration with the Institut Municipal d'Investigació Mèdica.

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Special thanks go to Dr. Kintz, Mr. Nakahara, Dr. Sachs and Dr. Tagliaro for the preparation and to Dr. Drummer for the editing of this manual.

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INTRODUCTION

A. Background

Over the last decade there has been an enormous increase not only in the production and supply of illicit drugs, reflected by the huge and mounting quantities of drugs seized by national and international authorities, but also in the rate of drug abuse, i.e. the illicit demand for drugs. Drugs seized are not only traditional drugs already under national and international control, but also include unexpected new illicit drugs, including designer drugs, or combinations of drugs prepared by chemists working in clandestine laboratories. At the same time there are reports of expanding misuse/abuse of drugs used for medical purposes, such as benzodiazepines, antidepressants and therapeutic substitutes for opiates.

What used to be traditionally a problem of developed countries, is no longer confined to these countries. Drug abuse is now a global problem affecting developed and developing countries alike such that today no nation is free from this threat.

The extent and diversity of abuse have placed increasing demands on nations to intensify regulatory and control efforts, in some cases with the introduction of stringent legislation which may have serious consequences on the individual charged with drug offences. Ultimately, the final outcome of legislative provisions and court proceedings rests upon the results of laboratory tests. This has placed greater pressures on national laboratories which are now required not only to identify seized materials but also to detect drug use by testing biological specimens.

In the field of drug abuse, laboratories must now be able to deal with more controlled substances and to use methods of detection and analysis which are faster and yet, at the same time, are more accurate and specific. The analysis of biological specimens presents additional challenges such as the need for much higher sensitivity and difficulties with interpretation. In addition, there is a need to separate target substances from interferences in complex biological matrices.

Furthermore, the international nature of the drug abuse problem requires speedy exchange of analytical data between laboratories as well as between the laboratories and law enforcement agencies on national and international levels. Development of internationally acceptable methods of detection and assay contributes greatly towards the achievement of these objectives and the United Nations has been instrumental in achieving this aim. Two series of manuals have been prepared for use by National Laboratories dealing with methods for the analysis of seized materials and biological specimens respectively and in addition, guidelines have been prepared on relevant topics such as quality assurance, good laboratory practices and validation of analytical methods. The United Nations International Drug Control Programme (UNDCP) is mandated by both the Commission on Narcotic Drugs and the United Nations General Assembly to assist member countries in the development of national programmes to combat drug trafficking and drug abuse and to advise

them on recent developments which should be considered when these programmes are revised and further developed.

The present publication was prepared in response to a recommendation made in the Consultative Meeting in Beijing in 1996 which reflected growing world-wide interest in the use of biological specimens other than urine and blood when testing for illicit drug use. It is timely to assist laboratories in monitoring developments in this field and to provide some guidelines concerning how these alternate specimens can be used, how to begin working with them, and to provide analytical methods which can yield results which are valuable from an interpretive point of view.

For this purpose, as well as to review other scientific aspects of the problems associated with drug manufacture, trafficking and abuse, a meeting was convened in Barcelona in November, 1997. The meeting was principally to evaluate the feasibility of using biological specimens other than blood and urine for drug detection and to recommend guidelines for use by national drug testing laboratories.

B. Purpose of the manual

Previous UNDCP Publications have been manuals dealing with established methods recommended to laboratories in developing countries as reliable starting points for creating their own standard operating procedures. This manual is the product of the meeting in Barcelona and is intended to be an introduction to the topic of alternative specimens. It has been designed to provide an overview of the field and give some practical guidance to national authorities and analysts in the determination of controlled drugs in hair.

The guidelines in the manual represent a summary of the experience of scientists from several reputable laboratories around the world and of how alternative specimens can be used effectively. In general, this publication is also an attempt to help promote and harmonize national efforts by providing internationally acceptable guidelines. In addition, it is important to note that the manual is provided to laboratories as an educational document within the training remit of the UNDCP and as a means of encouraging laboratories to collaborate and participate with the United Nations in the global effort to combat illicit drugs.

Attention is also drawn to the importance of the availability of textbooks on drugs of abuse, analytical techniques and quality assurance, including reference books on statistical procedures used in quality assurance. Furthermore, the analyst is expected to keep abreast of developments in the field by following current literature on the subject.

UNDCP's scientific section would welcome observations on the contents and usefulness of the present publication. Comments may be addressed to:

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C. Introduction to alternative specimen analysis for drugs of abuse

The presence in the body of drugs of abuse can be assessed in different biological samples offering specific diagnostic opportunities in terms of a time window.

Since 1979 [1], hair has been used to document chronic drug exposure. To date, more than 400 articles concerning hair analysis have been published reporting basic studies and applications in clinical and forensic toxicology. Comprehensive information on this subject can be found in several references [2-6].

The major practical advantage of hair testing compared with urine and blood testing for drugs is its larger detection window, which is weeks to months, depending on the length of hair shaft analysed, against a few days for urine. In practice, detection windows offered by urine and hair testing are complementary: urinalysis provides short-term information of an individual's drug use, whereas long term histories are accessible through hair analysis.

The number of drugs that are known to be detectable in hair is increasing steadily, however most publications are concerned with a small number of substances, including opiates, cocaine and the stimulants. More recently, particular attention has been focused on cannabis, benzodiazepines and doping agents.

Although there is a reasonable agreement that the qualitative results from hair analysis are valid, the interpretation of the results and the value of quantitative data are still under debate.

Sweat has relatively recently been shown to offer a possibility to detect drugs of abuse both in drug treatment centres and also for enforcement purposes and offers potential.

Saliva has been used to measure drug content for therapeutic drugs for many years, but have only recently been advocated for the detection of drugs of abuse.

Both sweat and saliva offer new non-invasive techniques requiring little specialist technical skills and show potential in complementing hair as alternative specimens to blood and urine.

This manual therefore provides readers an overview of the utility of these three alternative specimens for drugs of abuse testing.

I. HAIR

A. The hair matrix

Hair is a complex tissue, the biology and physiology of which are incompletely understood. Hair is an annex of skin, originated from the hair follicle in which the germination centre is formed by matrix cells. These cells give rise to the different layers of the hair shaft, including cuticle and medulla. In the root, cells are in active proliferation, while in the hair shaft the residual metabolism is negligible. The most important components of the hair stalk are fibrous proteins (keratins), melanins and lipids.

There are basic structural similarities between hair of different colour, ethnic origin and body region. The fundamentals of hair composition, anatomy and physiology have been summarised in articles by Harkey [7] and Cone and Joseph [8].

Normal hair grows in a series of stages: the anagen (growing stage), the catagen (transition stage) and the telogen (resting stage). The possible length of the hair and the density of a scalp depend on the duration of these stages and rate of growth. The duration of these stages varies from person to person and even in the scalp of one person. Average values for the anagen stage are 4-6 years, the catagen stage a few weeks and the telogen stage 4-6 months. The scalp hair growth rate reported in the literature ranges from 0.7 to 1.5 cm per month.

There are substantial differences in growth rate and the anagen/telogen ratio between scalp hair and hair from other body regions which have not been fully investigated yet. For example, the telogen phase of pubic hair accounts for about half of its lifetime.

