Employment-Related Drug Testing of Biological Matrices

URINE, ORAL FLUID AND HAIR COMPARISON

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Since 1986, RTI has supported the Department of Health and Human Services (HHS) by managing the National Laboratory Certification Program (NLCP). RTI conducts all aspects of the program, including assisting with the development of mandatory guidelines for federally regulated workplace drug testing, the review and assessment of laboratory applications; inspections of applicant and certified laboratories; design, preparation, and distribution, scoring performance testing samples; identifying problem areas, and monitoring corrective actions. These activities are designed to identify issues before they impact drug test results. We operate all aspects of the NLCP, including the inspection program for forensic drug testing laboratories and the manufacture, distribution, scoring, and reporting of proficiency testing (PT) samples. From the beginning of the program to 2022, the NLCP has accredited 147 Laboratories across the US, two in Canada, and one Initial Instrumented Testing Facility (IITF) in Canada and has performed over 4000 inspections and shipped more than 200,000 urine, oral fluid, and hair PT samples. The NLCP publishes a newsletter Drug Testing Matters (DTM) on topics written by subject matter experts that are of interest to the drug testing community. Portions of this article were previously published in the DTM series. More information on the NLCP can be found online at https://forensicrti.org/nlcp/

This is the first of a two-part series on drug testing of urine, oral fluid, and hair. This article provides background information describing the formation of each matrix and how drugs and/or drug metabolites are incorporated into that matrix.

Absorption, Distribution, Metabolism, and Elimination (ADME) of Drugs

A drug can be defined as a chemical substance that affects the processes of the mind or body or as any chemical compound that is administered as an aid in the diagnosis, treatment, or prevention of disease or other abnormal condition, for the relief of pain or suffering, or to control or improve any pathologic condition [1]. Because all drugs (depending on dose) can be considered poisons, drugs also can be termed toxicants, with the ultimate toxicant being the active form that binds to a specific target molecule and produces a pharmacodynamic effect.

Before a drug can have any effect, it must first be taken into the body. Common routes for the administration of drugs are oral, parenteral (intravenous, subcutaneous, and intramuscular), smoking (air pathway membranes and lungs), insufflation, transmucosal (oral and rectal or suppository), and transdermal. Most drugs have a limited number of absorption pathways, and numerous processes occur contemporaneously to prevent the formation of the ultimate toxicant and its binding to its target molecule (e.g., excretion, detoxication). For an in depth review and graphical illustration of ADME, see https://veteriankey.com/absorption-distribution-metabolism-and-elimination/ and https://lifechemicals.com/blog/computational-chemistry/424-importance-of-adme/tox-in-early-drug-discovery [2,3].

An example of a specific drug that may be administered for pain relief is codeine. Compounds produced during the metabolism of codeine are shown in Figure 1. If administered orally, codeine is absorbed and taken into systemic circulation. If administered intravenously (parenterally), codeine is taken into systemic circulation instantaneously. Intramuscular and subcutaneous administration delay codeine's entry into systemic circulation. Some of the codeine in systemic circulation is eliminated as the parent drug into urine and, possibly, bile, thus removing the codeine from being available to interact with an opiate receptor or convert into the more active morphine metabolite. This is sometimes known as "presystemic elimination." Although the parent drug codeine shows approximately 10% of the analgesic activity of its active metabolite morphine, in CYP2D6-competant individuals, codeine is converted partially into the more active morphine; also called "metabolic activation"). In this article, morphine is considered the major ultimate toxicant acutely [4,5]. Some codeine is deactivated by conversion into codeine glucuronide in the human liver. Distribution is a two-way street in most drug delivery processes, including that for codeine. Some codeine (along with any formed morphine, *vide supra*) is distributed to locations in the body where it can interact with an opiate receptor and some is transported to areas where receptor interaction and/or conversion cannot take place. The elimination of codeine glucuronide, morphine and its glucuronides and sulfates, and the small amounts of hydrocodone and formed hydromorphone through renal and biliary processes fall into the category of excretion. Counterbalancing excretion, eliminated drugs and their metabolites can be reabsorbed either by the renal tubules or, in the case of morphine glucuronide, through the intestinal wall and converted back into active morphine by intestinal glucuronidases by a process referred to as enterohepatic circulation). In the context of chronic morphine or codeine use, a small amount of the morphine may be metabolized into active hydromorphone. Notably, the overall distribution and metabolism depend on protein status, the presence or absence of mutant forms of metabolic enzymes and transporters, and the presence of small molecules other than the drugs/drug metabolites of immediate interest that may positively or negatively influence metabolism and transport. Renal and

