Clinical Issues Associated with Urine Testing of Substances of Abuse

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Several factors may affect the validity and outcome of urine testing for abused drugs such as amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, ethanol, opiates, and phencyclidine. Urine is used for large-scale testing because acquisition of the sample is noninvasive and because most abused drugs can be detected in urine for a reasonable duration after ingestion. Urine testing for drugs of abuse is a two-step process. In the first step, screening assays are used to identify presumably positive specimens. Common screening tests are radioimmunoassays, enzyme immunoassays, fluorescence polarization immunoassay, and thin layer chromatography. Since they may be subject to cross-reactivity, once a possible positive sample has been identified by a preliminary test, a second more specific methodology, gas chromatography with mass spectrometry, is done to confirm the results. Knowledge of the pharmacology and pharmacokinetics of abused drugs affects selection and interpretation of test results.
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Summary

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Most historians and drug abuse experts propose that we are in the declining phase of a drug epidemic that began some 30 years ago. Still, substance abuse remains a major national public health problem. Evidence continually mounts demonstrating its link to crime, neglect of children, domestic violence, illiteracy, and the acquired immunodeficiency syndrome (AIDS).[1]

The history of substance abuse in the United States dates back to at least 1620 when the Pilgrims landed at Plymouth Rock.[2] Illegal drug use since that time has increased here as well as abroad, and has reached epidemic proportions. In the early 1960s it was estimated that 5% of the population had experience with illicit drugs, and by 1970 this number had increased to 10%.[2] In 1988 the National Institute on Drug Abuse estimated that 19% of the United States population over age 12 years had used illicit drugs in the preceding year. The figure jumped to 44% for individuals age 18–25 years.[3] Currently, 13 million Americans use illicit drugs, and it is estimated that half of our youth will have an encounter with an illicit substance before high school graduation.[2,4]

This multifaceted problem has no simple answer. Recognizing and documenting substance abuse is extremely difficult; it is far easier to

Table 1. U.S. Department of Health and Human Services Testing Threshold Guidelines

Drug Class	Immunoassay Screening Threshold	GC-MS Confirmatory Threshold	Urinary Detection Period (days)
Amphetamines	1000 ng/ml		1–2
Amphetamine		500 ng/ml	
Methamphetamine[a]		500 ng/ml	
Cannabinoids	50 ng/ml		2–8, short-term use
11-nor-Δ9-carboxylic acid		15 ng/ml	14–42, long-term use
Cocaine	300 ng/ml		2–4, short-term use
Benzoylecgonine		150 ng/ml	up to 8, long-term, high dose
Opiates	300 ng/ml		1–2
Morphine		300 ng/ml	
Codeine		300 ng/ml	
Phencyclidine	25 ng/ml	25 ng/ml	2–8

Adapted from references 10, 11, and 63.
[a]The specimen must also contain amphetamine at a concentration of ≥ 200 ng/ml.

assess its results. As examples of the far-ranging consequences, an estimated 10–65% of individuals who are seropositive for the human immunodeficiency virus acquired the virus due to intravenous drug abuse,[5] and 50% of persons arrested for serious crimes in Washington, D.C., in 1992 had positive urine tests for illicit drugs.[6] Therefore, it is vital that health care providers be alert not only to the related signs and symptoms, but be familiar with laboratory tests that will help them differentiate problems associated with drug use from those due to other disease etiologies.

Urine testing for drugs of abuse has become increasingly popular. In addition to its use as a toxicologic screen in most emergency rooms, many employers mandate urine testing as a preemployment screen, guidelines have been established for a drug-free federal workplace that include urine testing, many companies perform for-cause testing when drug abuse is suspected, and random screens have long been performed on members of the armed services, athletes, and those in drug treatment facilities.[7] Positive results are interpreted as objective evidence that a person is using the agent. The consequences may include counseling, termination of employment, imprisonment, and other corrective or punitive measures.

Features of Urine Testing

Various body fluids can be tested for the presence of drugs, such as blood, saliva, hair, and urine.[8] The reason for the increased frequency of urine testing is primarily the fact that urine is easily obtainable (noninvasive) and contains relatively high concentrations of drugs and their metabolites.[8]

Urine has several disadvantages as the test specimen including the fact that it can be adulterated, diluted, or substituted. Drug concentrations in urine may vary depending on such factors as dose, route of administration, and time elapsed since administration. They also are influenced by urine flow, pH, and metabolism. Urine commonly indicates the presence or absence of drugs, but it cannot easily be used to quantify levels or to determine the time or duration of use. Thus it is not possible to establish whether an individual abuses a drug habitually or sporadically and casually. Also, it is not possible to correlate the presence of an abused substance in the urine with any specific degree of central nervous impairment in the user. However, some laboratories, if specifically requested, can provide quantitative levels that may be helpful to monitor cannabinoid levels after prolonged use.

