Microsegmental Analysis of a Single Hair Strand: Pushing the Envelope on Hair Drug Testing

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In this issue of *JALM*, Kuwayama et al. (*1*) present a method for estimation of drug-intake day through the microsegmental analysis of a single strand of hair. Over-the-counter drugs were used as indicator compounds and administered to the volunteers at specific intervals. Hair was analyzed for the indicator compounds in 0.4-mm segments, which corresponds with approximate daily hair growth. From the presence of drugs in different segments and known intervals of administration of the indicator compounds, the rate of hair growth and, thus, day of drug intake, was estimated. Although the segmental analysis of a single strand of hair has previously been done, this is a novel idea to calculate the drug-intake day within an error of ±2 days. This method provides a significant improvement over the current methods, which can estimate drug intake within an error of a few weeks to months (*2*–*4*). The method can be helpful in estimating the day of a crime such as robbery, battery, and sexual assault under the influence of a drug.

Use of hair as an alternate or complementary sample in forensic toxicology is well established (*2*–*4*). The major advantage of hair analysis is to reveal history of drug use. In contrast to conventional specimens such as urine and blood, which provide a shorter window of drug detection from hours to a few days, hair provides a longer window of drug detection from weeks to years—as long as the length of hair allows. To understand the full applications of hair analysis in forensic toxicology, it is important to understand the basics of drug incorporation in hair. Circulating drugs in blood capillaries first incorporate in hair follicles and then get entrapped in the core of the shaft as the hair grows out of the follicle. As the hair grows, drugs move along the growing hair shaft. Once in the hair shaft, under favorable conditions such as protection from light, heat, and moisture, drugs are stable for a long period. For example, cocaine metabolite, benzoylecgonine, has been detected in hairs from 4000-year-old mummies (*5*). In addition to providing history of drug use, other advantages of hair analysis include ease of specimen collection, noninvasiveness, and sample stability. Today, hair analysis is routinely used for drug detection in a variety of forensic and clinical applications, such as preemployment, random drug screening, return to duty, reissuance of driving license in traffic violations, correctional facilities, occupational medicine, and prenatal drug exposure (*2*–*4*, *6*).

Hair drug analysis is not very well standardized and varies significantly among laboratories. Currently, the common method of hair drug analysis uses multiple hair strands that are pooled and not cut into different segments. This type of pooled hair analysis provides information on drug exposure only, not on patterns of drug use. In contrast,

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segmental hair analysis can provide information on patterns of drug use and abstinence. This is based on the principle that drugs, when present in the bloodstream, incorporate into the hair matrix and move along the hair shaft as the hair grows. Drugs will be present in only those hair segments that came in contact with the drugs. Based on this principle, many studies have shown the usefulness of segmental hair in establishing periods of drug use and cessation. For example, studies on patients from rehabilitation or treatment centers have shown higher drug concentration in terminal parts and low drug concentration in proximal parts of hair (*7*, *8*). A study on a cocaine abuser showed the disappearance of cocaine in the hair sections closest to the root after 3 months of abstinence (*9*).

Length and amount of hair used in segmental analysis vary significantly among different methods. In routine segmental hair analysis, multiple strands of hair are cut into pieces several centimeters long and pooled before analysis. Because the average hair grows at a rate of approximately 1 cm/month, this type of analysis on relatively longer segments provides a time-resolution window of a few weeks to months. To get a higher time resolution, analysis of shorter hair length in the millimeter range is required; the shorter the length of the hair segments, the higher the time resolution will be. Because hair from different sites grows at different rates, analysis of short segments from a single hair strand can increase the accuracy and precision of time resolution. The analysis of a very short segment, in the submillimeter range, from a single hair strand is now possible with modern analyzers. Sensitive methods involving gas chromatography/tandem mass spectrometry, LC/MS/MS, and MALDI mass spectrometric imaging have been described for single-strand hair drug analysis (*10*–*12*). MALDI mass spectrometric imaging requires less sample preparation but is less sensitive than LC/MS/MS.

Kuwayama et al. (*1*, *11*), in what they call microsegmental analysis, used 0.4-mm segments of hair. This length of hair approximately corresponds to the average daily growth of human scalp hair. However, because hair in different areas grows at a different rate, microsegmental analysis cannot be used to calculate the exact date of drug intake. Kuwayama et al. (*1*) describe a novel approach to overcome this problem. They administered drugs as indicator compounds at different time intervals to calculate the rate of hair growth. The first indicator compound was administered at day 0, and it was supposed that the day of its administration is not known. This was followed by the administration of other indicator compounds at different time intervals. Hair samples were taken and analyzed in 0.4-mm segments. The rate of hair growth was calculated from the distance between the detection of indicator compounds and time intervals between administrations of these compounds. The day of drug intake was estimated from the rate of hair growth, distance between the proximal end of the hair and the detection of the first indicator compound, and the reference day. The application of this approach is to find the date of drugging a victim by the perpetrator.

Despite this novel approach of elucidating the day of a drug-assisted crime, there are several limitations of this method. First, to estimate the drug-intake date, the victim must take the indicator compounds at different intervals and, thus, must present several times to the investigating authorities. Also, different hair grows at variable rates, and approximately 85% of hair is in the growing phase while the remainder is in the resting-dormant phase. Depending on which hair strand is analyzed, time resolution and, thus, the resulting interpretation may be affected. Furthermore, despite many advantages of a single-hair strand microsegmental analysis, general limitations of hair drug testing are still applicable. There is a wide interindividual variation in hair drug incorporation. Both animal and human studies have shown that drug concentrations are higher in pigmented hair than nonpigmented hair. This is because of the higher

melanin concentration in pigmented hair. For example, Kronstrand et al. (*13*) measured methamphetamine and amphetamine levels in hair samples collected from gray-haired patients receiving selegiline, the drug that metabolizes to methamphetamine and amphetamine. Methamphetamine and amphetamine concentrations were higher in pigmented hairs than white hair. These findings raise the possibility of racial discrimination. It has also been shown that basic and neutral drugs incorporate more efficiently than acidic drugs. Therefore, if an acidic drug is used by a perpetrator, hair analysis may show false-negative results. External drug contamination and hair cosmetic treatments can influence drug concentrations and result interpretation. Although the presence of a drug in the hair can verify the exposure, it cannot differentiate between self-administration and drugging by a perpetrator.

In summary, despite many challenges, hair analysis provides many unique opportunities in the detection and deterrence of drug abuse. Microsegmental hair analysis can enhance the utility of hair drug testing by providing better time resolution.

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