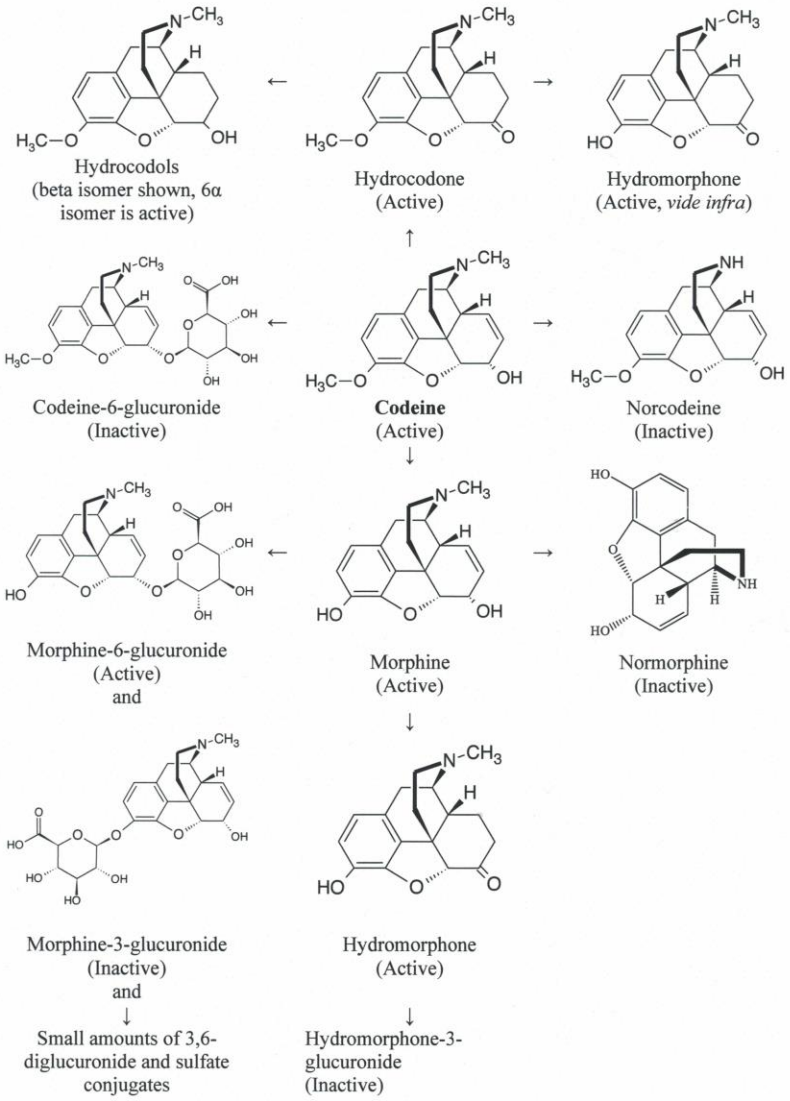

FIGURE 1. METABOLISM OF CODEINE.

hepatic elimination depend on the overall status and health of the respective organs.

Although parent codeine has some analgesic activity, the ultimate toxicant is morphine, with the 6-glucuronide conjugate and minor amounts of hydrocodone and hydromorphone also serving as ultimate toxicants (see Figure 1). After drug exposure, a random sample of blood, blood product, or oral fluid (*vide infra*) drawn from the body and analyzed for the drug and its metabolites will be a snapshot of all the forgoing described processes. Using codeine as the example drug, the extent to which each of the target analytes in Figure 1 is detected and resulted on a toxicology report is a function of the time between drug exposure and sample draw and the analytical capabilities of the laboratory. The drug and metabolites that an analytical toxicologist reports from a urine drug test are similar to blood, blood products and oral fluid but the relative concentrations are averaged over the time that the urine is being formed and collected in the bladder (minutes to hours). Blood, blood products, and oral fluid tend to be better matrices for finding active parent substances. As an excretory product, urine is better for finding metabolites, although metabolites and parent substances can usually be found in blood, blood products, and oral fluid. Unless a technique such as segmentation is employed, the results of hair testing usually demonstrate use over a much broader period of time. Both the parent drug and metabolites can be found in hair specimens.

