

Chapter 2

Specimens for Drugs-of-Abuse Testing

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SUMMARY

A wide variety of body fluid specimens have been utilized for analysis for the presence of drugs of abuse. Urine has been and remains the most widely used body fluid specimen for routine testing for drugs of abuse, but several alternative specimens are establishing their place as suitable for drug testing. Hair, sweat, and oral fluid have reached a sufficient level of scientific credibility to be considered for use in the federally regulated workplace drug-testing programs. Each specimen provides different information about time and extent of use and likelihood of impairment. Some of these specimens (e.g., urine and oral fluid) can even be analyzed with simple on-site, noninstrumented testing devices, as well as through standard laboratory methods. These drug-testing tools, as objective pieces of information identifying drug use, have proven highly useful in addressing our society's ongoing substance abuse challenges. This chapter reviews the use of these various body fluid specimens for drugs-of-abuse testing, addressing the balances between ease of specimen collection and handling, the ease and accuracy of analytical methods, the capability for sound interpretation of results, and, ultimately, legal defensibility.

1. INTRODUCTION

Drug abuse remains a significant public health issue worldwide. Although major advances have been made in our understanding of the neurobiology of addiction and the pharmacology of abused drugs, society still has few tools to

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effectively address drug abuse and addiction. However, drug testing has proven to be one of the key objective tools to at least identify those who have used and abused drugs of abuse. Furthermore, drug testing, with appropriate responses and sanctions for positive test results, has deterred drug use. These tests have demonstrated their utility in a wide variety of clinical and nonclinical settings, including emergency toxicology, perinatal testing, criminal justice, the workplace, schools, and drugged driving.

Urine is the most widely used specimen for such routine drugs-of-abuse testing, but several “alternative” specimens are establishing their place as suitable for drug testing (1–3). Hair, sweat, and oral fluid (*see* Chapters 7–11) have reached a sufficient level of scientific credibility to be considered for use in the federally regulated workplace drug-testing programs under the Substance Abuse and Mental Health Services Administration (SAMHSA) (4). Each of these specimens offers a different balance among ease of specimen collection and handling, ease and accuracy of analytical methods, capability for sound interpretation of results, and legal defensibility. Legal defensibility is important because tests for drugs of abuse are often utilized in a variety of criminal, civil, and administrative adversarial proceedings.

All of these specimens lend themselves to accurate analysis of drug and/or metabolite levels through conventional scientific techniques, i.e., immunoassays and chromatographic methods such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography–mass spectrometry (GC-MS; *see* Chapter 3). However, some specimens are clearly easier to analyze than others, with simple noninstrumented, on-site devices having been developed for urine and oral fluid. Furthermore, the analyte(s) in question varies between specimens, with the parent drug being predominant in some specimens (hair, sweat, oral fluid) whereas more polar metabolites are predominant in others (urine). Finally, issues of specimen collection, handling, transport, and stability also vary. Of importance are concerns about handling of biological specimens and the relative risks of exposure to, and transmission of, infectious agents. In 1991, the Occupational Safety and Health Administration (OSHA) published the Bloodborne Pathogen Standards at 29 CFR 1910.1030 (and subsequent OSHA Standards Interpretation and Compliance letters), which address the handling and infection risks of various biological specimens (5).

This chapter addresses the various body specimens that have been routinely utilized to identify use and abuse of drugs.

2. BLOOD

Blood is widely regarded as the specimen offering the best correlation between drug levels and likely dosing and likely concomitant pharmacological,

cognitive, and psychomotor effects. There have been many controlled dosing studies examining blood levels of drugs and concomitant effects. However, owing to ethical concerns, not all abused drugs have been subjected to such controlled dosing studies. Drug levels found in blood are often quite low (ng/mL) and often short-lived. The analysis of drugs in blood is time-consuming, generally requiring extraction procedures before further analyses can be performed. There have been several publications addressing the application of urine immunoassays to the analysis of blood specimens, after appropriate extraction protocols (6,7).

Although blood is widely used for drug testing in clinical and emergency toxicology settings, especially for alcohol, the invasiveness of the collection of blood specimens does not lend itself to routine testing in other nonclinical contexts, such as in workplace-, student-, and corrections-testing environments. Furthermore, there is much greater risk of transmission of infectious disease through handling of blood specimens than with many other routinely tested specimens for drugs-of-abuse testing. Accordingly, blood will not be further considered in this review.