Specimens testing positive are defined as containing drug in concentrations equal to or above the designated threshold. However, the threshold concentration (also referred to as cutoff) does not specify the sensitivity or detection limit of the assay and only designates whether specimens are positive or negative.[9] Cutoff concentrations are set by the Department of Health and Human Services (DHHS) to define a positive result (Table 1).[10, 11] They are within the detection limit of the assay to avoid imprecision related to technique.[9, 12] The cutoff value can be an actual or a calculated value.[13] It

Table 2. Advantages and Disadvantages of Analytic Methods[9]

Method	Advantages	Disadvantages
Preliminary tests		
Radioimmunoassay	Nominal personnel requirements Objective results Small sample requirements Minimal pretreatment	Radioactive isotope Heterogeneous assay Labor intensive
Enzyme immunoassay	Nominal personnel requirements Adaptation to batch or random-access analyzers Homogeneous Short analysis time	Reagents expensive Not specific for single drugs
Fluorescence polarization		Limited capacity
Thin-layer chromatography	Low equipment cost Rapid analysis Simultaneous determination of several drugs and metabolites	Skill dependent Labor intensive Sample pretreatment Subjective interpretation Nonreproducible raw data
Confirmation tests		
Gas chromatography	Reliable Sensitive analysis Reproducible results	Labor intensive Limited capacity Need to derivatize nonvolatile substances Expertise required High maintenance
High-performance liquid chromatography	Highly sensitive analysis	Similar to GC
Gas chromatography-mass spectrometry	Specificity in identification	Costly equipment Complex instrumentation High level of skill necessary to operate and interpret data Individual agents in a group where mass spectra are similar not discernable

infers the point at which a drug is not only detectable, but can be differentiated precisely from other, possibly endogenous, substances in the urine.[14] The specificity and sensitivity of a test are affected by cutoff concentrations. If the concentration of drug in the urine is above the cutoff value, the sample is regarded as positive, and if it is below the cutoff value, it is considered negative.

The goal of drug testing is to achieve accuracy with no false-positive or -negative results. A false-positive result is most commonly due to cross-reactivity of the assay with other substances that have structural similarity with the abused substance. False negatives may occur when the concentration of the substance in the urine is below the accepted threshold or when the sample has been diluted or otherwise adulterated to obscure the presence of a drug.

Types of Tests

Screening tests generally are performed to identify presumably positive specimens. These are followed by confirmation with a different, more specific, analytic test (Table 2).[9]

Preliminary Tests

Immunoassay is currently the preferred technique for initial urine tests.[7] Several immunoassays are available, including radioimmunoassay (RIA), enzyme immunoassay (EIA), and fluorescence polarization immunoassay (FPIA). Federal guidelines do not specify the assay of choice, but accuracy, precision, and sensitivity are equal, results are comparable, and differences in specificity are not significant for these tests.[9]

One advantage of immunoassays is that many specimens can be processed by automated instrumentation, minimizing personnel requirements. Also, results are objective because of computerized reporting.[9] The disadvantage is that these tests are often specific for a class of drugs rather than a single drug.[9] The systems are based on antibody binding to the drug, so that chemically related compounds or metabolites commonly cross-react, causing a false-positive

result. Therefore, a positive immunoassay requires confirmation by a more specific test.

Radioimmunoassay is based on a simple equilibrium equation. An antibody to the drug is generated usually in an animal such as a sheep or pig. Blood is drawn from the animal and the specific antibody is extracted and purified. When the extract is added to a test tube of sample, the antibody will bind to the drug. The drug-containing sample is then mixed with a radiolabeled ligand that competitively binds to the antibody. Once equilibrium is reached, the amount of the radiolabeled ligand that is not bound to the antibody directly correlates with the amount of drug in the sample.[15]

The enzyme multiplied immunoassay technique (EMIT) is the most common EIA. It is based on the principles of RIA but does not use radiolabeled antibodies. It is based on measurement of the enzymatic activity of glucose-6-phosphate dehydrogenase (G6PD) derived from the bacterium *Leuconostoc mesenteroides*. In the presence of a substrate such as glucose, this enzyme reduces the cofactor nicotinamide adenine dinucleotide (NAD) to reduced NAD (NADH). An enzyme-drug conjugate is prepared by combining the G6PD enzyme with molecules of the drug to be assayed.

To measure the concentration of drug in a sample, the enzyme-drug conjugate is added to the the sample fluid together with antibody to the drug, NAD, and a substrate for the G6PD enzyme. The drug on the enzyme-drug conjugate and the free drug in the sample fluid compete for binding sites on the drug antibody. If the enzyme-drug conjugate binds to the antibody, a steric hindrance results in a reduction of enzyme activity, such that the conversion of NAD to NADH is decreased. The greater the quantity of drug in the sample, the fewer antibodies will bind to the enzyme-drug conjugate, resulting in greater activity of enzyme. Therefore, greater production of enzymatic product correlates with higher drug concentration. Enzyme immunoassay is the most widely performed initial test because it is sensitive and specific as well as suitable for testing in high-volume laboratories and on a variety of batch or random access analyzers.[15]

The cloned enzyme donor immunoassay (CEDIA) is a newer EIA that is based on principles similar to the EMIT assay. It uses recombinant DNA technology to produce enzyme donor units to which a molecule of the assay drug is bound, and acceptor units that combine with drug antibodies to produce an antibody-enzyme-drug conjugate that cannot operate as an active enzyme. In the presence of assayable drug or metabolite, the antibody binds the free drug instead of the enzyme donor molecule, and an active enzyme produces a measurable product that correlates with drug concentration.[16]

The FPIA is based on fluorescence polarization and competitive binding. Antibodies to the drug and a tracer drug or similar chemical labeled with fluorescein are mixed with the specimen containing drug molecules that compete for antibody-binding sites. In specimens with low analyte (drug) concentration, a large amount of tracer binds to antibody with subsequent high polarization of fluorescence. Conversely, specimens with high analyte concentration result in low polarization of fluorescence.[15] Because of differences in cutoff values, comparison of results with other methods should be undertaken with caution.[13]

Thin-layer chromatography (TLC), a chromatographic procedure for preliminary testing, can be used to determine several drugs and their metabolites simultaneously.[9] The sample is placed on one end of a gel-covered plate and allowed to move up the plate by permeation. Large molecules move more slowly, and the sample can be separated into components with this method.[15] In contrast to immunoassay systems, TLC is labor intensive and the results are read by the operator, potentially leading to bias. Operator skill is therefore crucial in determining the quality of results. In addition, the results are not stored or easily retainable.[9] However, an advantage is its low equipment cost. The test can be done to confirm positive immunoassay results, although this is seldom done.[13]

Other newer simple screening systems, such as the Triage panel, use monoclonal antibodies to produce a color-coded reaction, similar to home pregnancy tests, in the presence of drugs in solution at threshold or greater concentrations.[17] These screening systems are fast and simple but tend to be expensive.