The metabolism of drugs can result in either toxication ("metabolic activation") or detoxication (sometimes referred to as "deactivation"). The enzymes for numerous drug metabolic pathways exist in multiple parts of the body. However, the liver is usually considered the primary site of drug metabolism. Although some common pathways exist between drugs, the number and types of metabolic products as a whole is typically specific to an individual drug and must be considered when reviewing and interpreting analytical results. Specialized processes, such as the formation of electrophiles, nucleophiles, and free radicals, are important to the general field of toxicology but usually play only minor roles in analytical drug toxicology and are beyond the scope of this limited discussion.

TABLE 1. IMPORTANT COMPONENTS OF KIDNEY FUNCTION.*

Function	Examples
Filtration	Preparation of an ultrafiltrate from plasma primarily to remove small organic compounds and inorganic ions from plasma
Reabsorptive	Water, glucose, amino acids, electrolytes, and proteins are returned to circulating blood thereby forming a hypertonic urine and conserving these vital plasma components included in the ultrafiltrate
Secretory	Non-glomerular addition of blood substances to forming urine
Homeostatic	Maintenance of extracellular volume, acid-base balance, blood pressure, and electrolytes
Metabolic	Synthesis of glutathione, gluconeogenesis, and generation of ammonia and ammonium
Endocrine (hormonal)	Erythropoietin synthesis, activation of vitamin D, and renin release

*Adapted from Reference 6.

Urine

The two most important routes for the elimination of non-volatile drugs are renal and biliary (hepatic). Analysis of fecal material is common only when meconium is used in neonatal drug testing. In adults, even though some drugs, such as Δ9-tetrahydrocannabinol (THC), are found in significant abundance in biliary excretion, the testing of fecal material is almost non-existent except for research purposes and will not be further addressed in this discussion. Urine is the most commonly employed matrix in clinical and forensic toxicology. Urine has historically been the only approved matrix for federally regulated workplace drug testing. A brief presentation on renal function and the application of the basic principles of renal function to drug and/or drug metabolite testing is provided below.

Generally, the kidneys are thought of as excretory organs, but they also have homeostatic, metabolic, and endocrine (hormonal) functions, as presented briefly in Table 1 [6]. Toxicologists usually only have an interest in the excretory (a combination of filtration and tubular physiologic activity) functions of the kidney both for drug and/or drug metabolite testing and specimen validity testing (SVT). The kidneys normally produce 400-2000 mL of urine per day.

The kidneys are ordinarily present as a pair of organs in the retroperitoneal space between the T-11 and L-3 vertebrae of the spinal column, with the right kidney being slightly lower than the left. The primary blood supply to the kidneys derives from the inferior vena cava. This blood supply splits into numerous afferent arterioles, each of which feeds a nephron, which is the functional unit of the kidney. Each human kidney contains approximately 0.6–1.5 million nephrons. The positioning of each nephron with respect to the renal vascularization and the macroscopic anatomy of a nephron are shown in Figure 2.

Blood filtration takes place in the glomerulus, where plasma molecules whose molecular weights are less than approximately 50,000 Da and plasma water are filtered from the blood in the capillaries that branch from the afferent arteriole in the glomerulus. Roughly 20% of the blood presented to the glomerulus is filtered [7]. Highly protein-bound (mostly albumin) molecules, such as THC, are less available for filtration than loosely bound substances, such as d-methamphetamine. Among charged species, the ultrafiltration process favors cationic species and discourages anionic species, especially polyanionic molecules such as albumin. Thus, the glomerulus acts as both a size and a charge filter, which is important for the conservation of proteins, such as albumin, and the elimination of small organic molecules, such as drugs and their metabolites, urea, creatinine, uric acid, and some steroids and their metabolites.