3. URINE

3.1. Urine Specimens

By far, urine is the most widely used specimen for drugs-of-abuse testing. It offers the advantages of large specimen volume and relatively high drug concentrations, because of the approx 100-fold concentrating effect of the kidneys (each minute, approx 125 mL of blood plasma is filtered in the kidneys by the glomeruli and concentrated to approx 1 mL of urine). There is an extensive body of literature addressing the detection of drugs and their metabolites in urine specimens, and much is known about the pharmacokinetics of drug and metabolite elimination in urine. There are several well-established guidelines and laboratory certification programs, most notably those originally established by the National Institute on Drug Abuse (NIDA) in 1988 for federally regulated workplace drug-testing programs. These guidelines, called the NIDA Guidelines, are now overseen by SAMHSA (8). These guidelines address testing for five drugs of abuse: cannabinoids (marijuana), cocaine metabolites, opiates, amphetamines, and phencyclidine (PCP). These workplace drug-testing guidelines are widely regarded as the “gold standard” in urine drug testing. As of March 2005, there were 49 laboratories (including 2 in Canada) certified under this program to perform such federally regulated workplace drug testing.

Urine is 95% water, with sodium chloride and urea in about equal amounts as the main dissolved substances, and with much smaller amounts of

a wide variety of other constituents. Urine is attractive as a specimen because it can be conveniently provided as a normal waste product in relatively large volumes. Typical urine production rates are about 1 mL/min during waking hours, so collection of a specimen of sufficient volume for both initial screening by immunoassay as well as any subsequent confirmation testing is generally not problematic. Furthermore, the large specimen volume allows the option of splitting the specimen into two portions at the time of collection for assurance of proper chain of custody and specimen integrity in the event of adversarial challenges to the test results. Drugs and their metabolites are reasonably stable in urine when specimens are refrigerated or frozen. As far as specimen handling is concerned, urine is generally not considered infectious unless visibly contaminated with blood (5).

3.2. Urine Analysis

Urine is relatively easy to collect and analyze. There are a wide variety of immunoassays available for detection of most common drugs of abuse and/or their metabolites in urine. Furthermore, in part because of the relatively high drug and/or metabolite concentrations in urine, simple noninstrumented, on-site immunoassays have been developed and are widely used in a variety of settings. There are numerous versions of these simple-to-use, noninstrumented immunoassays (i.e., visually read dipsticks, cassettes, cups) which allow the ready on-site testing of urine specimens outside of a formal laboratory. Some of these devices have even been cleared by the US Food and Drug Administration (FDA) for at-home use. Studies of the performance of these noninstrumented, on-site devices have demonstrated impressive performance for some devices at levels comparable to bench-top laboratory analyzers, even when performed by those without any formal laboratory experience (9,10).

3.3. Urine Issues

In spite of the well-established place of urine as a specimen for drug testing, its use is not without its challenges. One issue is the potential invasion of privacy involved in specimen collection. Unlike within the criminal justice testing programs (pretrial, probation, prisons, drug courts, and so on) (*see* Chapter 15), most workplace and other drug-testing programs do not allow direct observation of specimen collection, except under special circumstances. Without direct observation, the opportunity for specimen adulteration and substitution exists (*see* Chapters 13 and 14). To respond to such efforts to thwart the integrity of the testing, specimen collection and laboratory guidelines have been developed to minimize the opportunities for such tampering. Furthermore, laboratory procedures to detect such efforts have been established. Adulteration is generally

easily detected and is difficult at best when collection is performed under direct observation, as in criminal justice contexts. Although there are many adulterants available, there are also many simple test strips as well as laboratory methods to detect such adulterants.

Specimen dilution (in vivo), however, is a much greater challenge, as it is fairly easy to drink sufficient excess fluids prior to specimen donation and dilute one's urine by a factor of up to 10 or even more, thereby minimizing the chance of testing positive at conventional screening cut-offs. Although there are established criteria for what constitutes an excessively dilute specimen (e.g., creatinine less than 20 mg/dL and specific gravity less than 1.003), these regulatory criteria are undergoing scrutiny to ensure that false accusations of intentional efforts at dilution are not made.