B. Incorporation of drugs into hair

The mechanisms of drug incorporation into hair matrix are still not completely clarified. However, the first model proposed to explain this phenomenon was one in which drugs enter hair by passive diffusion from the blood into the growing cells in the hair follicle. More recent findings indicate that drugs may be incorporated from different sources, such as diffusion from blood into the follicle during formation of the hair shaft, diffusion from body secretions (e.g. sweat and sebum) and contamination from external environmental sources (after hair shaft formation) [9].

From a chemical point of view, there are three main factors for drug incorporation into hair: melanin affinity, lipophilicity and basicity. Scientific data show that melanin plays an important role for drug incorporation into hair [10-12]. In an animal study it was demonstrated that there was a good correlation between melanin affinity and drug incorporation into hair [10]. In a human study, it was confirmed that drug concentration in pigmented hair was much higher than that in nonpigmented hair [12, 13].

It has also been demonstrated that lipophilicity of drugs is one of the key factors for drug incorporation into hair. For example, cocaine and 6-acetylmorphine

are much better incorporated into hair than benzoylecgonine and morphine [14, 15]. Also, acetylamphetamine, which is no longer basic, is poorly incorporated into hair compared to methamphetamine. This fact suggests that basicity is an important factor for drug incorporation into hair [16]. A comparison of ratios of plasma AUC (area under curve) and hair concentration has shown that cocaine, PCP and MDMA are highly incorporated into hair, while THC acid is poorly incorporated [10].

Although the mechanisms of drug incorporation into hair still remain unclear, drug concentration in hair is nevertheless dependent on the ability to incorporate drugs and the ability to retain drugs in hair.

C. Specimen collection

According to the recommendations of the Society of Hair Testing [17], sample collection should be performed by a responsible authority respecting the legal, ethical and human rights of the person being tested. Hair samples should be obtained in a non drug contaminated environment by appropriately trained individuals, but not necessarily by a medical practitioner. A sufficient amount of sample should be collected, so that further analyses can be performed on the same sample if needed.

The following general collection procedures are recommended:

- ❒ Hair should be collected from the posterior vertex region of the scalp;
- ❒ Hairs should be tied together and cut as close to the skin as possible;
- ❒ In order to maintain a statistical significance of the sample and/or to perform a screening test as well as a confirmation test, the weight of the specimen should aim to be approximately 200 mg (a suitable amount of sample could be estimated by comparison with the diameter of a pencil);*
- ❒ For shipment and storage, the hair sample should be wrapped in aluminum foil to maintain integrity and to avoid contamination;
- ❒ Specimens can be stored under dry conditions at room temperature.

D. Sample preparation

Decontamination procedures

All hair samples must undergo a decontamination procedure. Different procedures have been reported in the literature. These include:

- ❒ First wash with an organic solvent, like methanol or dichloromethane, followed by an aqueous wash, and finally with an organic solvent such as dichloromethane or methanol [18, 19].
- ❒ Wash with 0.1 % sodium dodecylsulphate three times, and rinse with distilled water three times [20].

Final preparation stage

After washing, a measured segment of hair is dried, and then can be pulverized in a ball mill [19], or cut into small pieces [20]. Finally, the sample, or a portion of it, must be accurately weighed.

Particular attention must be paid to avoid contamination from the environment, especially by using an analytical balance other than that used for weighing drugs.

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*Note that this amount is not always attainable, in which case the specimen collector should aim for the maximum amount possible.

E. Extraction and clean-up

Several extraction procedures have been described. These include:

- ❒ Methanol incubation [21]: 4 ml methanol, sonication for 5 hours at 50 °C;
- ❒ Hydrochloric acid incubation [22]: 2 ml 0.25 M HCl overnight at 45 °C;
- ❒ Alkaline hydrolysis [23]: 1 ml of 1 M NaOH for 10 min at 95 °C;
- ❒ acidic methanol [15]: 2 ml of methanol-5 M HCl (20:1) sonication for 1 hour, stored at room temperature overnight;
- ❒ Soerensen buffer incubation [24]: pH 7.6 for 2 hours at 40 °C; and
- ❒ Enzymatic incubation [25]: 2 ml of acetate buffer of pH 4 and 50 μl β-glucuronidase-arylsulfatase for 2 hours at 40 °C.

Comparative efficiencies of the four extraction techniques has been described [26] and a review of specimen preparations has been published [27].

After evaporation of the methanol to dryness, specimens can be directly analysed if the selectivity of the analytical method is sufficient [28]. However to improve the signal-to-noise ratio, a sample clean up procedure and derivatization might be necessary. The clean-up step can be carried out either by liquid-liquid extraction or solid-phase extraction, according to the analytical procedure adopted. See below for examples.

F. Analytical techniques

Immunoassays, like ELISA [29] or RIA [30, 31] can be used for screening purposes. However, results from immunoassays must be confirmed by a chromatographic method or any other independent technique providing equivalent selectivity. The sensitivity of the confirmatory technique must be at least equal to the screening method for each individual compound.

Practical procedures

Four reliable methods routinely used in laboratories and incorporating different analytical features are summarised below. Full details on the analytical procedure can be found in the specific references.

Method 1 [21, 28]

Analytes:	Amphetamine, MDA, MDMA, MDEA, cocaethylene, dihydrocodeine, heroin, methadone, 6-monoacetylmorphine, morphine and tetrahydrocannabinol (THC).
Decontamination:	5 ml water (to be analysed), 5 ml acetone, 5 ml petroleum ether, then dried.
Preparation:	50-200 mg, cut in small sections.
Extraction:	4 ml methanol plus deuterated internal standards, ultrasonic bath (120 W) for 5 h at 50 °C.

Clean-up :	None.
Evaporation:	Add 3 drops acetic acid and evaporate under nitrogen at 100 °C.
Derivatization:	50 µl propionic acid anhydride for 1 h at 100 °C, evaporation to dryness, reconstitution in 50 µl ethyl acetate + 1 or 5 % propionic acid anhydride.
Chromatography:	HP-5 (amphetamines), DB-1 (other drugs), or equivalent, 20 m × 0.25 mm i.d. × 0.25 µm film thickness, temperature programme designed for drugs of interest. Use electron impact mode MS.

Method 2 [25, 32]

Analytes:	Amphetamine, methamphetamine, cocaine, benzoylecgonine, ecgonine methylester, dihydrocodeine, heroin, 6-monoacetylmorphine, acetylcodeine, morphine, methadone and EDDP (methadone metabolite).
Decontamination:	20 ml water twice, 20 ml acetone.
Preparation:	10-30 mg, pulverization with ball mill.
Extraction:	Add 100 ng deuterated internal standards, 2 ml phosphate buffer pH 7.6 and 75 µl β-glucuronidase (12 U/ml)/ arylsulfatase(60 U/ml) for 2 h at 40 °C.
Clean-up:	Add 2 ml phosphate buffer pH 7.6 and apply to 3 ml solid phase Chromabond C18 extraction column previously treated with 3 ml methanol and 3 ml water. The column was washed with 3 ml water, 3 ml 0.6 M $NaHCO_3$ and 3 ml water and eluted with 3 × 500 µl acetone-dichloromethane (3:1).
Evaporation:	Under nitrogen at 60 °C.
Derivatization:	100 µl pentafluoropropionic anhydride and 70 µl pentafluoropropanol for 30 min at 60 °C, evaporation to dryness, and reconstitution in 30 µl ethyl acetate.
Chromatography:	HP-Ultra 2 capillary column, or equivalent, 12 m × 0.2 mm i.d. × 0.33 µm film thickness, programmed from 70 °C (after 1 min hold) to 300 °C at 10 °C/min. Helium flow at 2 ml/min. Mass spectrometer at 70 eV, electron impact mode.

