Hair analysis has been an established procedure in forensic toxicology for many years, but serious commercial work started in the 1980s, when SureScreen analysed thioglycollate from a hair perming solution insurance disaster that left many women completely bald. We realised this could form an ideal method for extracting drugs from hair, and developed a method; however Psychemedics in America patented a similar digestion process shortly after, forcing all other interested labs to either cut the hair into segments, or mill the hair to a powder, followed by extraction in a solvent such as methanol. Some have also used supercritical solvent extraction in a reaction vessel with cut hair.

Drugs in the blood are deposited in the hair shaft as it grows, in proportion to the quantity ingested, leaving behind permanent traces in the hair shaft that toxicologists later release and analyse. Only the hair core should be analysed, so the surface which is exposed to cosmetics, shampoo and bleach will not affect the results of the test. Hair therefore cannot easily be adulterated; as a consequence the products sold on the internet for passing a hair drug test are largely ineffective.

**TEST METHODOLOGY**
The test methodology has only recently become available commercially since the development of sensitive screening immunoassay kits, (first designed for urine and oral fluid) and supported by highly-discriminating GC/MS and LC/MS instruments. In theory, if an immunoassay test can be produced, the test can be offered commercially, and if the method of extraction and chromatography process can handle it, then it can be analysed. Currently, over 300 different drugs can be detected in a hair sample.

As a system for detecting drug abuse, hair analysis is ideal, provided the window of detection is not too wide, and cost, and longer timescales are acceptable. However, these limitations have so far largely restricted commercial interest in hair analysis for illicit drugs.

As a consequence, the vast majority of employment drug screening is done by rapid testing on urine or oral fluid samples. Rapid tests instantly screen out all the negative samples cheaply and accurately. Positives can then be confirmed in the laboratory, although often the donor will admit drug use when presented with a positive rapid test result; a process now seen as acceptable by many, provided the donor will sign the consent form accordingly.

Hair analysis is more frequently chosen as the optimum pre-employment test because unlike urine or oral fluid, hair samples cannot be masked by abstaining prior to interview. Psychemedics, now the leader in this field in America, has analysed over two million samples; there is an increasing business with a number of other laboratories also offering this service. Around four times as many people are caught with hair samples compared to urine testing, largely due to drug abstinence.

In Britain, where company screening attitudes are more liberal, hair testing is still considered inconvenient, too expensive and too invasive of privacy by many customers, so it is mainly used in legal cases, or in occasional situations where a donor fails a urine drug test and declares their drink must have been spiked. Hair analysis will establish whether the donor is a drug user or not, because a one-off use will probably not be detected in hair, while regular use most certainly will. The process has also been used to track the history of the user, sometimes on a month-by-month basis, however this process usually requires a
considered approach accompanied by a toxicologist's interpretation. But hair testing deserves to be positioned along with the other methods described in this series of Bulletins, because each has its own advantages. This is why companies like SureScreen have developed a range of methodologies to suit all applications. Each has its place in drug screening.

**CUT OFF LEVELS**

It should be appreciated that the cut off levels applied to hair are totally different to those in urine and oral fluid, because they are related to hair weight (nanogram of drug detected in one milligram of hair, for example), and not fluid concentration (nanogram of drug per millilitre, for example). Laboratories can differ in how they report values, which are sometimes quoted as a concentration in ng/mg or in ng/10mg; the latter being ten times greater, so the two should not be confused. Others may quote cannabis (THC) as pg/10mg, which is 100 times the value of ng/mg.

The cut off value applied to drug testing is there to reflect a true positive and it is designed to avoid the possibility of a false positive from environmental contamination. A value above a cut off level can therefore be trusted with confidence. This is certainly true of drugs that are detected as the parent drug in hair, (for example, amphetamine), due to the possibility of environmental contamination. Other drugs such as cocaine and cannabis (which are coincidentally the most likely to produce environmental contamination because they are smoked) produce metabolites in hair. Positive results for these metabolites is a guarantee that the drug was ingested, since this is the only way that the metabolite can be incorporated in hair.