Another limitation to the utility of urine specimens in drug testing is the relative difficulty in correlating urine drug and/or metabolite levels with likely dosing and likelihood of impairment. Granted, the correlation between urine drug levels and time and extent of drug use and likelihood of impairment is weak. Unfortunately, some toxicologists claim that urine drug levels should *never* be interpreted, but this clearly is an extreme and incorrect position. In some situations, urine levels may clearly be commensurate with the claims of the user or not, and as such can be highly useful. Furthermore, very high urine levels can clearly demonstrate recent and significant use, whereas low drug levels are much more difficult to interpret. But to deny any value in urine drug levels for interpretation is incorrect.

4. ORAL FLUID

4.1. Oral Fluid Specimens

The alternative specimen receiving the most recent interest appears to be saliva or, more appropriately, oral fluid (11-15). Although *saliva* has been the commonly used term to describe fluid specimens from the oral cavity, this fluid, as collected by current simple swabbing or absorbent pad devices, is really a complex mixture of several different oral fluids, including saliva. Accordingly, the broader term *oral fluid* is preferred. Oral fluid represents a mixture of not only the saliva from the three oral salivary glands (parotid, submandibular, and submaxillary), but other oral fluids as well (e.g., gingival crevicular fluid).

The first experiments to measure biological analytes in saliva were performed in the mid-19th century. Further experiments in the 1930s demonstrated the role of lipid solubility and ionizability in the partitioning of solutes into saliva. Oral fluid has been used for a wide variety of analytes, including

steroids, hormones, enzymes, antibodies, DNA typing, therapeutic drugs, and drugs of abuse. From the earliest days of immunoassay development for drug testing in the early 1970s, saliva has been considered a suitable specimen. In fact, one of the first papers published on the use of homogeneous immunoassays for the detection of drugs (a spin immunoassay developed by Syva Company, called free radical assay technique [FRAT], a forerunner of the now well-established enzyme-multiplied immunoassay technique [EMIT] assay), specified in the title the use of both urine and saliva for morphine testing (16).

The key advantage of oral fluid for drugs-of-abuse testing is the ease of specimen collection, without invoking privacy or gender concerns. Oral fluid for drug testing offers great promise for roadside driving-under-the-influence scenarios, which prove prohibitive for the collection of a urine specimen (*see* Chapters 8 and 17). Furthermore, there is the potential for immediate test results with on-site, noninstrumented immunoassays already developed. In addition, there has been the promise that saliva drug levels may correlate better with blood levels than urine, thereby allowing for better assessment of the level of likely impairment. Unfortunately, a close review of the literature indicates that although oral-fluid levels may correlate better with blood levels than urine drug levels, the correlation is not so strong that a clear relationship with impairment exists. Finally, the possibility of specimen adulteration appears to be minimal. Some limitations are the very low specimen volume and the low analyte levels. Although oral fluid as a specimen for drugs-of-abuse testing is receiving active research interest, oral fluid has been widely studied for use in therapeutic drug monitoring (17).

Ethanol was apparently first reported in saliva in 1875. Saliva ethanol levels have been shown to demonstrate excellent correlation with blood alcohol levels, with a saliva/blood ratio close to 1; this is why saliva as a specimen for initial alcohol testing is authorized under the Department of Transportation (DOT) program as well as under several state driving statutes (18–20). The DOT regulations detail saliva collection and testing procedures. In conjunction with the DOT rulemaking, the National Highway Traffic and Safety Administration (NHTSA) included performance evaluations of nonevidential alcohol-screening devices for saliva for use in the DOT testing program. Those devices fulfilling NHTSA's criteria are listed in their Conforming Products List (CPL), periodically published in the Federal Register (20). In addition, there is ongoing review by SAMHSA's Division of Workplace Testing of the use of saliva for federally regulated workplace testing for other drugs of abuse as well (4). There is also a program in Europe called Roadside Testing Assessment (ROSITA), which examines a variety of specimens and technologies for their suitability in roadside testing, with much attention paid to saliva (21).

Saliva is effectively an ultrafiltrate of blood. All of the organic compounds present in plasma have been detected in saliva, albeit in trace amounts for some analytes. Saliva is about 98% water, with a specific gravity of 1.00–1.02. Saliva contains both electrolytes (primarily Na, K, Cl, and HCO_3) and proteins, and its osmolality is less than or equal to that of plasma. The electrolyte concentrations and pH are markedly dependent upon saliva flow rate. Accordingly, stimulating saliva flow for speed of specimen collection can alter the partitioning of drugs between blood and oral fluid and thus affect the saliva: blood ratio. The protein concentration in saliva is less than 1% of that in plasma. However, saliva has proven a suitable specimen for forensic DNA analysis as well as antibody testing for human immunodeficiency virus (HIV).