Method 3 [18]

Analytes:	Cocaine, benzoylecgonine, ecgonine methylester, anhydroecgonine methylester, cocaethylene, 6-monoacetylmorphine, morphine and codeine.
Decontamination	Twice with 5 ml dichloromethane.
Preparation:	50 mg, pulverization with ball mill.
Extraction:	1 ml 0.1 MHCl and deuterated internal standards, incubated overnight at 56 °C.

Method 3 [18] *(continued)*

Clean-up:	1 ml 0.1 M NaOH and phosphate buffer (1 M) at pH 8.4 and chloroform-isopropanol-n-heptane (50:17:33); purification of the organic phase with 5 ml 0.2 M HCl; back extraction at pH 8.4 with 2 ml phosphate buffer (1 M) into 5 ml chloroform.
Evaporation:	Speed vac concentrator at 45 °C.
Derivatization:	30 µl BSTFA + 1 % TMCS 20 min at 70 °C.
Chromatography:	BP-5 capillary column, or equivalent, 12 m × 0.22 mm i.d., with helium at 1.8 ml/min, temperature programme from 60 °C to 310 °C at 30 °C/min. Electron impact mode MS, 70 eV.

Method 4 [14, 20, 33, 34]

Analytes:	Cocaine, cocaethylene, benzoylecgonine, ecgonine methyl ester, methamphetamine, amphetamine, ephedrine, cathine, propanolamine, MDA, MDMA, MDEA, PCP.
Decontamination:	Wash with 1 ml 0.1 % SDS 3 times and rinse with 1 ml water.
Preparation:	10-20 mg, cut in small sections.
Extraction:	2 ml methanol-5 M HCl (20:1) and deuterated internal standards, ultrasonication for 1 h at <20 °C, stored overnight at room temperature and filtrate evaporated off under nitrogen.
Clean-up:	Bond Elut Certify columns; residue dissolved in 0.1 M potassium phosphate buffer (pH 6.0), applied to activated column, washed with 1 ml volumes of water, 0.1 M acetic acid and water, successively, dried and eluted with 3 ml methanol- 5 M HCl (20:1). Columns were activated with methanol and phosphate buffer [33].

For PCP and metabolites drugs were eluted with methanol-28 % ammonia solution (98:2) [34].

Evaporation:	Nitrogen at 40 °C.
Derivatization:	*Amphetamines:* 200 µl pentafluoropropionic acid anhydride/ethylacetate (1:1) for 20 min at 60 °C; evaporate off reagents under nitrogen and reconstitute into 50 µl ethyl acetate [33]. *PCP and metabolites:* 50 µl N,O-bis-trimethylsilylacetamide (BSA) for 20 min at 90 °C; direct injection of cool solution into GC/MS [34]. *Cocaine specimens:* 100 µl pentafluoropropionic acid anhydride and 50 µl pentafluoropropanol for 20 min at 60 °C, evaporation to dryness, reconstitution in 50 µl ethyl acetate and injection into GC/MS.
Chromatography:	Use conditions mentioned previously, designed for separation.

Standards for calibration curves can be prepared by adding drugs in methanol to drug free hair. A suitable concentration range for analysis of opiates, cocaine and amphetamines is in the range 0.1–10 ng/mg. For cannabis the concentration range of the standard curve should be 0.01-10 ng/mg [28].

Reviews of the current analytical methods for hair analysis for drugs of abuse have recently been published [35-38].

G. Analytical problems

Because of the high sensitivity requirements of hair analysis and the potential interferences from the hair matrix, which is substantially different from more common samples like urine and blood, selective instrumentation is needed for providing accurate and precise results.

Hair is a solid, heterogeneous matrix which makes calibration particularly complicated, compared with homogeneous liquid samples. Spiked samples do not mimic exactly the real samples, in which drugs are incorporated during hair growth. Standard material is also difficult to produce and is available only from institutions with experience in hair analysis.

Because of its high surface to volume ratio, hair samples are easily susceptible to contamination from environmental sources [39, 40]. This must be taken into account especially in laboratories where seized street drugs are analysed.

Although methanol incubation, after a decontamination procedure, is almost a universal extraction method, which allows a high number of analyses per day, it tends to produce chromatograms with a high background. If necessary, as for other specimens, a specific sample clean-up procedure can be performed. See previous section for specific examples.

H. Interpretation of drug concentrations in hair

Because of differences in hair growth rate depending on anatomical region, gender, age, race and interindividual variability, there are difficulties in the interpretation of drug concentrations in hair.

One theory of drug incorporation into the hair matrix is that it takes place at the root level. If no other mechanism operated, this would allow details of the time and dose to be obtained from segmental analysis of hair. Some reports support this hypothesis, but on this point recent research has raised serious deficiencies with this theory and provide evidence of drug uptake into hair from skin secretions and from external contamination [9, 41, 42]. It is now clear that the mechanism of drug incorporation into hair is complex.

It is difficult to estimate drug doses from their concentrations in hair, because there are large inter-individual differences regarding hair texture, hair colour, metabolism, bioavailability of drugs and hair growth rate. Nevertheless, some studies have shown a certain correlation in estimated drug consumption, i.e. high, medium or low, from hair concentrations obtained from hair segments close to the root [43-46].

Some other important issues in hair analysis are still open. First of all, it has been demonstrated that hair can be contaminated from the environment, if exposed to solutions or from airborne particles of drugs. Several attempts have been made to distinguish external contamination from the presence of drugs in hair due to active use [47], but this point is still highly controversial [48-51].

In order to evaluate the possibility of passive contamination, four criteria are recommended to confirm drug use by the individual concerned:

- ❒ Identification of metabolites;
- ❒ Use of metabolite-to-parent drug ratios;
- ❒ Assay of decontamination washes; and
- ❒ Examination of specimens collected from other body regions.

The stability of drugs in hair, although generally considered good, has been found compromised by hair cosmetic treatments like bleaching and dyeing [52, 53].

Colour of hair, age, gender and differences in hair structure may also affect drug concentration in hair [46, 54-56].

A further variable is the choice of cut-off levels by the laboratory for screening and confirmation. Depending on whether cut-off values are used for specific analytes, they will affect the analytical detection process [36, 57].