Confirmation Tests

Although gas chromatography with a mass spectrometry (GC-MS) detector is the best available analytical method for confirmatory testing, GC has been used with other types of detectors as a confirmatory technique.[9, 18] However, MS is the most specific technique used

for identification. Gas chromatography is used to separate vaporized analytes carried by a gas across a separation column where they are retained for varying periods of time, depending on their relative affinities for the column. The detector then identifies a spectrum or pattern that is specific for a particular analyte. Mass spectrometry provides a fragmentation pattern that is usually characteristic of a specific compound.[11]

Combined GC-MS is regarded as the only legally defensible and most reliable method of testing urine for drugs of abuse.[18] The GC component separates analytes and MS identifies them, resulting in both sensitivity and specificity. The disadvantage is that technical skill and experience are necessary to operate the instrument and interpret the results, making the system labor intensive and expensive.

High-performance liquid chromatography (HPLC) is also done for confirmation testing. It is similar to GC, but the analytes do not require vaporization, and the carrier is a liquid rather than a gas.[15] Although the test is highly sensitive, specific confirmation requires a method such as MS to determine the actual component of each HPLC peak. In addition, HPLC is labor intensive and time consuming because only one sample can be analyzed at a time.

Commonly Assayed Drugs of Abuse

Alcohol, barbiturates, and benzodiazepines are commonly abused. Drugs that are included in the DHHS guidelines for testing in the workplace include amphetamines, cannabinoids, cocaine, opiates, and phencyclidine (PCP).[10] In some cases the drug itself is measured in urine testing, in other cases it is a metabolite. Knowledge of the pharmacology of these drugs is important for interpreting urine tests.

Alcohol

Despite the ubiquity and legal availability of alcohol (ethanol), urine testing for the agent is not commonly performed. Several reasons account for this. First, it is not one of the five drugs mandated for testing by the DHHS, although the Department of Transportation guidelines now require breath alcohol testing. Second, urine alcohol concentrations correlate poorly with blood concentrations. Breath and blood alcohol concentrations correlate more closely with central nervous system (CNS) effects and are used to determine whether an individual is operating a vehicle while impaired due to alcohol.

After oral ingestion alcohol is metabolized, principally by alcohol dehydrogenase, to acetaldehyde and ultimately to carbon dioxide and water by acetaldehyde dehydrogenase. A small amount is also metabolized by the microsomal enzyme system. Alcohol's pharmacokinetics are complicated, and it is commonly stated that its elimination is zero order.[19] However, this is somewhat less than true because its elimination conforms to Michaelis-Menton pharmacokinetics, which means that the speed of elimination is concentration dependent.[20] Consequently, blood alcohol levels over time in a specific individual are not as predictable as was once believed.

Pharmacologic effects of alcohol are well known and range from stimulation and disinhibition at lower concentrations to impairment of coordination and speech, respiratory depression, and possibly death at high concentrations.[21] Blood alcohol concentrations 0.05 g% and above are associated with measurable impairment in complex tasks requiring divided attention, such as driving.[22]

Blood is the preferred matrix for testing alcohol concentrations and is usually preferred to urine. Breath alcohol concentrations also correlate more closely with blood alcohol concentrations than do urine concentrations. For urine testing a biochemical method is usually performed in which alcohol dehydrogenase oxidizes any alcohol that is present to acetaldehyde. The reaction producing acetaldehyde results in conversion of NAD to NADH. The concentration of NADH is measured spectrophotometrically and correlated to alcohol concentration.[23]

It is important to recognize that a positive test establishes that alcohol has been ingested, but it does not indicate that the amount ingested was sufficient to cause impairment. In addition, unlike PCP, cannabinoids, or cocaine, alcohol is widely and legally available. Therefore, persons with positive urine alcohol tests have not necessarily done anything illegal. Consequently, determining the significance of a positive result is not always straightforward.

Amphetamines

Amphetamine consists of not only racemic amphetamine, dextroamphetamine, and methamphetamine, but also chemically related compounds such as the phenethylamines pseudoephedrine, phentermine, ephedrine,

phenylpropanolamine, and phenylephrine. Many of the last compounds are available in a variety of over-the-counter products and have the potential to interfere with preliminary urine tests, resulting in false-positive results. Notably, some other illegal substances, such as methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA, ecstasy), have the potential to produce positive results in some immunoassays used for screening for amphetamines.[23]

Selegiline, a monoamine oxidase inhibitor used to treat Parkinson's disease, is metabolized to both *l*-amphetamine and *l*-methamphetamine.[24] Since the *l*-isomers do not produce the euphoria seen with the *d*-isomers, selegiline is not commonly abused, although it tests positive in many immunoassays. Chiral GC-MS procedures that can distinguish between the *d*- and *l*-isomers should be applied when positive amphetamine urine tests may be due to selegiline.[25] Large doses of Vicks nasal inhaler can result in measurable quantities of *l*-methamphetamine.[25] However, only the older EMIT-d.a.u. assay is affected by this interaction. Even twice the recommended dose of Vicks inhaler causes no false positive by EMIT monoclonal amphetamine-methamphetamine or EMIT II amphetamine-methamphetamine assays.