Typical daily saliva secretion is 500–1500 mL—average 0.6 mL/min (range 0.1–1.8; during sleep, 0.05 mL/min). Production rates for stimulated saliva have been reported to average about 2 mL/min but have reached as high as 7 mL/min. Saliva pH is typically 6.7 (5.6–7.9, flow rate dependent), vs 7.4 for plasma.

The mechanism by which drugs are found in saliva is passive diffusion, although there are examples of active secretion (e.g., lithium). The major factors affecting drug entry into saliva are lipid solubility and degree of ionization at saliva pH.

Unfortunately, oral fluid has not proven very sensitive for detection of cannabis use, as it appears that cannabinoids are not secreted from the blood into oral fluid. Rather, it is only from contamination of the oral cavity after smoking or oral ingestion of cannabis that cannabinoids may be detected in oral fluid. Accordingly, detection of cannabis use is likely only for several hours after use (22,23). However, this may prove beneficial in testing programs where the goal is to demonstrate a likelihood of impairment. If an appropriate threshold cut-off is established, then a positive result in oral fluid would clearly represent use within the past few hours, with demonstrated cognitive and psychomotor deficits.

4.2. Oral Fluid Analysis

Analysis of oral fluid for drugs is relatively straightforward. However, there are limitations in repeat and multiple confirmation tests as a result of low specimen volumes. Both on-site and laboratory-based methods have been developed (24,25).

Specimens may be collected through a variety of techniques, although simple expectoration (spitting) into plastic (polypropylene) tubes (either stimulated or unstimulated) or absorption of oral fluid with an absorbent material (foam pad, cotton fiber wad) are the most common. Spitting causes

a saliva secretion rate of approx 0.5 mL/min. The flow of saliva can be stimulated through a variety of techniques, such as chewing paraffin, or through the use of chemical stimulants, such as citric acid or sour candy drops. Of course, the use of any foreign material to stimulate saliva must be carefully considered so that the specimen is not altered or contaminated in a way that might limit subsequent analyses or interpretations. Chewing paraffin will cause secretion rates of 1–3 mL/min, but paraffin may absorb highly lipophilic compounds, causing a reduction in measured saliva levels. Citric acid candies are potent stimulators, leading to secretion rates of 5–10 mL/min. Stimulated saliva appears to have a fairly narrow pH range (approx 7.4) relative to the broader range for unstimulated saliva. Again, the variability in pH may be important for the saliva/plasma ratios of weakly basic or weakly acidic compounds. The pH of saliva increases from approx 6.2 to 7.4 as the secretion rate increases. It is generally approx pH 7 for stimulated saliva, whereas unstimulated saliva shows a greater pH variation. This variation in saliva pH resulting from variations in secretion rates can have a significant impact on the saliva/plasma ratio for certain drugs, depending on their pKa.

Generally, a specimen is collected with an absorbent pad placed in the mouth for a few minutes. After the pad is saturated with oral fluid or a specific amount has been absorbed, the pad is placed in a tube of buffer for shipment to the laboratory. On-site methods may similarly collect the specimen with an absorbent pad from which the specimen is applied to a noninstrumented or instrumented immunoassay device. There is even a device that aspirates a specimen directly into a bench-top analyzer. However, drug levels in oral fluid are generally much lower than those found in urine specimens, except when there is direct contamination of the oral cavity.

Specimen handling is relatively straightforward. Saliva has been shown to be source of infectious microorganisms, and appropriate precautions should be taken in the handling of oral fluid (5). Court cases have addressed the relative infectivity of saliva when one subject has been bitten by another.