Reabsorption of drugs and their metabolites from the glomerular ultrafiltrate happens mostly in the tubules while the ultrafiltrate from the glomerulus is being concentrated into what will ultimately be collected in the bladder as urine. Although the glomerular filtration process is generally viewed as the source of drugs and their metabolites in formed urine, some drugs and their metabolites are both filtered and secreted by the tubules. Although numerous small organic molecules are excreted, other molecules and ionic species that need to be conserved are re-absorbed, as shown in Table 2.

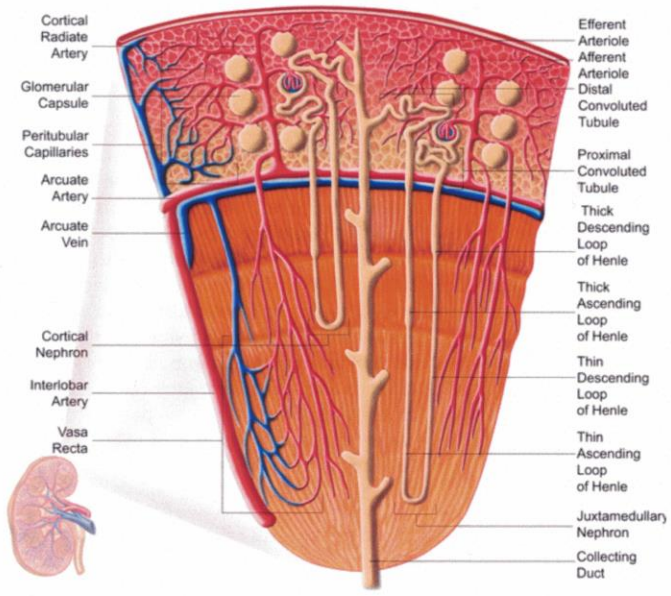

FIGURE 2. VASCULARIZATION AND POSITIONING OF NEPHRONS.

Formed urine is used extensively by clinical and forensic analytical toxicologists to both identify substance use (e.g., the confirmed finding of benzoylecgonine in a urine specimen indicates cocaine use within the past 3–5 days) and confirm compliance with drug therapy qualitatively (e.g., finding oxycodone and/or oxymorphone and no other opiates or analgesics in a patient who has been prescribed oxycodone for chronic pain). Formed urine also contains naturally occurring small organic compounds, such as urea, creatinine, uric acid, cortisol, and 17-ketosteroids, that can be employed to great advantage in urine SVT.

The initial and confirmatory drug test analytes and cutoffs from the 2017 Mandatory Guidelines for Federal Workplace Drug Testing Programs using Urine are listed in Table 3 [8]. High-dose drugs, such as acetaminophen and meprobamate, are generally found in urine in higher concentrations than those in Table 3, whereas drugs such as lysergic acid diethylamide (LSD) are found at lower concentrations.

TABLE 2. FILTRATION, REABSORPTION, AND EXCRETION RATES OF DIFFERENT SUBSTANCES BY THE KIDNEYS.*

	FILTERED (meq/24 h)	REABSORBED (meq/24 h)	EXCRETED (meq/24 h)	REABSORBED (%)
Glucose (g/day)	180	180	0	100
Bicarbonate (meq/day)	4,320	4,318	2	>99.9
Sodium (meq/day)	25,560	25,410	150	99.4
Chloride (meq/day)	19,440	19,260	180	99.1
Water (L/day)	169	167.5	1.5	99.1
Urea (g/day)	48	24	24	50
Creatinine (g/day)	1.8	0	1.8	0

**Glomerular filtration rate: 125 mL/min = 180 L/24 h.*

*Adapted from Reference 5 (page 668)

Oral Fluid

Oral fluid, which is commonly mis-referenced as whole saliva or mixed saliva, is saliva that is mixed with crevicular fluid, nasopharyngeal secretions, oral cavity bacteria, and oral cavity debris such as food particles. For a more complete discussion of oral fluids, including the effects of disease states and drugs on oral fluid itself, please consult Chapter 1 in Reference 9 [9].