**COMMON DRUGS AND CUT OFF LEVELS FOR ANALYSIS**

Amphetamines  
Methamphetamines and Ecstasy (MDMA)  
Cocaine (cocaine and cocaine metabolite)  
Cocaethylene (cocaine in conjunction with alcohol)  
Opiates (codeine, morphine, heroin metabolite)  
Extended opiates: Hydrocodeone (Vicodin, Lorcet, Lortab)  
Extended opiates: Hydromorphone (Dilauidid), Oxycodone (Percolet)  
Phencyclidine (PCP)  
THC metabolite (Cannabis and Marijuana)

‘SPECIALS’

Benzodiazepines (including flunitrazepam (Rohypnol))  
Ketamine  
Barbiturates  
GHB

**DRUGS**

<table>
<thead>
<tr>
<th>DRUG</th>
<th>SCREEN</th>
<th>CONFIRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines</td>
<td>5ng/10mg</td>
<td>5ng/10mg</td>
</tr>
<tr>
<td>Cocaine/metabolite</td>
<td>5ng/10mg</td>
<td>5ng/10mg</td>
</tr>
<tr>
<td>Opiates</td>
<td>3ng/10mg</td>
<td>3ng/10mg</td>
</tr>
<tr>
<td>Extended opiates</td>
<td>3ng/10mg</td>
<td>3ng/10mg</td>
</tr>
<tr>
<td>PCP</td>
<td>3ng/10mg</td>
<td>3ng/10mg</td>
</tr>
<tr>
<td>THC metabolite</td>
<td>0.010ng/10mg</td>
<td>0.003ng/10mg</td>
</tr>
<tr>
<td>Benzoylcegonine</td>
<td>-</td>
<td>0.5ng/10mg</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>-</td>
<td>0.5ng/10mg</td>
</tr>
<tr>
<td>Norcocaine</td>
<td>-</td>
<td>0.5ng/10mg</td>
</tr>
<tr>
<td>Codeine</td>
<td>-</td>
<td>2ng/10mg</td>
</tr>
<tr>
<td>6MAM</td>
<td>-</td>
<td>2ng/10mg</td>
</tr>
<tr>
<td>THC Limit of Detection</td>
<td>-</td>
<td>0.2pg/10mg</td>
</tr>
</tbody>
</table>

For these metabolites, it is acceptable to increase the sensitivity of the test to LOD or ‘limits of detection’. Most laboratories have a limit of detection at least five times more sensitive than the stated cut-off level – this can be useful when analysis has to be very sensitive, such as in drug rape cases. SureScreen’s pioneering forensic work on drug rape cases has also shown that hair sebum contains significant traces of recently ingested drug, and analysing an unwashed hair sample often makes all the difference between detection and a negative result. This procedure can be acceptable if the unfortunate victim clearly does not take drugs routinely.

SureScreen also often acts as an independent expert in hair analysis cases and has noticed that results from different laboratories on essentially the same sample can be surprisingly variable. However this should be expected because extraction processes, analytical techniques, instruments and the like are never quite the same, and as we shall see, hair samples taken from the same donor are not as uniform as might be expected. It does mean, however, that hair analysis results should not be taken as absolute. Clients should appreciate that hair analysis is still mainly intended to be a yes/no test unless special clinical assessments are made on the results.

**DRUG METABOLITES**

Cocaine is usually detected in hair as the parent drug and its metabolite, benzoylcegonine, may also be detected, though not always. The combined use of alcohol and cocaine frequently results in the metabolite cocaethylene being detected.

When crack cocaine is smoked the drug undergoes pyrolysis, forming anhydrocegonine methyl ester (AEME) and ecgonine. The presence of AEME in hair indicates a positive association with crack abuse.

Heroin use produces significant amounts of the metabolite 6-monooacetylmorphine (6-MAM) in hair, along with the final decomposition product, morphine. Sometimes acetylcodine is also detected. This is a by-product of heroin production, and not the result of consuming codeine. Taking codeine simply produces traces of codeine, the rest metabolising to morphine.

Cannabis is much more difficult to analyse, and it is desirable to detect the primary metabolite of the active ingredient, THC, which is Δ9-THC carboxylic acid (sometimes written Δ9-THC-COOH) which ensures the drug was ingested. Other constituents cannabinol and cannabidiol may also be detected, along with the unmetabolised THC and 11-hydroxy and 11-nor derivatives.

It used to be said that cannabis migrated along the hair shaft, but this effect seems more likely to be due to slow clearance of cannabis from the body, so cannabis metabolite continues to deposit in hair for some time after use. This same reasoning is behind THC positive urine samples that occur long after the donor has abstained from cannabis. We have known prisoners test THC positive for 3 months after admission, though presumably isolated from the drug during this time.

**HAIR GROWTH RATES**

One major benefit to hair analysis is the history locked up in the hair shaft, sometimes called the ‘tape recorder effect’. Hair is assumed to grow at a rate of 1.3cm per month, though the actual growth rate on the crown (the point furthest away from the chin) is 1.3cm per month of growth the timescale and their metabolism. So for a sample representing one month of growth the timescale can be out by plus or minus one week.

Hair from other parts of the body can also be used, but hair growth rates vary (see the table on page 4). When someone is bald, body hair is acceptable but underarm, chest or arm hair is generally considered to approximate to one year’s growth. Public
hair is only acceptable in special circumstances because collection is intrusive and exposure to contamination from drugs in urine, if the donor is a drug user, could cause uncertainties in concentration levels.