4.3. Oral Fluid Issues

One promise of oral fluid testing is a supposed better correlation with blood levels and, accordingly, impairment. Unfortunately, a detailed review of the literature indicates that although oral fluid levels generally correlate better with blood levels than, for example, urine, the correlation is not so close as to allow a strong prediction of blood levels. This is especially so shortly after drug use by oral ingestion, smoking, or nasal insufflations, when contamination of the oral cavity by drug can lead to dramatically elevated drug levels, much greater than corresponding blood levels, at least for several hours. Another issue

is that oral fluid testing is relatively insensitive for the detection of cannabis use, as it appears that cannabinoids are not secreted from the blood into oral fluid. Rather, detection of cannabis use is possible only as long as there is contamination of the oral cavity with cannabinoids. This period of detection is much shorter than the several days for detection of cannabinoids in urine. However, by choosing an appropriate cut-off, one can insure that a positive cannabis result in oral fluid can occur only within a few hours of use, and thus provides a clear indication of likely impairment.

5. HAIR

5.1. Hair Specimens

It has been demonstrated that a very wide variety of ingested drugs and/or their metabolites may be found in hair specimens. Hair specimens from ancient mummies have been demonstrated to contain cocaine. Several famous deceased persons have also had their hair analyzed for drug exposure (Napoleon Bonaparte, Ludwig van Beethoven, William Butler Yeats) (26,27).

Hair testing has gained interest because of its ability to provide a history of drug use, dependent on the length of hair tested (*see* Chapter 11). Unlike other conventional biological specimens used for drug testing with detection times measured in days, drugs have been demonstrated to remain in hair for extended periods of time: years, decades, and even longer. Current hair-testing protocols examine segments of hair representing about 3 mo of growth (head hair typically grows approx 1 cm/mo). That drug residues may be detected in hair over extended periods of time has been amply demonstrated in a large number of published studies. Hair specimens examined include not only head hair, but also beard hair, axillary hair, body hair, and even pubic hair. Furthermore, even neonatal hair has been analyzed to demonstrate possible prenatal drug exposure (28).

There have been numerous national and international scientific meetings specifically addressing hair testing, with the establishment of a few professional societies dedicated to hair testing. In 1990, there was a small conference addressing this new technology convened in Washington, DC, by the Society of Forensic Toxicology, the National Institute on Drug Abuse, and the National Institute of Justice. Although most attendees were critical of hair testing, subsequent research has demonstrated its utility. The first international meeting addressing hair testing was held in Genoa in 1992 (29); an international workshop was held in 1994 in Strasbourg (30); a joint The International Association of Forensic Toxicologists (TIAFT)/Society of Forensic Toxicologists (SOFT) meeting was held in 1994 in Tampa with a special session dedicated to hair testing; another international meeting held in 1995

in Abu Dhabi; and the first European meeting held in Genoa in 1996 (31). The proceedings of several of these meetings have been published as full issues of the journal *Forensic Science International*. Since then, hair-testing science and technology has been extensively addressed at numerous forensic science and toxicology meetings. A large body of experimental and epidemiological scientific data has been published (32–36).

The mechanism of drug incorporation in hair has been found to be not as simple as originally proposed. It was thought that drugs within the blood capillaries bathing the follicle were transferred into the growing hair shaft and effectively locked in place. However, it has been shown that such a simple mechanism does not account for all the experimental observations. It has been demonstrated that drugs can also enter the hair shaft via sweat and sebum. Also, environmental contamination of the hair has been demonstrated. One question is whether hair analysis can differentiate between drugs in hair from actual drug use as opposed to environmental exposure (*see* Chapter 11).

5.2. Hair Analysis

Hair analysis is performed by cutting a segment of hair from close to the scalp, generally representing about three months' growth. Hair typically grows approx 1 cm/mo, although there are inter-individual differences in hair-growth rates. The cut hair specimen is washed to remove potential external contamination and then digested. The digest solution is tested by immunoassay and GC-MS. In addition, some laboratories also test the initial wash solutions for an assessment of the possibility of environmental contamination and its likely contribution to the subsequent test results.

Unlike the multitude of laboratories offering urine drug-testing services, there are only a few laboratories offering hair-testing services. There are currently no formal hair-testing laboratory regulations or guidelines, although there are a few professional societies as well as some proficiency-testing programs (37). However, hair testing is on the list of alternative specimens proposed for federally regulated workplace testing, with some laboratory and testing standards established, at least in draft form (4).

5.3. Hair Issues

The main issues facing hair testing are (1) distinguishing environmental exposure/contamination of the hair from drug incorporation in the hair shaft from use and (2) addressing the possibility of hair-color bias. Both of these issues have been reasonably well investigated but still appear to remain subjects of controversy (38).