About 90% of all saliva is produced by three pairs of major salivary glands (parotid, sublingual, and submandibular) whose locations are shown in Figure 3 (labeled as 1, 2 and 3 respectively). Less than 10% of formed saliva is synthesized from numerous (about 1,000) minor or accessory salivary glands [10]. The production of saliva starts in the acinar cells and moves through intercalated ducts into striated ducts, and finally into excretory ducts where the saliva flows into the oral cavity. As a comparison to the kidneys, the salivary glands usually produce 500-1500 mL of saliva per day. Almost all oral fluid is swallowed.

Routine salivary fluid formation starts in secretory units called acinar cells. An acinar cell contains four ion transporters important for saliva production and flow. The Na^+/K^+ adenosine triphosphatase (ATPase; "sodium-potassium pump"), a Na^+-K^+-$2Cl^-$ cotransporter (NKCCl), and a Ca^{+2}-activated K^+ channel are all located in the basolateral membrane of the acinar cell. A Ca^{+2}-activated Cl^- channel is located on the apical membrane. The expulsion of Cl^- from the acinar cell into the lumen of the salivary duct creates an excess negative charge. The excess negative charge is cancelled out by Na^+ that passes between or through the acinar cells. True to common mammalian physiology, osmotically obligated water passes between the acinar cells or through specific aquaporin channels in the acinar cells and follows Na^+ creating an isotonic saliva in the lumen of the salivary duct. Although the duct wall through which the formed saliva travels is relatively impermeable to water, the saliva can be modified by removing Na^+ and adding K^+ creating a hypotonic saliva that passes into the oral cavity. The myeloproliferative cells which are wrapped around the ducts facilitate movement of formed saliva by squeezing on the duct. Bicarbonate (HCO_3^-) enters into the saliva-producing process by diffusion of CO_2 into the acinar cells and production of carbonic acid (H_2CO_3) by the action of carbonic anhydrase (CA). Formed carbonic acid ionizes into H^+ and HCO_3^-. Formed HCO_3 then either displaces more Cl^- into the lumen since both Cl^- and HCO_3^- are anions or the HCO_3^- itself is expelled into the lumen. An illustration of this process can be viewed at https://www.researchgate.net/figure/Fluid-secretion-model-in-human-parotid-acinar-cells-Acinar-cell-secretion-model-based-on_fig2_6460326 [11]. Formed saliva also contains non-enzyme proteins such as sIgA (secretory IgA; not monomeric IgA as found in blood serum or plasma), enzymes such as amylase, and small molecules that might be found in normal plasma. As saliva is formed and travels down a duct, it may acquire more proteins such as enzymes, mucins, and immunoglobulins and other common analytes that are found in the oral cavity [12].

The amount of saliva formed by the mechanisms described above appears in a ratio of about 16:3:2. Common substances found in the saliva produced by each major salivary gland are presented in Table 4.

The transfer of molecules from capillary plasma to the lumen of the salivary ducts, naturally occurring analytes that would be anticipated in oral fluid are DNA (primarily breakdown of sloughed buccal epithelial cells), RNA (mRNA or messenger RNA, miRNA or microRNA, and non-coding RNA's), epidermal growth factor, nerve growth factor, IgM, IgG, electrolytes, lipids, uric acid, glucose, proteins, enzymes, and hormones. Normal plasma components, some of which are listed in the previous sentence, are expected to enter forming saliva by passive or active diffusion. The minor contribution from Von Ebner's glands adds a small amount of lipase activity to oral fluid [12].

The composition of saliva is highly dependent on flow rate. When the flow rate of saliva increases, salivary Na^+, Cl^-, Ca^{+2}, and HCO_3^- increase while K^+ decreases. The higher amount of HCO_3^- with increasing flow rate enhances buffering capacity and raises pH. Table 5 sums up properties of saliva with flow rate. "Stimulation" in the table

TABLE 3. URINE DRUG TEST ANALYTES AND CUTOFFS FROM THE 2017 HHS MANDATORY GUIDELINES.