I. Conclusions and recommendations

For twenty years, scientists have improved analytical procedures to test drugs in hair and documented interpretation of their findings [58]. It is clear that a great deal of research still has to be performed before scientific questions and problems surrounding hair drug testing are completely satisfied. It is generally accepted however, that routine analysis of drugs in hair can provide much useful information, provided analytical tests are appropriate and adequate quality control is included in the testing process.

Hair testing has already been accepted in many countries, particularly in Europe and in North America. At present, hair analysis is routinely used as a tool for the detection of drug use in clinical toxicology and forensic sciences, as well as traffic-related and occupational medicine.

Primarily, hair testing is a complementary test for urinalysis. Advantages over urinalysis include a longer detection window, a stable specimen to store and non-invasive sampling. Disadvantages include the relatively low concentrations usually detected in hair and the relatively complicated procedures required for analysis.

It is recommended that a high efficiency separation analytical technique be used at least for the confirmation of both negative and positive results. Capillary gas chromatography combined with mass spectrometry is the most common technique used for this purpose. Other suitable methods can be based on high-performance liquid chromatography with selective detectors (including diode array, electrochemical and mass spectrometric detectors) or capillary electrophoresis. A final confirmation by a mass spectrometric technique is recommended, when technically available.

Laboratories where hair analysis is performed should be totally separated from laboratories where street drugs are analysed or stored.

To prevent breakdown of analytes, such as heroin and/or 6-monoacetylmorphine to morphine, and cocaine to ecgonine, alkaline hydrolysis should be avoided. Acid extraction conditions should be also avoided to avert formation of benzophenones from benzodiazepines.

If results from a first hair sample are contested by the subject claiming external contamination of the sample or analytical mistakes, a second sample may be collected within a reasonable time (no more than few weeks) from the first specimen. The analytical results from this specimen should match the initial testing process to ensure the reliability of the overall analytical procedure.

The approaches to the analysis of alternative specimens reviewed in this publication have been selected on the basis of proven usefulness and value. However, while several are provided, which can in part be used directly, it is recommended that the manager of each laboratory supervise the validation of any method(s) to be used. Of course, the final choice of method used remains in the hands of the laboratory manager.

Attention is drawn to the importance of adequately trained staff where matters of method development and validation are concerned: implementation of a written or formalized standard operating procedure as required by an internal quality system and an external accreditation system. Proper and efficient laboratory practices will be more effective when carried out in co-operation with informed and trained staff.

An important adjunct to the development of a validated method is participation in collaborative studies and, if possible, an external proficiency testing programme. At present, programmes for hair testing are provided by the National Institute for Standards and Technology, Gaithersburg, Maryland, USA and by the Society of Hair Testing, Strasbourg, France. Participation in an external proficiency programme is also a means to improve the educational level of the staff involved in drugs of abuse analysis and to provide laboratories with means of checking their own performance by comparing it with other laboratories carrying out similar analyses.

II. SWEAT

A. Anatomy and physiology

Sweat is a natural secretion in all humans particularly at higher ambient temperatures. Two types of sweat glands are found in humans: eccrine glands of about 4 to 6 million, distributed over nearly the entire body surface and particularly abundant on palms, soles, axillae, forehead and chest; and apocrine glands located in the skin of axillae, areola of nipple and anogenital areas. Sebaceous glands, found everywhere except on palms and soles, are particularly abundant on the face. They produce sebum and not sweat.

Approximately 50 % of all sweat is produced by the trunk, 20 % by the legs, and 30 % by the head and upper extremities. The rate of sweating is usually ~20 ml/h but can be much higher [59].

The major role of eccrine sweat is the control of body temperature through evaporative heat loss. Sweat is also involved in immunological protection and hydration of the skin. Sweat is composed at 99 % of hypotonic aqueous solution (50 mM Na^+), lactate, urea, ammonium ions and some enzymes. The pH ranges from 4 to 6.

B. Specimen collection

Researchers have known since 1911 [60] that drugs are excreted by the body in sweat, but it was not until recently that a practical method of capturing sweat was developed. An occlusive bandage consisting of 1 to 3 layers of filter paper [61] or pieces of cotton, gauze, or towel [59] were previously proposed to collect sweat. Thermal [62] or pharmacological stimulation was proposed to excrete a usually large amount of sweat [63].

Significant advances have been made during the past years to develop a sweat patch technology such as that recently developed by Pharmchem Laboratories [64]. The sweat patch acts as a specimen container for non-volatile liquid components of sweat, including drugs of abuse. Sweat components are collected on a special absorbent pad of 14 cm^2, located in the centre of the patch. Non-volatile substances from the environment cannot penetrate the transparent film, however a semi-permeable membrane over the pad allows oxygen, water and carbon dioxide to pass through the patch, leaving the skin underneath healthy. Over a period of several days of contact with skin, sweat saturates the pad and slowly concentrates drugs present in sweat.

The sweat patch is applied to the outer portion of the upper arm or back, after the skin area has been cleaned with an isopropyl alcohol swab. The patch can be removed several days later (3-10 days) by pulling an edge of the adhesive backing. After removal of the patch, the absorbent part is stored in a plastic tube at –20 °C.

C. Analysis of sweat

Sweat analysis is relatively straightforward, since matrix effects seen in blood, urine and hair analysis are essentially absent in sweat.

Target compounds can be extracted from the pad with 2.5 ml of 0.2 M (pH 5.0) acetate buffer with methanol (25:75, v/v) [65] or with 5 ml methanol [66] or acetonitrile [67].

A solid phase enzyme immunoassay using microlitre plates can be used for screening, but confirmation must be by GC/MS. Most of the studies described in the literature use GC/MS with deuterated internal standards, without using a prior methanol cleaning step.

D. Results

As with hair, the parent drug is the major component found in sweat [68, 69].

For example, in a study conducted on 14 subjects receiving heroin-HCl intravenously daily in the range 80 to 1,000 mg, concentrations (ng/patch) ranged from 2.1 to 96.3 for heroin, 0 to 24.6 for 6-monoacetylmorphine, and 0 to 11.2 for morphine [67].

Opiates generally can be detected in sweat using sweat patches [69].

Cocaine appeared in sweat within 1 to 2 h and peaked within 24 h, in an apparent dose-dependent manner. A low dose of cocaine (approximately 1 to 5 mg) produced detectable amounts in sweat [70]. Crack use can be identified in sweat (and in urine, hair and saliva) through the detection of anhydroecgonine methylester [71].

When 8 volunteers participating in a methadone treatment were monitored, the substitute drug could always be detected in the patch, associated with a minor concentration of methadone metabolite (EDDP) [72].

In another study [66] conducted in a detoxification centre, sweat patches (PharmCheck™) were applied to 20 known heroin abusers for 5 days. During the same period, 2 urine specimens were also collected. Heroin (37-175 ng/patch) and 6-monoacetylmorphine (60-2386 ng/patch) were identified in 8 cases and codeine exposure (67-4018 ng/patch) was found in 4 cases. Cocaine (324 ng/patch) was found in only one case. Δ^9-THC (4-38 ng/patch) was identified in 9 cases. MDEA (121 ng/patch) and its metabolite MDA (22 ng/patch) were detected in one case. Buprenorphine, which was administered as therapy under close medical supervision, was detected in the range 1-153 ng/patch, with no apparent relationship between the daily dose and amount excreted in sweat. All urine test results were consistent with the sweat analysis findings. However, in order to identify the same drugs, it was necessary to test two urine specimens where only one sweat specimen was required to detect drug use.