**DORMANT PHASE**

Hair strands go through phases of growth and dormancy, so a tuft of hair may contain active and dormant strands. This is because not all the hair on your head grows at the same time. Most follicles are growing, in what is called the ‘anagen’ phase, which lasts between two and six years. After a short transitional phase, the dormant ‘telogen’ phase begins and lasts about six weeks. Then when the anagen phase starts, the follicle falls out and a new hair begins to grow.

This telogen phase can somewhat ‘blur’ the results of a month-by-month analysis used to determine if a donor has abstained or taken up their habit again. Such results must therefore be used with caution.

**QUANTITY CONSUMED**

Similarly, the amount found in the hair is related to the quantity that was consumed, but this relationship depends on the dormant phase, metabolism of the user, and extraction ratio achieved by the laboratory. So the quantity detected should only be used as a guide. This is particularly true when historical sectioning month-by-month is used to decide whether someone is increasing or decreasing their usage. The technique of hair analysis is frequently used for this purpose by drug workers and solicitors but it is not a technique that readily lends itself to this sort of comparative analysis. At the very least, we strongly recommend that a clinical assessment by an expert is sought in such cases.

We routinely offer this sort of independent expertise to our clients. However, having said that, the following is an approximate guide for cocaine values based on typical extraction rates.

<table>
<thead>
<tr>
<th>Cocaine / metabolite</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low use (weekend recreational)</td>
<td>5-10 ng/10mg</td>
</tr>
<tr>
<td>Medium use (daily and/or weekend)</td>
<td>20-100 ng/10mg</td>
</tr>
<tr>
<td>High use (constant, regular)</td>
<td>&gt;100 ng/10mg</td>
</tr>
</tbody>
</table>

**ENVIRONMENTAL CONTAMINATION – A CAUTION**

A recent case involving child custody required us to investigate low levels of cocaine present in a child’s hair. It was claimed that the parent had deliberately given low levels of cocaine to the child. Research showed that as much as 4 or 5 ng/10mg of cocaine is routinely found in young children from the homes of cocaine users, simply from exposure. What makes all the difference to the quantity detected is the relatively low weight of the child, which accentuates the amount of substance that is captured in the hair, but this factor is frequently overlooked.

**ALCOHOL IN HAIR**

Alcohol is not as easily detectable as other drugs in hair. Ethanol derivatives are present in all hair including those of teetotallers, so these traces of ethanol do not correlate to alcohol which has been consumed. Zero alcohol does not produce a zero result.

In contrast to other drugs consumed, alcohol is not deposited directly in the hair. The analysis looks for direct products of ethanol metabolism; fatty acids that produce esters, and a secondary metabolite, ethyl glucuronide (Etg). Either Etg on its own, or the sum of the four fatty acid ethyl esters (FAEEs: ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate), are used as indicators of the alcohol consumption. Currently, FAEEs appear to have better correlation with long term detection qualities, and have recently been accepted in court proceedings in child custody situations.

In addition to their presence in the hair medulla, these products enter the hair through sebum deposited on the hair surface, resulting in a slight increase in concentration in recent hair compared to old hair, much in the same way that SureScreen found in cases of drug assisted assault, where detection limits were enhanced if the hair was not washed with solvent prior to extraction. Because of this, the company that has pioneered this system has gained valuable data that now allows them to predict levels of alcohol over six months history.

These processes for hair alcohol are only recent developments in hair testing, but have now become mainstream, showing this is an area where further development is likely.

**MEDICAL TESTS**

The internet is dominated by hair tests for their mineral content, and although this is considered by some to have a tenuous relationship to health, there have certainly been some remarkable studies relating hair analysis to breast cancer markers, passive smoking, and a range of medical conditions; such as cancers, Crohn’s disease and rheumatoid arthritis. It should certainly be possible to detect tumour and inflammatory markers such as cytokines from hair strand analysis.

**TYPICAL PROCEDURE FOR COLLECTING HAIR**

Get the donor to sit with their back to you. Decide where the hair sample is going to be collected from, depending on hairstyle and quantity available.

If the donor has hair on their head, it must be used for the sample. If the donor has no head hair or it is very sparse, and collecting it would leave a visible bald spot, hair should be collected from a different area. One option is body hair, which can be collected from the legs, underarms, chest and arms, and any of these may be combined to provide sufficient sample. Another alternative is beard or moustache hair but only sample this if the collection is going to be cosmetically acceptable to the donor.