Racemic mixtures or *d*-isomers of amphetamines cause mood elevation, increased alertness, and decreased appetite, and may be prescribed to treat hyperactive children or narcolepsy.[26] Central nervous system effects are mediated by release of dopamine and norepinephrine from central neurons. The typical abuser combines amphetamines with alcohol, barbiturates, benzodiazepines, or opiates to minimize the undesirable effects of amphetamines. Tablets frequently are obtained from legal sources but diverted to the illicit market; they may be taken orally or crushed and injected intravenously to obtain the euphoric effects. Alternatively, amphetamines may be synthesized by illicit laboratories in a form that can be easily dissolved and injected or smoked. Common street names for amphetamines are uppers, pep pills, bennies, meth, crank, dexies, hearts, whites, black beauties, speed, crystal, and ice, a smokeable form of methamphetamine.[9,26]

Peak plasma concentrations are achieved very rapidly after intravenous administration of an amphetamine, and maximum concentrations after oral ingestion usually occur within 2 hours.[26] Half-lives of the different amphetamines vary, but generally range from 8–12 hours.[26] Amphetamine sulfate is metabolized in the liver by aromatic hydroxylation, *N*-dealkylation, and deamination, whereas methamphetamine is slowly metabolized to amphetamine.[27] Approximately half the dose of either agent is excreted in urine unchanged.[28–30] Urinary excretion of unchanged drug is pH dependent; urinary acidification to pH less than 5.6 yields a plasma half-life of 7–8 hours, whereas alkalinization may increase the half-life to up to 33.6 hours.[26,27]

Both TLC and the immunoassays are used for preliminary testing for amphetamines, but TLC is more specific. The immunoassays vary in their specificity for amphetamine; RIA appears to be the most specific followed by FPIA and EIA.[31,32] The newer EMIT assays differ in their specificity for the agents. The EMIT amphetamine class assay contains polyclonal antibodies that cross-react with many drugs that have an amphetamine-like structure.[33] The EMIT-d.a.u. monoclonal amphetamine-methamphetamine assay is much more specific for amphetamine and methamphetamine but it also detects MDA and MDMA.[34,35] The EMIT II amphetamine-methamphetamine assay also contains monoclonal antibodies and is even more specific for detecting only amphetamine and methamphetamine.[36] The lack of specificity of many preliminary tests emphasizes the need to confirm a positive result by either GC or GC-MS.[9,37–40]

Barbiturates

Barbiturates can produce CNS mood alterations ranging from excitation to mild sedation, hypnosis, and deep coma. At the lower end of the dosage range the agents usually produce relaxation and euphoria. Barbiturates are classified based on duration of action and specific clinical use.[7] Common street names are barbs, downers, and goofballs; blues or blue heavens (amobarbital); yellow jackets (pentobarbital); red birds or red devils (secobarbital); and rainbows and red-and-blues (secobarbital and amobarbital).[7,9]

These drugs are absorbed to varying degrees after oral ingestion, with onsets of action ranging from 20–60 minutes.[41] They are weak acids that are primarily in the nonionized state in the blood, resulting in a high degree of lipid solubility. They are rapidly distributed to all tissues and fluids, with high concentrations in the brain, liver, and kidneys. Barbiturates are metabolized primarily by the hepatic microsomal

enzyme system, and metabolites are excreted in urine and feces.[42] Approximately 25–50% of phenobarbital, a long-acting agent, is excreted unchanged in urine, whereas only negligible amounts of other short- and intermediate-acting barbiturates are excreted in the urine.[33] Total elimination is more rapid in young than in elderly persons; the elimination rate in infants is 2–5 times faster than in adults due to the metabolic process.[9]

Barbiturates can be detected in for varying amounts of time urine depending on the half-life of the specific compound .[9] In contrast to short- and intermediate-acting agents, which may test positive for 1–3 days, long-acting barbiturates may be detected for 10–14 days after a single dose and for several weeks after long-term use.[11,23]

As with the amphetamines, TLC and immunoassay are common preliminary tests for barbiturates. Although TLC has greater specificity, it cannot differentiate among agents in the same class (short-, intermediate-, long-acting). Immunoassay cannot differentiate among classes of compounds, although some of them are sensitive for specific barbiturates. Presumptive positive results are usually confirmed by HPLC, GC, or GC-MS.

Benzodiazepines

Benzodiazepines are marketed and prescribed worldwide as sedatives, anxiolytics, muscle relaxants, and anticonvulsants. Since they have many legitimate uses, it is sometimes difficult to determine whether they are being abused. The prescriber or dispenser can ask several questions to identify abuse; for example, whether the drug appears to be causing impairment, the dose is being increased without direction of the prescriber, and prescriptions are being refilled more frequently than necessary.