Similarly, methamphetamine was readily detected in sweat using a sweat collection device (PharmCheck™) as was amphetamine in users wearing the patch for 1-7 days using confirmation cut-off values [73].

E. Drugwipe device

Drugwipe™ is an immunochemical test for the detection of traces of controlled drugs on any kind of surface that can be performed in 2 minutes. Recently, the use of this device has been proposed for the collection of sweat from the armpits and to

test for opiates, cocaine and cannabinoids [74]. Comparisons of findings with blood and/or urine obtained from drivers controlled by the police, results of the Drugwipe showed positive reactions for opioids and cannabis in 70-80 % of cases.

Further studies are necessary to document the usefulness of this and other similar devices, particularly for on-site drug testing. However, such devices do not prove drug use; they only give evidence of exposure of a subject to drugs, which can be used for further investigation.

III. SALIVA

A. The physiology of saliva production

Saliva refers to any fluid that discharges into the oral cavity. Saliva acts to moisten the mucous membranes of the upper digestive tract, to facilitate speech, to control bacterial flora of the mouth, to assist in digestion, and to supply certain hormones and other active substances into the oral cavity. Saliva is excreted primarily by three glands; the parotid, submaxillary and sublingual and by other small glands such as labial, buccal and palatal glands [75]. Mixed saliva used for drug analysis consists approximately of 65 % submandibular, 23 % parotid, 4 % sublingual; the remaining 8 % from the other 3 glands.

The daily flow of saliva in an adult ranges from 500 to 1500 ml. Saliva flow is mediated by a number of physiological factors particularly emotional factors, age, gender and food intake. The flow of saliva is dependent on neurotransmitter stimulation and can vary from zero to several ml/min; although flow rates for the parotid and submaxillary are < 1 ml/min. Craving for food is clearly an important factor. Flow rates are also dependent on age of person and seasonal influences. The pH ranges from 6.2 to 7.4 [76].

Stimulation of saliva flow can lead to a change in pH. A review on the physiology and other analytical aspects of saliva are available [76, 77].

Drugs can affect the production of saliva. The tricyclic antidepressants cause dry mouth due to their anticholinergic activity.

The fluids secreted by the various salivary glands differ considerably from each other. Their composition is affected by time of day, food, age, gender, state of health, and by drugs. In general saliva is made of the usual electrolytes as well as mucus and amylase. Protein content is less than 5 % of plasma.

Salivary glands have a high blood flow from the carotid arteries and its branches in the submandibular and sublingual glands. For drugs circulating in the blood they must pass through the capillary walls, the basement membrane and the membrane of the glandular epithelial cells. The rate determining step is the passage of drug through the epithelial membranes. Physiochemical principles generally determine the rate and extent of transport. Saliva is not an ultrafiltrate of blood, as is sometimes been suggested, rather a complex fluid formed by different mechanisms against a concentration gradient, by pinocytosis, by ultrafiltration through pores in the membrane and by active transport [76]. Passive diffusion is apparently the dominant mechanism.

B. Specimen collection

Specimen collection should be performed by a person with responsible authority respecting the legal, ethical and human rights of the person being tested.

The four most common methods [78] are:

Draining method: saliva is allowed to drip off the lower lip into a preweighed tube,

Spitting method: saliva is allowed to accumulate in the floor of the mouth and the subject spits the collected saliva into a preweighed tube,

Suction method: saliva is continually aspirated from the floor of the mouth into a tube by a aspiration device or saliva ejector, and

Absorbent method: saliva is absorbed onto a swab or similar absorbent material which is removed for reweighing at the end of the collection period.

Saliva is best taken while the subject is sitting upright with the head tilted forward and the eyes open. Subjects should refrain from smoking, eating or drinking for at least 30 minutes prior to the sampling [78]. It is necessary to ensure the oral cavity is free from food material and other objects prior to collection.

Saliva should be taken by appropriately trained individuals, but not necessarily a physician. Specimens can be stored frozen at –20 °C, unless analysis will be conducted shortly after collection.

Samples should be centrifuged to ensure particulate and other material does not interfere with sampling.

C. Saliva collection devices

A number of devices are available to stimulate saliva formation and facilitate the collection [78-80]. These are recommended over passive collection of saliva. These will also provide less opportunity for contamination than other means of collecting saliva. Four examples are briefly described below.

Salivette®

The Salivette® device uses dental cotton wool contained in a roll to absorb saliva. Typically a roll is chewed for 30-45 seconds with or without stimulation. The roll is then placed into a container which is centrifuged for 3 minutes at 1000 RPM. The saliva is collected as a fluid at the bottom of the tube. Cellular particles are contained in a sink below the fluid. Volumes collected are about 1.5 ml.

Orasure®

The Orasure device® uses a pad which is placed between check and gums. This collects a mixture of gingival fluid and saliva. Total volume collected is 1.0 ml.

Saliva Sampler®

This device uses a sterile collection pad which is placed under the tongue. When indicator litmus turns blue the collector is placed into a special container. Volume collected is 0.8-1.3 ml which takes from 1 to 7 minutes.

Saliva Lollipop®

The saliva lollipop is an absorbent device which is used to collect a sample of saliva. This specimen is then transferred to a hand-held reader which tests up to eight classes of drug on a single 0.15 ml saliva. The process takes 3-5 minutes.

D. Extraction and analytical techniques

Saliva can be extracted and analysed as other biological fluids such as blood. In general there will be less interference with saliva from endogenous compounds than with blood or urine. However, one should ensure that all necessary validation of drug-free and drug-spiked saliva is carried out prior to conducting case work.

Care should be exercised in the use of screening and confirmation tests designed for other matrices, since cut-off levels may be different.

E. Problems of testing

Since the concentrations of drugs are often less than in blood, higher analytical sensitivity is required. This may test the instrumentation being used.

The use of devices to stimulate saliva flow will also change the level of drug due to osmotic and possibly also pH changes. There is also a potential problem of the device interfering with the analytical test [81].

Saliva volumes will usually be small, hence there will be limited ability to repeat analyses. Additionally, not all subjects will be able to provide saliva on demand.

Immunoassays are also directed to metabolites of drugs. This could limit its applicability since profiles of drug metabolites may be different in saliva. Laboratories should validate immunoassay kits properly before use.

F. Interpretation of drug concentrations in saliva

Interpretation of saliva drug concentrations is more difficult than for blood, since saliva concentrations are subject to more variables than blood, such as degree of protein binding and pK_a of drug and pH of saliva.

Saliva has been widely used to detect drugs and has been shown to correlate well with the non protein-bound fraction in blood.

Contamination of saliva by recently ingested drug is a real problem with saliva (e.g. cannabis), although this may be useful if evidence of recent exposure to a drug (through inhalation or oral use) is required.