Initial test analyte	Initial test cutoff	Confirmatory test analyte	Confirmatory test cutoff concentration
Marijuana metabolites (Δ-9-tetrahydrocannabinol-9-carboxylic acid [THCA])	50 ng/mL	THCA	15 ng/mL
Cocaine metabolite (Benzoylecgonine)	150 ng/mL	Benzoylecgonine	100 ng/mL
Codeine/Morphine	2,000 ng/mL	Codeine Morphine	2,000 ng/mL 2,000 ng/mL
Hydrocodone/Hydromorphone	300 ng/mL	Hydrocodone Hydromorphone	100 ng/mL 100 ng/mL
Oxycodone/Oxymorphone	100 ng/mL	Oxycodone Oxymorphone	100 ng/mL 100 ng/mL
6-Acetylmorphine	10 ng/mL	6-Acetylmorphine	10 ng/mL
Phencyclidine	25 ng/mL	Phencyclidine	25 ng/mL
Amphetamine/Methamphetamine	500 ng/mL	Amphetamine Methamphetamine	250 ng/mL 250 ng/mL
Methylenedioxymethamphetamine (MDMA)/Methylenedioxyamphetamine (MDA)	500 ng/mL	MDMA MDA	250 ng/mL 250 ng/mL

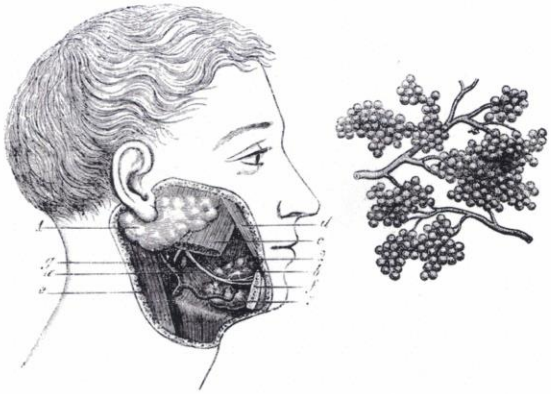

FIGURE 3. LOCATION AND STRUCTURE OF MAJOR SALIVARY GLANDS.

may be gustatory, masticatory, and/or pharmacological. It is notable that the values presented are subject to numerous other factors including, but not limited to, body position, circadian rhythm, circannual rhythm, and degree of hydration [12].

Mucins, which are listed in Table 4. as routine components of saliva, are a class of protein specific to saliva and oral fluid and have a protein backbone that is covered with branching glycans. The glycans give mucins an unusually large water-holding capacity. Mucin ability to hold water gives saliva a property called Spinnbarkeit, which means that it is stretchy or stringy. Mucin's properties are essential to the maintenance of oral cavity health. However, the same properties that are essential for health make neat oral fluid very difficult to handle analytically. A complete discussion of mucins and their properties is beyond the scope of this article [12].

Drugs and their metabolites can enter saliva as it is being formed as described above or can enter the final product (oral fluid) by a number of pathways. These include

TABLE 4. COMMON SUBSTANCES IN SALIVA.

Gland	Substance
Parotid (Serous Saliva)	Amylase
	Proline-rich Proteins
	Agglutinins
	Cystatins
	Lysozymes
	IgA
Submandibular (Mixed Saliva)	Cystatins
	Amylase
	IgA
	Mucins (MG1)
Sublingual (Mucous Saliva)	Mucins (MG1 and MG2)
	Amylase
	Lysozymes
	IgA

Adapted from Reference 10.

- transfer from whole blood that is in contact with forming saliva (primarily basic drugs, except when direct transport is possible);
- as a nasopharyngeal contaminant from the insufflation of drugs (e.g., cocaine);
- when smoke passes through the oral cavity on its way to the lungs (e.g., from a marijuana cigarette);
- when drugs leach from uncoated tablets, pills, or powders while the formulation is in the oral cavity;
- from gastric reflux when the gastric contents contain drug and/or metabolite (e.g., benzoylecgonine after oral or nasal cocaine ingestion);
- from pulmonary efflux (e.g., smoked methamphetamine); and
- from the intake of dust from a solid drug form or an aerosol from a liquid form of a drug.