The abusability of these drugs was studied in individuals with a history of drug abuse.[43] In general, benzodiazepines were not rated as highly desirable by such individuals compared with other commonly abused substances. Most likely to be abused are those with high lipophilicity and rapid absorption and onset of effect, such as flunitrazepam and diazepam. Although flunitrazepam (Rohypnol) is not currently marketed in the United States, it is available in Europe and Latin America and has developed a following as an illicit drug in states such as Florida and Texas, which are close to or border other countries.[44]

Pharmacologic effects associated with benzodiazepines include sedation, muscle relaxation, disinhibition, ataxia, and amnesia. Most benzodiazepines are extensively biotransformed by oxidation or reduction, or undergo conjugation with glucuronic acid.[45] They have structural similarities, and the majority can cause positive results of EIA urine screens due to cross-reactivity. The duration of detection in urine depends on elimination half-life, dose, and duration of use of the specific agent. Some rapidly eliminated drugs such as alprazolam may be detected in urine for only a few days, whereas more slowly eliminated ones may be detectable for weeks or even months after long-term use.[33]

Immunoassays for urine screening are often calibrated with oxazepam or nordiazepam, which are common metabolites of many of the compounds.[23,45] Benzodiazepines as a class may be detected by immunoassay, but determining which specific agent is present requires GC-MS. Unfortunately, many laboratories do not routinely determine specific agents by GC-MS other than oxazepam, nordiazepam or diazepam. Also, since cutoff levels are not stipulated by the DHHS, the threshold for reporting a positive result varies depending on the laboratory.[23]

Cannabinoids

Cannabinoids include more than 60 C-21 compounds found in the plant *Cannabis sativa*, commonly known as marijuana. Marijuana is also known as pot, Mary Jane, grass, weed, hash, hashish, and bhang.[9] Its effects are probably the result of a sum of different active compounds. Delta-9-tetrahydrocannabinol (THC) is the major intoxicant and is the most extensively studied cannabinoid.[46] The content of THC in marijuana increased slightly from 2% in the 1970s to 4% in 1988; carefully cultivated varieties such as Sinsemilla may have an average THC content of around 7%.[46]

The immediate effects of smoking cannabis are euphoria, altered sense of time, keener sense of hearing, exaggerated visual imagery, alteration in short- and long-term memory, impaired motor function, and possibly hallucinations and paranoia. Symptoms are usually accompanied by tachycardia, conjunctival reddening of the eyes, a feeling of hunger, and dry throat and mouth.[46]

Marijuana is better absorbed after smoking than after oral ingestion, primarily due to first-pass elimination in the liver. Factors that alter its bioavailability and onset of effect include the potency of THC, puff duration, volume of smoke

inhaled, and amount of time the smoke is held in the lungs. The average bioavailability of THC in heavy smokers is 13% higher than in light smokers.[9] The substance is highly lipophilic with a large volume of distribution (10 L/kg), and accumulates in many body tissues, especially the liver and lungs. When smoked, the peak plasma THC concentration is usually achieved within minutes. The levels are lower and more erratic after oral ingestion than after smoking or intravenous administration.[47] The concentrations of metabolites are usually higher after oral ingestion.[47–49]

The initial half-life of marijuana is 3.1–4.5 minutes, reflecting rapid distribution into tissues including the brain.[50] After redistribution, marijuana has a longer elimination half-life of 19–36 hours.[46, 48, 50] After THC is transformed by the cytochrome P-450 system to a psychoactive metabolite, it is oxidized by alcohol dehydrogenase to an inactive compound. It undergoes extensive enterohepatic circulation.[51] One-third of the metabolites are eliminated by the kidneys and the remaining two-thirds are excreted in feces. The metabolites of THC appear in urine for 2–8 days after short-term use and 14–42 days after long-term use.[11]

The time after marijuana use during which a positive urine test may result depends on route of administration, potency of THC, frequency of use, and assay sensitivity.[9] Several tests are effective for preliminary testing of THC and its metabolites, such as EMIT, RIA, and FPIA. Although each of these is sensitive enough for preliminary testing, none is specific for the carboxy-THC metabolite itself. Since chromatographic assays (TLC, HPLC, GC, GC-MS) specifically measure the carboxy-THC metabolite, they are often used for the confirmation test after immunoassay. The threshold for an immunoassay system is usually set at 50 ng/ml for preliminary testing and 15 ng/ml for confirmatory analysis by GC-MS.[9] Although any of the chromatographic methods may be effective, GC-MS is considered the assay of choice for confirmation. Immunoassay and GC-MS may detect urinary cannabinoid metabolites, but they cannot quantify the amount ingested or smoked.

Anecdotal reports describe nonsmokers testing positive for cannabinoids after being exposed to passive inhalation.[52–56] It is possible that this could occur, but the exposure would have to be extensive and in a very small closed, nonventilated area. The likelihood of a positive urine test due to passive inhalation is miniscule.

In a report of three studies, 80 urine specimens from 12 subjects were collected separately over 24 hours after passive exposure to marijuana.[52] In the first study, two nonsmokers were placed in a confined area with two experienced subjects who smoked two marijuana cigarettes containing 2.5% THC for 1 hour. This was repeated several weeks later with the THC content increased to 2.8%. In the second study, nonsmokers were placed on two separate occasions in a medium-size station wagon for 1 hour after four people started smoking marijuana cigarettes containing 2.8% THC. In the final study, four smokers were instructed to smoke one marijuana cigarette as little as possible in the presence of two nonsmokers. All samples collected from nonsmokers were analyzed by EMIT for the presence of cannabinoids. Of the 80 samples, only 2 tested positive for THC with a value equal to or greater than 20 ng/ml (the threshold for positive urine testing is 50 ng/ml). Of interest, both of these samples were collected in the first void, 5 and 6 hours, respectively, after exposure. The results indicate that it is highly unlikely that a positive cannabinoid urine assay would result if individuals were not confined to small, nonventilated areas and marijuana cigarettes were not smoked simultaneously.[52]

Cocaine

The abuse of cocaine increased tremendously in middle and late 1980s, and received much attention in the mass media and at federal and local law enforcement levels. Commonly known as coke, toot, dandruff of the gods, brain Tabasco, snow, and lady, it is extracted from the leaves of the South American shrub *Erythroxylon coca*.[26, 57] The hydrochloride form is prepared by dissolving the cocaine base in hydrochloric acid, forming a water-soluble salt.[9] Although it is convenient for legal medicinal purposes, cocaine hydrochloride is heat labile.