Predictions of saliva to plasma ratios can be calculated from the following equations [79].

$$S/P = \frac{1 + 10^{(pH_s - pK_a)}}{1 + 10^{(pH_p - pK_a)}} \times \frac{f_p}{f_s}$$

Acidic drugs

$$S/P = \frac{1 + 10^{(pK_a - pH_s)}}{1 + 10^{(pK_a - pH_p)}} \times \frac{f_p}{f_s}$$

Basic drugs

Where S = concentration of drug in saliva
P = concentration of drug in plasma
pK_a = pK_a of drug
pH_s = saliva pH
pH_p = plasma pH
f_p = free (unbound) fraction of drug in plasma
f_s = free (unbound) fraction of drug in saliva

With these equations, the pH of plasma is generally constant at 7.4 and the degree of protein binding in saliva is close to zero. Basic drugs have high S/P ratios due to pH effects. Amphetamine, cocaine and codeine have S/P values exceeding unity.

Salivary concentrations of THC, cannabinol (CBN) and cannabidiol (CBD) after oral administration of 5 mg of each (peaked at 1 h) were 135, 178 and 158 ng/ml, respectively [82]. Detection times in saliva were 6 to 10 h, compared to under 6 h for plasma. A good correlation was found for saliva and plasma THC concentrations following smoking standard marijuana cigarettes. Interestingly, the S/P ratio increased with time [83].

Cocaine and benzoylecgonine have been detected in saliva of subjects taking the drug by smoking and nasal insufflation for at least 16 h after administration. Levels were higher in saliva than in serum and urine and the authors concluded that saliva was suitable for screening for recent cocaine use [84]. Cocaine was detected in saliva of chronic cocaine users for 5-10 days during abstinence suggesting that deep tissue stores of cocaine exist [85]. The smoking of crack also leads to formation of anhydroecgonine methylester which can be targeted in saliva, as well as in hair and sweat [71].

Salivary levels of amphetamine were 2-3 fold higher than plasma levels and in the postabsorptive phase were predictably proportional to plasma drug levels [86]. Amphetamines were detected in saliva for 48 h after drug administration (10 mg).

Barbiturates are found in saliva for up to 48 h, however concentrations are less than unity compared to plasma. Examples include amobarbital, secobarbital, phenobarbital, and hexobarbital, all of which have S/P ratios around 0.3 [83]. Methaqualone has also been detected at a ratio of 0.1. Chloral hydrate and meprobamate are also present in saliva.

Benzodiazepines have also been detected in saliva; however, because of their extensive protein binding (often >95 %), little drug is present in saliva. For example, chronic users of diazepam have salivary concentrations of diazepam and its metabolite nordiazepam ranging from 1 to 23 ng/ml. Corresponding plasma levels range up to 1000 ng/ml [87]. Consequently, sensitive assays are needed to measure benzodiazepines in saliva.

In general when contamination of the oral cavity was excluded there was a good correlation between saliva and plasma concentrations for most drugs.

Reviews published on this topic include references 76 and 88 through 90. Papers detailing use of saliva in therapeutic drug monitoring include references 77 and 91, and the Bremen workshop held in 1988 [92].

G. Conclusions and recommendations

Saliva analysis for drugs of abuse has not received as much attention as other alternative specimens in the recent literature. However, saliva is being evaluated in the area of drugs and driving, since saliva can be taken non invasively.

There is also substantial literature on saliva for therapeutic drug monitoring [76, 77, 81, 88, 91, 92]. Readers are also referred to standard references.

REFERENCES

1. Baumgartner, A. M., *et al.*, *Radioimmunoassay of hair determining opiate-abuse histories.* Journal of Nuclear Medicine, 1979. **20**: p. 748-752.
2. Forensic Science International. Vol. 63. 1993: Elsevier. 1-316.
3. Forensic Science International. Vol. 70. 1995: Elsevier. 1-222.
4. Forensic Science International. Vol. 84. 1997: Elsevier. 1-313.
5. Cone, E. J., J. Welch, and M. B. Grigson-Babecki, *Hair testing for drugs of abuse.* International Research on Standards and Technology, NIH Publication No. 95-3727. 1995, Rockville, MD: National Institute on Drugs Abuse.
6. Kintz, P., ed. *Drug testing in hair.* 1st ed. 1996, CRC Press: Boca Raton, Florida. 293.
7. Harkey, M. R., *Anatomy and physiology of hair.* Forensic Science International, 1993. **63**: p. 9-18.
8. Cone, E. J. and R. E. Joseph, *The potential for bias in hair testing for drugs of abuse*, in *Drug Testing in Hair*, P. Kintz, Editor. 1996, CRC Press: Boca Raton, Florida. p. 69-93.
9. Henderson, G. L., *Mechanisms of drug incorporation in hair.* Forensic Science International, 1993. **63**: p. 19-29.
10. Nakahara, Y., K. Takahashi, and R. Kikura, *Hair analysis for drugs of abuse. X. Effect of physicochemical properties of drugs on the incorporation rates into hair.* Biol Pharm Bull, 1995. **18**(9): p. 1223-1227.
11. Harrison, W. H., R. M. Gray, and L. M. Solomon, *Incorporation of d-amphetamine into pigmented guinea pigs.* British Journal of Dermatology, 1974. **91**: p. 415-418.
12. Slawson, M. H., *et al.*, *Quantitative determination of phencyclidine in pigmented and nonpigmented hair by ion-trap mass spectrometry.* Journal of Analytical Toxicology, 1996. **20**: p. 355-361.
13. Uematsu, T., *et al.*, *Possible effect of pigment on the pharmacokinetics of ofloxacin and its excretion in hair.* Journal of Pharmaceutical Sciences, 1992. **81**: p. 45-48.
14. Nakahara, Y. and R. Kikura, *Hair analysis for drugs of abuse. VII. The incorporation rates of cocaine, benzoylecgonine and ecgonine methyl ester into rat hair and hydrolysis of cocaine in rat hair.* Arch Toxicol, 1994. **68**(1): p. 54-9.
15. Nakahara, Y., R. Kikura, and K. Takahashi, *Hair analysis for drugs of abuse. VIII. Effective extraction and determination of 6-acetylmorphine and morphine in hair with trifluoroacetic acid-methanol for the confirmation of retrospective heroin use by gas chromatography-mass spectrometry.* J Chromatogr B Biomed Appl, 1994. **657**(1): p. 93-101.
16. Nakahara, Y. and R. Kikura, *Hair analysis for drugs of abuse. XIII. Effect of structural factors on incorporation of drugs into hair: the incorporation rates of amphetamine analogs.* Arch Toxicol, 1996. **70**(12): p. 841-9.

17. *Society of Hair Testing.* Forensic Science International, 1997. **84**: p. 3-6.

18. Kintz, P. and P. Mangin, *Simultaneous determination of opiates, cocaine and major metabolites of cocaine in human hair by gas chromotography/mass spectrometry (GC/MS).* Forensic Science International, 1995. **73**: p. 93-100.

19. Cirimele, V., *et al.*, *Testing human hair for flunitrazepam and 7-amino-flunitrazepam by GC/MS- NCI.* Forensic Sci Int, 1997. **84**: p. 189-200.