Hair-testing laboratories claim that they can distinguish between actual drug use and contaminating environmental exposure by a comparison of the levels of drugs that might be found in the preliminary wash solutions and the level of drugs found in the actual hair digest. If there are high levels of drugs in the wash solutions relative to those found in the digest, external contamination is considered likely. However, there appears to remain some controversy surrounding these claims.

It has been well demonstrated that drugs bind to hair differentially, dependent upon the physicochemical properties of the drug in question and those of the hair. It is known that many drugs bind preferentially to dark-pigmented hair over fair-colored hair, leading some to make claims of a hair-color bias in hair testing, unfairly identifying those with heavily pigmented hair over those with fair hair. Some have even called this a racial bias (39–42).

Another issue is the possibility of specimen adulteration (43–45). It has been demonstrated that hair color plays a significant role in binding of drugs to hair and that bleaching or other treatments can dramatically reduce the amount of drug found in hair. In addition, there are shampoos being sold on the Internet claiming that they can rid the hair of drugs (43). It seems clear that the opportunity to thwart hair testing through such chemical treatments exists. Of course, drug users could also shave their heads and even other body hair to prevent testers from obtaining an incriminating specimen.

Some claim that by segmental analysis of the hair shaft, a time profile of drug use may be obtained, although others challenge the scientific validity of such segmental analysis (46–48). Some experimental studies have challenged the simple view that drugs are neatly deposited along the hair shaft from the blood capillaries bathing the hair follicle, and remain in place as the hair shaft grows to provide a timeline of drug use. It has been demonstrated that this model of drug deposition and incorporation into hair is too simplistic and that drugs may be incorporated into and onto the hair shaft by a variety of mechanisms, including sweat and sebum excretion.

There have been numerous court challenges to the admissibility, probative value, and interpretation of hair drug tests. On balance, it now appears that hair testing has been generally accepted by the courts (49).

Another issue to consider when using hair testing in the workplace setting is that by examining *prior* drug use where there may not be *current* drug use, any sanctions may run afoul of the Americans with Disabilities Act. This act precludes employers from discriminating against otherwise qualified applicants or employees based on *prior* drug use, as long as they are not *currently* using drugs. Whether a positive hair test that looks back 3 mo in time represents *current* use appears not to have been conclusively decided in the courts.

6. SWEAT

6.1. Sweat Specimens

Drugs of abuse and their metabolites have long been known to be excreted in sweat. Quinine was detected in sweat in 1844, morphine in 1942, and amphetamines in 1972. The development and patenting of a sweat patch collection device by PharmChem Laboratories (Haltom City, TX) in the 1990s has allowed for the ready detection of drug use over a period of approx 1 wk of patch wear (50–54). The sweat patch is a simple Band-Aid®-like device consisting of a small 3×5 cm absorbent cellulose pad covered by a gas-permeable polyurethane membrane that allows water vapor to pass through while trapping in the absorbent pad any drugs and/or their metabolites excreted in sweat. The patch is held in place on the torso or arm by a special Band-Aid-like adhesive layer surrounding and covering the cellulose collection pad. After a wear period of approx 1 wk, the analysis of the sweat patch is relatively straightforward—drugs are eluted from the collection pad and the extract subsequently analyzed by immunoassay and GC-MS.

The criminal justice community has shown great interest in sweat-patch testing for drugs of abuse. The patch offers the primary advantage of constantly monitoring for any drug use over a period of approx 1 wk, obviating the need for multiple urinalyses to effectively monitor for any drug use over that period. The patch cannot be removed or tampered with without it being apparent to a trained technician. There has also been interest in the use of the sweat patch for federally regulated workplace drug-testing applications (4). However, given the invasion-of-privacy implications of an employer monitoring the off-duty behavior of an employee, the sweat patch would likely be used only as a last-chance agreement between an employee and employer after a prior failed drug test or other evidence of workplace drug use.

Sweat arises from both eccrine and apocrine sweat glands. The eccrine sweat glands are found on most parts of the body, whereas apocrine sweat glands are found primarily in the axillary, inguinal, and perineal areas. The apocrine sweat glands open directly onto the hair follicle and are less well studied and understood than eccrine sweat glands. Eccrine sweat-gland density varies widely, from $60/\text{cm}^2$ on the back to $600/\text{cm}^2$ on the sole of the foot. Glandular sweat production is approx 1–5 nL/min/gland. Insensible sweat amounts to 400–700 mL/d. Sweat is 99% water, originally isotonic with plasma, but water re-absorption makes it hypotonic. Sweat pH when resting is 5.8, but exercise increases sweat pH to 6.1–6.4.