Cocaine produces a more intense euphoria after smoking than after insufflation (snorting) or oral intake, so smokers convert it to the freebase form, an alkaloid base that is not destroyed by heat. Traditionally, freebase was prepared by extracting cocaine into an organic solvent such as ether, but this method lost favor since ether is extremely flammable.[26] Freebase can be prepared more safely from the hydrochloride form by mixing it with baking soda and water.[9, 26] The resulting alkaline solution is evaporated to form a

rocklike substance commonly known as crack.[9]

There is no doubt that cocaine causes severe psychologic dependence, particularly in those who inject it or inhale the vapors. The drug produces increased alertness, depressed appetite, decreased perception of the need for rest, and intense euphoria. Acute cocaine intoxication leads to profound CNS stimulation that may progress to seizures and cardiac arrest.

Coca leaves are traditionally chewed or sucked, which results in slow absorption through the mucous membranes, low blood levels, and a relatively slow onset of action. Cocaine hydrochloride is a fine powder that may be insufflated high into the nasal membrane mucosa, producing relatively quick absorption and onset of effects. Intravenous use leads to a quick and powerful but brief effect. Smoking crack results in the most profound effects and has become the method of choice for many users. The large surface area of the lungs is ideal for absorption, and circulation of blood between the lungs and brain is rapid, producing effects almost immediately.[26]

Cocaine is metabolized by ester hydrolysis and *N*-demethylation in the liver and serum.[58, 59] It is converted in the blood to benzoylecgonine at alkaline and neutral pH by nonenzymatic hydrolysis, and to ecgonine methyl ester by pseudocholinesterase. Both metabolites are excreted in urine, and the quantity and proportion of each do not differ with route of administration.[58, 60, 61] Cocaine itself is renally excreted to an appreciable degree with an estimated half-life of about 1 hour. The benzoylecgonine and ecgonine methyl ester metabolites have plasma half-lives of 7.5 and 3.6 hours, respectively; they can be detected for up to 3 days after occasional use, and up to 8 days after repeated high doses.[62, 63]

Immunoassays are favored for preliminary testing of urine for cocaine and do not require sample preparation or stabilization.[62, 64–66] Immunoassays target benzoylecgonine and have little cross-reactivity with the remaining metabolites, except RIA, which cross-reacts with cocaine.[9] Immunoassays can detect benzoylecgonine at a concentration of 300 ng/ml or less. Chromatographic techniques can also be used to detect cocaine or its metabolites in urine, but sample preparation is somewhat complicated. Gas chromatography-MS has the highest sensitivity and specificity in detecting cocaine and benzoylecgonine and is used to confirm preliminary tests.[67]

Opiates

Opium is derived from the *Papaver somniferum* plant.[68] During harvest, the seed pod produces a resinlike material from which morphine is extracted and heroin is derived. Raw opium contains about 10% morphine by weight. Heroin is synthesized by diacetylating morphine.[68] It is 2–3 times more potent than morphine and, due to the added acetyl groups, has better penetration across the blood-brain barrier. Street names for heroin include skag, dope, shill, horse, H, white stuff, and Lady Jane.[9] Although chemists have developed several synthetic compounds that produce the same narcotic effects as morphine, an agent that has the same analgesic effects without causing physical dependence has yet to be synthesized.

Opiates act by binding and activating specific receptors in the brain, mimicking the activity of enkephalins and endorphins. These naturally occurring peptides have potent analgesic effects and reside in the neurons that innervate the midbrain gray area involved in pain perception. The major indication for the narcotic analgesics is pain control. Pain is generally relieved after appropriate doses, but other effects also occur, including euphoria, decreased number of peristaltic contractions in the gastrointestinal tract, and an antitussive effect. Tolerance develops to the analgesic as well as the euphoric effects, and consequently it becomes necessary to increase the dose to produce or maintain the same effect.[68]

Due to the increase in purity of available heroin supplies on the street and the fear of contracting AIDS, many heroin users smoke heroin. Even so, since heroin is partially destroyed by pyrrolysis, the intravenous route is still preferred by many. Intravenous administration results in immediate high drug concentrations in plasma and rapid distribution into tissue.[69] Heroin bioavailability is compromised when it is taken orally due to significant first-pass biotransformation. The plasma half-life is about 3 minutes due to its rapid conversion to morphine by hydrolysis.[70]

Morphine has oral bioavailability of approximately 30% due to significant first-pass hepatic metabolism.[71, 72] In contrast, intramuscular administration results in nearly complete bioavailability. Morphine undergoes rapid distribution into the tissues; the volume of distribution varies between 1.0 and 6.2 L/kg.[9] The maximum plasma concentration is reached

7–20 minutes after intramuscular administration compared with 15–60 minutes after oral ingestion.[9]

Codeine undergoes fairly rapid absorption after oral administration, with peak plasma concentrations occurring in about 1 hour. The majority of its analgesic effect is due to conversion to morphine; 10% of a codeine dose is transformed to morphine and the remaining 90% undergoes metabolism to norcodeine. The glucuronide conjugates of codeine, norcodeine, and morphine appear in urine.[9]

Opiate agonists are metabolized mainly in the endoplasmic reticulum in hepatocytes by microsomal pathways, but also in the CNS, kidneys, lungs, and placenta. In addition to hydrolysis, oxidation, and *N*-dealkylation, opiate agonists undergo conjugation with glucuronic acid. The drugs are excreted principally in urine in the unchanged form and as metabolites; small amounts are excreted in feces.[73]