20. Nakahara, Y., *et al.*, *Hair analysis for drugs abuse: I. Determination of methamphetamine and amphetamine in hair by stable isotope dilution gas chromatography/mass spectrometry method.* J Forensic Sci, 1991. **36**(1): p. 70-78.

21. Roehrich, J. and G. Kauert, *Determination of amphetamine and methylenedioxy- amphetamine-derivatives in hair.* Forensic Science International, 1997. **84**: p. 179-188.

22. Tagliaro, F., *et al.*, *Integrated use of hair analysis to investigate the physical fitness to obtain the driving licence: a case study.* Forensic Science International, 1997. **84**: p. 129-135.

23. Cirimele, V., *et al.*, *Testing human hair for cannabis. III. Rapid screening procedure for the simultaneous identification of delta-9-tetrahydrocannabinol, cannabinol and cannabidiol.* Journal of Analytical Toxicology, 1996. **20**: p. 13-16.

24. Kintz, P., *et al.*, *Hair analysis for nordiazepam and oxazepam by gas chromatography-negative-ion chemical ionization mass spectrometry.* Journal of Chromatography B, 1996. **677**: p. 241-244.

25. Moeller, M. R. and C. Mueller, *The detection of 6-monoacetylmorphine in urine, serum and hair by GC/MS and RIA.* Forensic Science International, 1995. **70**: p. 125-133.

26. Couper, F. J., I. M. McIntyre, and O.H. Drummer, *Extraction of psychotropic drugs from human scalp hair.* J Forensic Sci, 1995. **40**(1): p. 83-6.

27. Chiarotti, M., *Overview on extraction procedures.* Forensic Science International, 1993. **63**: p. 161-170.

28. Kauert, G. and J. Roehrich, *Concentration of delta9-tetrahydrocannabinol, cocaine and 6-monoacetylmorphine in hair of drug abusers.* International Journal of Legal Medicine, 1996. **108**(294-299).

29. Segura, J., *Possibilities of ELISA methodologies for hair analysis*, in *Hair Analysis in Forensic Toxicology. Proceedings of the 1995 International Conference and Workshop*, R.e.a. de Zeuw, Editor. 1995: Abu Dhabi. p. 351-369.

30. Cassani, M. and V. Spiehler, *Analytical requirements, perspectives and limits of immunological methods for drugs in hair.* Forensic Science International, 1993. **63**: p. 175-184.

31. Baumgartner, W. A., *et al.*, *Detection of drug use by analysis of hair.* Journal of Nuclear Medicine, 1988. **29**: p. 980.

32. Moeller, M. R., P. Fey, and R. Wennig, *Simulataneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program.* Forensic Science International, 1993. **63**: p. 185-206.

33. Kikura, R., *et al.*, *Hair analysis for drugs of abuse. XV. Disposition of 3,4-methylenedioxymethamphetamine (MDMA) and its related compounds into rat hair: application to hair analysis for MDMA abuse.* Forensic Science International, 1997. **84**: p. 165-177.

34. Nakahara, Y., *et al.*, *Hair analysis for drugs of abuse. XVII. Simultaneous detection of PCP, PCHP, and PCPdiol in human hair for confirmation of PCP use.* Journal of Analytical Toxicology, 1997. **21**: p. 356-362.

35. Moeller, M. R., *Drug detection in hair by chromatographic procedures.* Journal of Chromatography B, 1992. **580**: p. 125-134.

36. Cairns, T., D. J. Kippenberger, and A. Gordon, *Hair analysis for the detection of drugs of abuse*, in *Handbook of Analytical Therapeutic Drug Monitoring and Toxicology*. 1997, CRC Press: Boca Raton, Florida. p. 237-251.

37. Moeller, M. R. and H. P. Eser, *The analytical tools for hair testing*, in *Drug testing in hair*, P. Kintz, Editor. 1996, CRC Press: Boca Raton, Florida. p. 95-120.

38. Tagliaro, F., *et al.*, *Hair analysis, a novel tool in forensic and biomedical sciences: new chromatographic and electrophoretic/electrokinetic analtyical strategies.* Journal of Chromatography B, 1997. **689**: p. 261-271.

39. Cone, E. J., *et al.*, *Testing human hair for drugs of abuse. II. Identification of unique cocaine metabolites in hair of drug abusers and evaluation of decontamination procedures.* Journal of Analytical Toxicology, 1991. **15**: p. 250-255.

40. Welch, M. J., *et al.*, *Hair analysis for drugs of abuse: evaluation of analytical methods, environmental issues, and development of reference materials.* Journal of Toxicology, 1993. **17**: p. 389-397.

41. Cone, E. J., *Mechanisms of drug incorporation into hair.* Theraeutic Drug Monitoring, 1996. **18**: p. 438-443.

42. Henderson, G. L., *et al.*, *Incorporation of isotopically labelled cocaine and metabolites in the human hair: 1. Dose-response relationships.* Journal of Analytical Toxicology, 1996. **20**: p. 1-12.

43. Nakahara, Y. and R. Kikura, *Hair analysis for drugs of abuse. XVIII. 3,4-Methylenedioxymethamphetamine (MDMA) disposition in hair roots and use in identification of acute MDMA poisoning.* Biol Pharm Bull, 1997. **20**(9): p. 969-72.

44. Nakahara, Y., *Detection and diagnostic interpretation of amphetamines in hair.* Forensic Sci Int, 1995. **70**(1-3): p. 135-53.

45. Nakahara, Y., M. Shimamine, and K. Takahashi, *Hair analysis for drugs of abuse. III. Movement and stability of methoxyphenamine (as a model compound of methamphetamine) along hair shaft with hair growth.* J Anal Toxicol, 1992. **16**(4): p. 253-7.

46. Wilkins, D. G., *et al.*, *Disposition of codeine in female human hair after multiple-dose administration.* Journal of Analytical Toxicology, 1995. **19**: p. 492-497.

47. Baumgartner, W. A. and V. A. Hill, *Sample preparation techniques.* Forensic Science International, 1993. **63**: p. 121-135.

48. Kidwell, D. A. and D. L. Blank, *Comments on the paper by W. A. Baumgartner and V. A. Hill: sample preparation techniques.* Forensic Science International, 1993. **63**: p. 137-143.

49. Kidwell, D. A. and D. L. Blank, *Environmental exposure—The stumbling block of hair testing*, in *Drug Testing in Hair*, P. Kintz, Editor. 1996, CRC Press: Boca Raton, Florida. p. 17-68.

50. Blank, D. L. and D. A. Kidwell, *External contamination of hair by cocaine: an issue in forensic interpretation.* Forensic Science International, 1993. **63**: p. 145-156.

51. Baumgartner, W. A. and V. A. Hill, *Comments on paper by D. L. Blank and D. A. Kidwell. External contamination of hair by cocaine: an issue in forensic interpretation.* Forensic Science International, 1993. **63**: p. 157-160.

52. Cirimele, V., P. Kintz, and P. Mangin, *Drug concentration in human hair after bleaching.* Journal of Analytical Toxicology, 1995. **19**: p. 331-332.

53. Jurado, C., *et al.*, *Influence of the cosmetic treatment of hair on drug testing.* International Journal of Legal Medicine, 1997. **110**: p. 159-163.