6.2. Sweat Analysis

The patch is applied to the torso or arm after precleaning the skin with alcohol wipes. The cleaning is designed to not only remove any possible

surface contaminants but also to ensure an effective seal of the adhesive. The patch is worn for about a week, absorbing sweat and any drug and metabolites present in that sweat. The patch absorbs approx 300 μ L of sweat each day, or approx 2 mL/wk. After approx 1 wk, the patch is removed and sent to Pharm-Chem Laboratories for elution and analysis by immunoassay and GC-MS. Elution is performed using an aqueous methanol/acetate buffer. Cut-off levels for reporting a positive result are on the order of 25 ng/patch. The Administrative Office of the US Federal Courts has also administratively required that to report positive test results for cocaine or methamphetamine, their respective metabolites must also be present at their limits of detection.

6.3. Sweat Issues

The patch has been demonstrated to be sensitive and accurate, although its use has not been without challenges. There have been claims that the patch may be contaminated from exposure to drugs, both from the environment and from residual levels of drug in the skin from prior use (55,56). It has been demonstrated that under certain laboratory conditions, drugs applied in certain pH solutions to the outside of the patch can migrate through the polyurethane outer membrane into the underlying collection pad when the underlying pad is also soaked with certain pH buffers. However, these laboratory experimental conditions are not likely to occur in a real-world setting. In addition, it has been demonstrated that when alcohol-precleaned skin is doped with drug in solution, the preplacement cleaning procedure does not remove all of the drugs from the skin and can generate a positive test result. Again, these conditions are not likely to occur in a real-world setting. Although at least one federal court has recognized the possibility for such external contamination, these contamination challenges have been effectively rebutted in several subsequent cases. Further research would be welcome to define more accurately whether the patch may be prone to the possibility of contamination under more real-world drug-exposure conditions.

7. OTHER SPECIMENS

A wide variety of additional body specimens have been analyzed for drugs of abuse. These are not widely used, but a few will be addressed briefly.

7.1. Meconium

Meconium is a newborn's first stool. It is formed over the last trimester of pregnancy, and thus can represent exposure to drugs over 16–20 wk prepartum. Meconium is collected from the neonate's diaper and extracted with solvents. The extract is then analyzed by conventional immunoassay and GC-MS techniques (57).

7.2. Breast Milk

Drugs and alcohol have also been detected in mother's breast milk, with implications for healthcare concerns about neonatal exposure. It has been demonstrated that alcohol is eliminated in breast milk, and that newborns can detect the flavor of alcohol in breast milk and actually suck harder but obtain less milk as a result of alcohol's inhibition of prolactin secretion, thereby inhibiting milk release. More dramatically, methamphetamine and cocaine have been found in mother's breast milk and linked to adverse neonatal health effects (58,59).

7.3. Vernix Caseosa

Vernix caseosa is a white deposit of sebum and desquamated cells covering the skin of neonates and has been analyzed for evidence of prenatal cocaine exposure (60).

7.4. Semen

Semen has been demonstrated to have measurable levels of drug after drug use. It is occasionally proposed as a basis to explain positive drug tests as a result of exposure to drugs through sexual relations. However, the absolute amount of drug present in semen is very low and could not account for significant exposure (61).

7.5. Nails

Drugs are found in nails, as they are in hair. Nails have been used to detect drugs in both adults and neonates (62–65).

7.6. Vitreous Humor

Vitreous humor is the gel that fills the eye. It is obtained at autopsy and analyzed for drugs (66).

8. CONCLUSIONS

A wide variety of body fluid specimens have been analyzed for the presence of drugs of abuse. The analytical methods are sound and well developed. Each specimen provides different information about time and extent of use and likelihood of impairment. However, the interpretation of test results from each of these types of specimen offers its own challenges. Formal regulatory criteria have been established for several of these specimens, and case law addressing their admissibility and probative value has been developed for some. These drug-testing tools, as an objective piece of information identifying drug use, have proven highly useful in addressing the ongoing challenge of substance abuse.

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