For a more complete discussion of these processes, please see Chapters 1, 2, and 9 in Reference 9.

TABLE 5. SALIVA FLOW RATE AND PROPERTIES.

Unstimulated		Stimulated
Flow (mL/min)	0.3-0.4	Up to 7
Osmolality (mOsmol/kg)	50-70	Not Measured
pH	6.0-7.0	7.4
Buffer Capacity (mmol H^+/L)	3.1-6.0	3.3-8.5

In the transfer of normal plasma constituents from capillary arterial blood to saliva and the reabsorption of some constituents back into capillary venous blood, formed saliva at the ductus or exit from the salivary gland is vastly different in composition from the blood plasma from which it was created. Similarities between the formation of urine and the generation of saliva are obvious, although the products differ in their function. The so-called "ion trapping" of drugs (i.e., preferential transfer of basic drugs from the almost neutral environment of blood plasma to the more acidic environment typical of saliva) is highly dependent on a number of factors discussed in Reference 9, unless active transport of a drug is possible.

Since the drug and other small molecule composition of oral fluid, which is primarily saliva, is essentially an ultrafiltrate of blood plasma without the concentrative ability of the kidney, it might be anticipated that the drug/drug metabolite composition of oral fluid would be found in lower levels than that found in urine. Although a direct comparison is not possible due to differences in the drug or drug metabolite measured in oral fluid and urine, Table 6, below, is presented for an approximate comparison of the measured drug/drug metabolite concentration measured for employment-related purposes [13].

As stated above, urine is a fairly stable matrix. However, collected neat oral fluid generally behaves differently from urine when stored at room or refrigerated temperature. Neat oral fluid may remain completely liquid with no macroscopic evidence of inclusions or precipitates, form a gel that may or may not revert to the liquid state on repeated inversion or vortexing, remain liquid but form precipitates, or remain liquid but form string-like inclusions. What neat oral fluid does upon storage differs from donor to donor and may even change day-to-day for a given donor [9].

TABLE 6. ORAL FLUID DRUG TEST ANALYTES AND CUTOFFS FROM THE 2019 HHS MANDATORY GUIDELINES.

Initial test analyte	Initial test cutoff	Confirmatory test analyte	Confirmatory test cutoff concentration
Marijuana (THC)	4 ng/mL	THC	2 ng/mL
Cocaine metabolite (Benzoylecgonine)	15 ng/mL	Cocaine Benzoylecgonine	8 ng/mL 8 ng/mL
Codeine/Morphine	30 ng/mL	Codeine Morphine	15 ng/mL 15 ng/mL
Hydrocodone/Hydromorphone	30 ng/mL	Hydrocodone Hydromorphone	15 ng/mL 15 ng/mL
Oxycodone/Oxymorphone	30 ng/mL	Oxycodone Oxymorphone	15 ng/mL 15 ng/mL
6-Acetylmorphine	4 ng/mL	6-Acetylmorphine	2 ng/mL
Phencyclidine	10 ng/mL	Phencyclidine	10 ng/mL
Amphetamine/Methamphetamine	50 ng/mL	Amphetamine Methamphetamine	25 ng/mL 25 ng/mL
Methylenedioxymethamphetamine (MDMA)/Methylenedioxyamphetamine (MDA)	50 ng/mL	MDMA MDA	25 ng/mL 25 ng/mL

Although neat oral fluid may be employed as a drug and/or drug metabolite testing matrix, at the time of this writing, the use of a so-called pad-type oral fluid collector (e.g., Intercept, Quantisal, NeoSal, Oral-Eze), which employs a fiber pad for collection and a buffer-preservative to avoid matrix changes such as those previously described and to preserve drugs and their metabolites, appears to be a desirable approach. For a pad-type collector, an indicator that shows when an oral fluid collection is complete is a necessity. Unlike urine collection, all collections of oral fluid should be witnessed collections [9].