54. Skopp, G., L. Potsch, and M. R. Moeller, *On cosmetically treated hair—aspects and pitfalls of interpretation.* Forensic Sci Int, 1997. **84**(1-3): p. 43-52.

55. Offidani, C., S. Strano Rossi, and M. Chiarotti, *Drug distribution in the head, axillary and pubic hair of chronic addicts.* Forensic Science International, 1993. **63**: p. 105-108.

56. Green, S. J. and J. F. Wilson, *The effect of hair color on the incorporation of methadone int hair in the rat.* Journal of Analytical Toxicology, 1996. **20**: p. 121-123.

57. Kintz, P. and P. Mangin, *What constitutes a positive result in hair analysis: proposal for the establishment of cut-off values.* Forensic Science International, 1995. **70**: p. 3-11.

58. Sachs, H., *History of hair analysis.* Forensic Science International, 1997. **84**: p. 7-16.

59. Ishiyama, I., *et al.*, *The significance of the drug analysis in sweat in respect to rapid screening for drug abuse.* Zeitschrift fuer Rechtsmedizin, 1979. **92**: p. 251-256.

60. Tachau, H., *Ueber den Uebergang von Arzneimitteln in der Schweiss.* Archive fuer Experimentelle Pathologie und Pharmakologie, 1911. **66**: p. 224-246.

61. Parnas, J., *et al.*, *Excretion of antiepileptic drugs in sweat.* Acta Neurologica Scandinavia, 1978. **58**: p. 197-204.

62. Fox, R. H., *et al.*, *The nature of the increase in sweating capacity produced by heat acclimatization.* Journal of Physiology, 1964. **171**: p. 368-396.

63. Balabanova, S., *et al.*, *Die Bedeutung der Drogenbestimmung in Pilocarpinschweiss fuer dwn Nachweis eines zurueckliegenen Drogenkonsums.* Beitrage ueber Gerichtliche Medizin, 1992. **50**: p. 111-115.

64. Sutliff, J., *Detecting drugs in perspiration*, . 1993, Pharmchem Laboratories: Menlo Park, CA.

65. Spiehler, V., *et al.*, *Enzyme immunoassay validation for qualitative detection of cocaine and sweat.* Clinical Chemistry, 1996. **42**: p. 34-38.

66. Kintz, P., *et al.*, *Sweat testing in opioid users with a sweat patch.* Journal of Analytical Toxicology, 1996. **20**: p. 393-397.

67. Kintz, P., *et al.*, *Sweat testing for heroin metabolites in a heroin maintenance program.* Clinical Chemistry, 1997. **43**: p. 736-739.

68. Kintz, P., *Drug testing in addicts: a comparison between urine, sweat and hair.* Therapeutic Drug Monitoring, 1996. **18**: p. 450-455.

69. Fogerson, R., *et al.*, *Qualitiative detection of opiates in sweat by EIA and GC-MS.* Journal of Analytical Toxicology, 1997. **21**: p. 451-458.

70. Cone, E. J., *et al.*, *Sweat testing for heroin, cocaine and metabolites.* Journal of Analytical Toxicology, 1994. **18**: p. 298-305.

71. Kintz, P., *et al.*, *Evidence of crack use by anhydroecgonine methyl ester identification.* Human and Experimental Toxicology, 1997. **16**: p. 123-127.

72. Skopp, G., *et al.*, *Preliminary practical findings on drug monitoring by a transcutaneous collection device.* Journal of Forensic Sciences, 1996. **41**: p. 933-937.

73. Fay, J., *et al.*, *Detection of methamphetamine in sweat by EIA and GC-MS.* Journal of Analytical Toxicology, 1996. **20**: p. 398-403.

74. Sachs, H. *Detection of illegal narcotics on the skin of drug users with drugwipe.* in *International Congress of Therapeutic Drug Monitoring in Clinical Toxicology.* 1997. Vancouver, Canada.

75. Schneyer, L. H., J. A. Young, and C. A. Schneyer, *Salivary secretion of electrolytes.* Physiological Reviews, 1972. **52**: p. 720-777.

76. Hold, K. M., *et al.*, *Saliva as an analytical tool in toxicology*, in *International Journal of Drug Testing (http://big.stpt.usf.edu/~journal/volume1.html).* 1996. p. 1-29.

77. Danhof, M. and D. D. Breimer, *Therapeutic drug monitoring in saliva.* Clinical Pharmacokinetics, 1978. **3**: p. 39-57.

78. Navazesh, M., *Methods for collecting saliva.* Annals of the New York Academy of Science, 1993. **694**: p. 73-77.

79. Rasmussen, F., *Salivary excretion of sulfonamides and barbiturates by cows and goats.* Acta Pharmacologica et Toxicologica, 1964. **21**: p. 11-19.

80. *Saliva Sampler*, in *Internet address http://www.sav.com/.* 1998.

81. Cone, E. J. and A. Jenkins, J., *Saliva Drug Analysis*, in *Handbook of Analytical Therapeutic Drug Monitoring and Toxicology*, S.H.Y. Wong and I. Sunshine, Editors. 1997, CRC Press: Roca Baton, Florida. p. 303-333.

82. Kircher, V. and H. Parlar, *Determination of delta-9-tetrahydrocannabinol from human saliva by tandem immunoafinity chromatography-high performance liquid chromatography.* Journal of Chromatography B, 1996. **677**: p. 245-255.

83. Cone, E. J., *Saliva testing for drugs of abuse.* Annals of the New York Academy of Science, 1993. **694**: p. 91-127.

84. Schramm, W., *et al.*, *Cocaine and benzoylecgonine in saliva, serum and urine.* Clinical Chemistry, 1993. **39**: p. 481-487.

85. Cone, E. J. and W. W. Weddington, *Prolonged occurrence of cocaine in human saliva and urine after chronic use.* Journal of Analytical Toxicology, 1989. **13**: p. 65-68.

86. Wan, S. K., S. H. Matin, and D. L. Azarnoff, *Kinetics, salivary excretion of amphetamine isomers and effect of urinary pH.* Clinical Therapeutic Monitoring, 1978. **23**: p. 585-590.

87. De Gier, J. J., *et al.*, *Psychomotor performance and real driving performance of outpatients receiving diazepam.* Psychopharmacology, 1981. **73**: p. 340-344.

88. Schramm, W., *et al.*, *Drugs of abuse in saliva: a review.* Journal of Analytical Toxicology, 1992. **16**: p. 1-9.

89. Kidwell, D. A., *Discussion: caveats in testing for drugs of abuse*, . 1992, NIDA.

90. Cone, E. J., *The validity of self-reported drug use: improving the accuracy of survey estimates*, 1997, NIDA.

91. Mucklow, J. C., *et al.*, *Drug concentration in saliva.* Clinical Pharmacology and Therapeutics, 1978. **24**: p. 563-570.

92. *The application of saliva in laboratory medicine*, in *Journal of Clinical Chemistry and Clinical Biochemistry*. 1989, German Society for Clinical Chemistry: Bremen, Germany. p. 221-252.