The head hair sample should be taken from an undereather layer at the crown of the head. The crown is that part of the head that is furthest from the chin. If the donor has a ponytail, dreadlocks, a special hair style or a transplant, refer to the separate chart for collection advice. Make sure you are not sampling a wig!

Using tweezers or the scissors blade, lift a flap of hair from the crown and gently grasp a small lock of hair from the portion below the flap. Taking a sample from here will not show as a missing piece of hair. The lock of hair should be about half an inch wide and a few strands deep. The width should be about the distance between your second and third joint of your index finger. This should be about 100 hairs.

Make sure this area will not leave a bald spot once the flap of hair is replaced. If it will, choose another area, or sample from several places. Position the scissors as close to the scalp as possible, with the blades lying on the scalp. Cut all the hair you are holding. Be sure to keep the cut ends aligned. Put the scissors down, and place the sample into the foil with the root ends firmly grasped to make sure the hair remains aligned. Position the hair on the foil so that the root ends extend about ⅛ inch beyond the tapered end of the foil.

You need to collect at least 50 hairs for drugs and 100 or more hairs for alcohol testing.
If the person has thin or short hair, note that more hair may need to be collected from elsewhere in order to provide us with enough sample for testing. You may collect from more than one area on the head provided the root ends are kept aligned in the foil. Press the sides of the foil together and pinch tightly, trapping the hair inside the foil. If the hair is long, wrap it round the outside of the foil, DON’T cut the excess off.

Put the foil into the bag, press the seal edge together, and put the security seal over this edge. Place this bag into the evidence bag. Lay the bag flat, pull off the self seal strip and fold the edges of the bag together to seal it. Put the bag in the outer container (some of our services include a self addressed box) and post it to us using first class registered mail or via your courier.

Since chemical digestion of the sample is needed, the testing takes around 5 days but could be a little longer depending on workload.

COMMENTS ABOUT CHAIN OF CUSTODY
For tests to be evidential, certain rules need to be followed when collecting the sample. The process of proving your sample hasn’t been tampered with or substituted is called ‘chain of custody’. The easiest way to ensure this is to use pre-printed forms, follow best practice procedures, and use items such as tamper evident bags with unique reference numbers to ensure that the sample that is analysed is the same sample that left your sight. If we were to receive a sample where the chain of custody was not intact, we would contact the customer to question the integrity and not proceed with analysis. All of our confirmation kits contain all that you need to ensure sample credibility.

At collection, the donor has to identify any medication they are taking, including herbal forms, and a medical review can be carried out on these if you should get a positive result.

SUMMARY
Hair analysis is one part of the comprehensive system of drug tests that also include oral fluid and urine. Each one has its own unique insight into a person’s drug use:

- Oral testing for immediate and impairment
- Urine for recent drug use over days and weeks
- Hair to provide that person’s long term history.

Hair cannot identify drug use in the last few weeks, but it provides a unique historical breakdown of drug use over months and even years. If you missed our Definitive Guides on Oral Fluid and Urine there are details below of how you can have copies emailed to you.

OVERVIEW
ADVANTAGES OF HAIR ANALYSIS
- 90 day + window of detection
- Increased detection of positive heroin, PCP and cocaine when compared to other test mediums
- No known adulterants
- No known methods of dilution
- No known specimen validity issues
- Non-intrusive and observed collection
- Resistance to evasion
- Patterns of use (segmental analysis)
- No refrigeration needed
- Easy handling
- Non biohazardous

DISADVANTAGES OF HAIR ANALYSIS
- Strict chain of custody
- Training before collection
- Remote analysis by specific laboratories
- Expensive cost per test compared with rapid screening
- Time delay for results

UNSUITABLE SAMPLES FOR ANALYSIS
- Excess surface contamination (found in wash)
- Quantity not sufficient - QNS
- Packaging problem, eg. no chain of custody
- Head lice

GROWTH CHARACTERISTICS OF HUMAN HAIR

<table>
<thead>
<tr>
<th>Location</th>
<th>(mm/day)</th>
<th>(inches/month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chin</td>
<td>0.38</td>
<td>0.44</td>
</tr>
<tr>
<td>Crown</td>
<td>0.35</td>
<td>0.41</td>
</tr>
<tr>
<td>Axilla</td>
<td>0.30</td>
<td>0.35</td>
</tr>
<tr>
<td>Thigh</td>
<td>0.20</td>
<td>0.23</td>
</tr>
<tr>
<td>Eyebrow</td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>Chest</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>Beard</td>
<td>0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>Vertex</td>
<td>0.44</td>
<td>0.51</td>
</tr>
</tbody>
</table>

It takes approximately two weeks for the hair growing in the root to become available on the scalp. Therefore the last 2 weeks of any substance abuse will not be present in a hair sample sent for analysis.