Immunoassays for preliminary testing have adequate sensitivity but do not distinguish morphine from other opiates.[74, 75] They can detect both free and conjugated forms of morphine and codeine, as well as dihydrocodeine, hydrocodone, hydromorphone, and oxycodone.[9] Because they cannot distinguish among these compounds, confirmation must be performed with a more specific method. Chromatographic procedures appear best, although TLC is occasionally done. Since codeine is metabolized to morphine, a procedure that can simultaneously detect and differentiate the two is necessary. Although HPLC, GC, and GC-MS are capable of this, GC-MS is preferred because it identifies the agent unequivocally.[9]

Phencyclidine

Phencyclidine is among the five drugs required by the DHHS to be tested in the workplace, but it is not consistently abused throughout this country. Its abuse appears to occur in pockets, primarily in selected urban areas.[76]

The agent was initially synthesized in the 1950s as an anesthetic, but it was quickly removed from the market due to unpredictable behavioral reactions that occurred during recovery from anesthesia. It is usually abused due to its euphoric and hallucinogenic effects. However, these effects can be quite erratic, with violent or bizarre behavior occurring in up to 35% of patients who come to hospital emergency departments after using PCP. The sensorium in these patients can vary from alert and oriented to stuporous.[77] Physical manifestations of PCP use are also not consistent. Tachycardia, hypertension, nystagmus, diaphoresis, seizures, skeletal muscle rigidity, and dystonias have been seen but are not universally present. Possible disturbing results of the combination of psychotic or violent behavior and anesthetic actions are severe injuries such as broken limbs, and severe wounds self-inflicted by people who are feeling "no pain" and are too violent and agitated for medical staff to treat.[77]

The drug exerts its pharmacologic effects by binding to a site within the *N*-methyl-D-aspartic acid (NMDA) subtype of glutamate receptors. Binding of PCP results in blockade of the NMDA calcium channel.[78] The drug is highly lipophilic and can be administered by oral and intravenous routes, as well as by smoking. A formerly popular route was smoking after spreading PCP on parsley or marijuana.

The majority of PCP is hydroxylated and then glucuronidated; the metabolites are excreted renally. Up to 15% is eliminated unchanged in urine.[79] The elimination half-life is usually about 3 days, but urinary acidification can increase the speed of elimination and decrease the half-life to 1 day.[23] Usually PCP can be detected in urine for about 1 week, but this may increase to 2–4 weeks in long-term users. Few substances cross-react with PCP in immunoassays, although there is one anecdotal report that large doses of thioridazine can cross-react.[80] The presence of either thioridazine or PCP can be confirmed by GC-MS.

Alteration of the Urine Sample

The drug-abusing population has devised many ways to prevent detection of illicit drugs in urine. These range from ingestion of legal substances known to cross-react with the assays to in vitro adulteration by adding substances that interfere with the enzymatic methodology of the assay. Other methods are substituting a sample from a drug-free individual and diluting the urine by ingesting diuretics.[81, 82]

In Vivo Cross-Reactants

Several over-the-counter agents and foods cross-react with assays causing false-positive results for intoxicating drugs. Common cold and allergy preparations may interfere with various urine assays for amphetamines.[13] For example, diphenhydramine can cause a false positive for methadone, and phenylpropanolamine may test

positive for amphetamine.[57] Treating urine with a reagent designed to eliminate these substances may overcome this problem, yet some immunoassays will still test positive. Preliminary assays should be followed by confirmation tests specific enough to alleviate all false positives.

Poppy seeds have long been known to cause potentially false-positive results for opiates. Poppy seeds ingested in pastries, rolls, or bagels contain both morphine and codeine, both of which are excreted in urine.[13, 83, 84] This situation has led to the "poppy seed defense" and creates a dilemma for those involved in urine drug testing for opiates.[83] Since the opiate most commonly abused today is heroin, it was suggested that urine be analyzed for a metabolite that is present after heroin use but not after poppy seed ingestion, to identify samples that are positive due to abuse.

The presence of 6-monoacetylmorphine (6-MAM) is unequivocal evidence of heroin use because it is not a product of morphine or codeine metabolism. Thus, samples that are positive for opiates (codeine or morphine) by preliminary tests should be confirmed by a second procedure such as GC-MS to detect the metabolite.[83] Unfortunately, 6-MAM is detectable in urine for only up to 8 hours after heroin administration, and no method is currently able to differentiate or distinguish morphine or codeine ingestion from poppy seed ingestion.[23, 83]

In the early 1980s Health Inca Tea (HIT) was imported into the United States from South America. It was purported to cause false positives when testing urine for cocaine,[12, 85] probably due to the fact that it contains material from the plant *Erythroxylon novogranatense*, var *truxillense*. The average amount of cocaine in 1-g HIT bags reportedly is 4.8 mg. If consumers drink the recommended 2 cups/day (range 1–4 cups) less cocaine is ingested than after a single intranasal dose.[86] However, GC-MS detects benzoylecgonine in the urine of consumers of HIT. With this in mind, it is safe to conclude that normal patterns of consumption do not constitute abuse, but those overseeing urine-testing programs should be advised of the content of HIT.[85, 86] In 1986 the U.S. Drug Enforcement Agency banned the sale of this product in the United States, although it is reportedly available elsewhere.[12, 85, 86]

It is widely believed that certain substances can be ingested that will result in a negative urine test even when drugs have been taken, for example, vitamin C, vinegar, and the herb golden seal (capsules or tea). There is currently no scientific evidence that any of these methods is an effective way to escape detection of drug use.[82]