Hair

Human hair grows from the matrix cells (melanocytes and keratinocytes) in the germination center, which is the papilla of the anagen follicle. Mitotic cell division forces the layers above the germination center toward the outside. As the forming hair is pushed upward, keratin expression occurs, followed by pigment incorporation, if any pigment will be incorporated. Subsequently, hardening and dehydration of the hair take place, followed by removal of the inner root sheath before the mature hair protrudes from the skin. The time for an individual hair to grow from the papilla and emerge from the skin is estimated at 8-35 days. This variable is dependent on both the growth rate and the distance from the papilla to the skin surface. As the germination center in the papilla is well vascularized (#6 in Figure 4), there exists a distinct opportunity for drug and/or drug metabolite to be incorporated into growing hair. In addition to the deposition of drugs and their metabolites into forming hair in the papilla, the germination center possesses all of the enzymes required for metabolism, such as

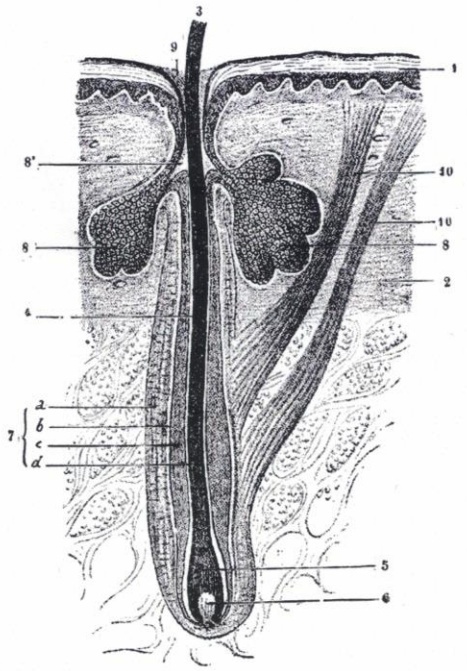

FIGURE 4. GENERAL HUMAN HAIR FOLLICLE AND ACCOMPANYING GLANDS.

phase II glucuronidation, which may explain the presence of highly polar glucuronides in the formed hair shaft [14,15].

Formed or even partially formed hair shafts are usually exposed to eccrine and apocrine sweat, where an apocrine gland (#10 in Figure 4) is present, and sebum (produced by sebaceous

TABLE 7. PROPOSED HAIR TESTING CUTOFFS FROM THE 2020 HHS MANDATORY GUIDELINES.

Initial test analyte	Initial test cutoff	Confirmatory test analyte	Confirmatory test cutoff concentration
Marijuana metabolites (Δ-9-tetrahydrocannabinol-9-carboxylic acid [THCA])	1 pg/mg	THCA	0.05 pg/mg
Cocaine/Benzoylecgonine	500 pg/mg	Cocaine Benzoylecgonine	500 pg/mg 50 pg/mg
Codeine/Morphine	200 pg/mg	Codeine Morphine	200 pg/mg 200 pg/mg
Hydrocodone/Hydromorphone	200 pg/mg	Hydrocodone Hydromorphone	200 pg/mg 200 pg/mg
Oxycodone/Oxymorphone	200 pg/mg	Oxycodone Oxymorphone	200 pg/mg 200 pg/mg
6-Acetylmorphine	200 pg/mg	6-Acetylmorphine	200 pg/mg
Phencyclidine	300 pg/mg	Phencyclidine	300 pg/mg
Amphetamine/Methamphetamine	500 pg/mg	Amphetamine Methamphetamine	300 pg/mg 300 pg/mg
Methylenedioxymethamphetamine (MDMA)/Methylenedioxyamphetamine (MDA)	500 pg/mg	MDMA MDA	300 pg/mg 300 pg/mg

glands, #8 in Figure 4). Such contact with sweat and sebum also provides an opportunity for drug deposition into hair shafts. For a more complete discussion, please see References 14 and 15.

Drugs and their metabolites in hair are found at much lower concentrations than in other matrices and must be analyzed using techniques that are extremely sensitive (e.g., LC-MS/MS or GC-MS/MS for confirmatory testing). Proposed initial and confirmatory drug test cutoffs for hair testing were published in 2020 and are listed in Table 7 [16]. At the time this article was written, the final technical requirements for hair testing in federally regulated drug testing were still being discussed.

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