In Vitro Adulteration

Attempts by illicit drug users to falsify urine test results resulted in the need to test samples for pH, specific gravity, osmolarity, temperature, and appearance to screen for samples that are not authentic. In vitro manipulation of a urine specimen is attractive because those subject to drug screens usually have minimal to no advance notice and little opportunity to do in vivo manipulation. The most common adulterants reported in the literature are household vinegar, table salt, liquid laundry bleach, concentrated lemon juice, caustic household cleansers, golden seal tea, liquid hand soap, and Visine (tetrahydrozoline hydrochloride) eye drops.[81, 82, 87]

In 1988, 222 EIA-positive specimens confirmed by GC-MS were evaluated to determine if these additives invalidate enzyme immunoassay results and if the adulterant could be identified at several different concentrations.[87] The authors concluded that the adulterants interfered differently with each of the assays. In vitro addition of all except lemon juice was capable of producing false-negative results when added to urine before testing. The authors postulated that the interference was most likely due to reactions of both the drugs and metabolites with the adulterants.

A concentration-dependent effect was seen when either liquid Drano or liquid chlorine bleach was mixed with amphetamine-positive samples.[87] Sodium chloride concentrations of 75 g/L urine produced false-negative results with amphetamine concentrations up to 1.42 mg/L. Urine samples containing 0.52 mg/L of amphetamine tested falsely negative when Drano or bleach was added at a concentration of 12 ml/L of urine. When the Drano or bleach concentration was increased to 23 ml/L of urine, amphetamine concentrations of 1.80 mg/L became negative. Samples with barbiturate concentrations of less than 1.45 mg/L were adulterated by three additives. Sodium chloride 75 g/L, liquid hand soap, Drano, and bleach 125 ml/L produced false-negative barbiturate test results. The assay for THC was the most sensitive to manipulation. All of the additives with the exception of lemon juice had an effect on the positive THC samples. Golden seal tea and vinegar produced a concentration-dependent

interference, whereas sodium chloride 25 g/L, Visine 125 ml/L, Drano, and bleach 12 ml/L interfered at all THC concentrations studied.

Samples containing cocaine were assayed for the benzoylecgonine metabolite. Drano, bleach, and sodium chloride interfered with the assay in a concentration-dependent fashion. Urine samples with benzoylecgonine concentrations up to 1.18 mg/L were altered by Drano or bleach concentrations of 42 ml/L. If the concentration of Drano was increased to 125 ml/L, samples with benzoylecgonine concentrations up to 1.82 mg/L were altered. Two additives interfered with the assay for opiates. Sodium chloride interfered with urine opiate concentrations of less than 0.78 mg/L, and Drano or bleach 125 mL/L interfered with samples with up to 2.7 mg/L.[87]

Detecting Adulteration

Attempts to adulterate urine samples can be detected in many instances. Guidelines for the federal civilian employee drug program mandate that those being tested should not have access to water or chemicals that could be used for adulteration at the collection site. Bluing can be added to toilet water to help identify attempts to dilute the specimen. In addition, excess or bulky clothing should be removed and handbags or briefcases should be isolated. The specimen should be tested for temperature within 4 minutes of collection (temperature should fall between 32.5 and 37.7°C). The appearance of the sample should also be noted. Foaming or turbidity might indicate the addition of a detergent or other adulterant. Unusual smells should be noted, such as a perfume or a strong ammonia odor (unusual in a fresh urine sample).

In the laboratory, pH and specific gravity determinations should detect the addition of strong acid or alkaline substances, as well as dilution with water. Analysis of the sample for urine sodium or chloride would detect the addition of sodium chloride. When adulteration is suspected, the individual may be asked to provide another specimen under direct observation.[82]

Summary

Urine testing is a method to deter those who use drugs of abuse. Because substance abuse may alter cognitive performance and behavior, its repercussions are far reaching and might include decreased performance on the job. This in turn may decrease production or subject the drug user or others to physical danger, alter performance in sports, give rise to legal problems, make it difficult to obtain or hold a job, and decrease parenting ability.

It is estimated that each year, occupational absenteeism and medical expenses related to drug use average approximately $60 billion.[4] A relationship between these costs and urine drug screen results has been established.[88, 89] Individuals who test positive for marijuana and cocaine in preemployment screening have markedly higher accident rates, injury rates, and absenteeism than those who test negative. Consequently, urine drug testing can serve a useful purpose in certain instances.

It should be stressed, however, that urine tests reveal only that an individual has used a substance; they cannot establish the length of time and extent of impairment associated with drug use. In fact, drug use may occur without a positive result. Since a threshold concentration of the abused substance (or its metabolite) must be present in the specimen, positive test results depend on the amount of time between drug use and urine collection, as well as the time course over which the particular drug or metabolite is eliminated from the body.

It is usually an administrative decision to perform urine drug testing as a preemployment screen, in athletes, in the emergency room, or as a condition of employment or parole. Federal regulations established that federal workplaces retain a medical review officer who is a licensed physician, has knowledge of substance abuse disorders, and is trained to interpret drug test results. In other places, however, the job of interpreting results may fall to a clinician who has little training in this area.

Health care providers must be cautions in interpreting results because of intrinsic problems in sensitivity, specificity, accuracy, reliability, and validity of the available tests. Understanding these limitations as well as the pharmacology and pharmacokinetics of drugs is important for identifying possible abusers. Such information also allows the health care provider to identify sequelae of long-term drug use or to acquaint casual users with the possible repercussions of the practice.

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