CHAPTER

14

Limitations of Drugs of Abuse Testing

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INTRODUCTION

Substance abuse is a serious public health and safety issue not only in the United States but also worldwide. According to the *2010 National Survey on Drug Use and Health* by the Office of Applied Science of the Substance Abuse and Mental Health Services Administration (SAMHSA), an estimated 22.6 million Americans 12 years or older used an illicit drug during the month prior to the survey interview (Figure 14.1). This represented 8.9% of the U.S. population aged 12 years or older. Marijuana was the most commonly used drug, with an estimated 17.4 million people using it in the month prior to the survey. In addition, there were 1.5 million cocaine users, and an alarming 7.0 million people abused prescription medications. Illicit drug abuse was more common among unemployed people (17.5% among unemployed people vs. 8.4% among employed people). Another alarming finding was that an estimated 10.6 million people aged 12 years or older reported driving under the influence of an illicit drug [1]. Thus, December has been designated National Impaired Driving Prevention Month.

Because of widespread use of drugs and alcohol, drug testing using urine specimens and blood alcohol testing are commonly performed in emergency room patients who are involved in accidents, especially car accidents, as well as in anyone with clinical symptoms indicating drug or alcohol overdose or both. In many hospital laboratories, blood alcohol testing is performed using enzymatic assays that can be easily adopted on automated analyzers. However, such enzymatic methods are subject to interference most notably from elevated blood lactate and lactate dehydrogenase. This topic is discussed in Chapter 16. This chapter focuses on limitations of drugs of abuse testing conducted in clinical laboratories, with a brief discussion on the challenges of legal drug testing, especially the issue of urine adulteration.

DRUGS OF ABUSE TESTING: MEDICAL VERSUS LEGAL

Whereas medical drug testing has been practiced for a long time, especially for patients admitted to emergency departments, legal drug testing was initiated by President Reagan, who issued Executive Order 12564 on September 15, 1986. This executive order directed drug testing for all federal employees who are involved in law enforcement, national security, protection of life and property, public health, and other services requiring a high degree of public trust. Following this executive order, the National Institute of Drug of Abuse (NIDA) was given the responsibility of developing guidelines for federal drug testing. Currently, SAMHSA, an agency under the U.S. Department of Health and Human Services, is responsible for providing mandatory guidelines for federal workplace drug testing. Although Reagan's executive order was not intended for private employers, currently a majority of *Fortune* 500 companies have policies for workplace drug testing. Drug abuse costs American industry and the public an estimated $100 billion a year, but workplace drug testing can contribute to a better work environment, improves morale of employees, and is very effective in preventing work-related accidents. Therefore, developing a cost-effective corporate workplace drug testing program that meets federal guidelines, capable of standing legal challenge and also accepted by employees, is the objective of all workplace drug testing programs [2]. Several publications have established large negative

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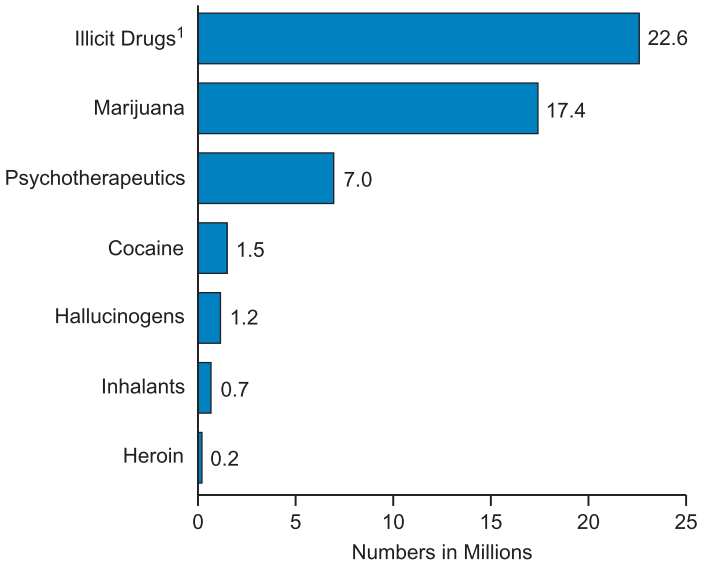

FIGURE 14.1 Abuse of various illicit drugs and inhalants in the United States. Illicit drugs include marijuana/hashish, cocaine (including crack), heroin, hallucinogens, inhalants, and prescription-type psychotherapeutics used nonmedically. Source: *Data from the 2010 National Survey on Drug Use and Health: National Findings. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration (SAMHSA), Office of Applied Studies.*

correlations between workplace drug testing and employee substance abuse [3].

In medical drug testing, informed consent may not be obtained from a patient. An overdosed patient admitted to the emergency department may not be able to grant an informed consent anyway. In contrast, in a workplace or any other legal drug testing program, obtaining an informed consent prior to testing is mandatory. Another major difference between medical and legal drug testing is that in medical testing, an initial positive screening result obtained by using immunoassays may not be confirmed by using gas chromatography combined with mass spectrometry (GC-MS), but in legal drug testing, GC-MS confirmation is mandatory. In addition, a chain of custody must be maintained in legal drug testing indicating all personnel who have possession of the specimen from the time of collection to the time of reporting results. Chain of custody is not usually initiated in medical drug testing. Therefore, a medical drug testing result may not stand a legal challenge.

DRUGS TESTED IN DRUGS OF ABUSE TESTING PROTOCOLS

The fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* distinguishes 11 categories of abused substances, including amphetamines, cocaine, marijuana, hallucinogens, inhalants, opioids, phencyclidine (PCP), sedative hypnotics, anxiolytics, and alcohol. Despite this guideline, many drugs of abuse programs may screen only five drugs, also known as NIDA-5 or SAMHSA-5 drugs. The five SAMHSA-mandated drugs for federal workplace drug testing are amphetamine, cocaine (tested as benzoylecgonine, the inactive metabolite), opiates, PCP, and marijuana (tested as 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol, the inactive metabolite). Although these drugs are more frequently tested, there is no industry standard and terms such as "routine drug screen" or "comprehensive drug screen" are used by different laboratories, but the drugs included in each protocol may vary from one laboratory to another [4]. Some private employers may test for additional drugs in their workplace drug testing protocols, and such comprehensive drug panel may include barbiturates, benzodiazepines, oxycodone, methadone, methaqualone, and propoxyphene.

DRUG TESTING METHODOLOGIES

Immunoassays are widely used as the first step of drug screening in both medical and workplace drug testing programs. Immunoassays can be easily automated, several drugs can be analyzed using one specimen, and results can be directly downloaded in the laboratory information system. The main component of the immunoassay reagents is the analyte-specific antibody, which can be either polyclonal or monoclonal in nature. In general, monoclonal antibody-based immunoassays are more specific to the target analyte than are the polyclonal antibody-based assays. All immunoassay methods used for drugs of abuse screening require no specimen pretreatment. The assays use very small amounts of sample volumes (most $<100\ \mu L$), reagents are stored in the analyzer, and most assays employ stored calibration curves in the automated analyzer. With respect to assay design, there are two formats of immunoassays: competition

and immunometric (commonly referred to as "sandwich"). Competition immunoassays work best for drugs that are small molecules such as therapeutic drugs and drugs of abuse requiring a single analyte-specific antibody. In the competition format, the analyte molecules in the specimen compete with analyte (or its analogs), labeled with a suitable tag and provided in the reagent, for a limited number of binding sites provided by an analyte-specific antibody (also provided in the reagent). Thus, in these types of assays, the higher the analyte concentration in the sample, the less label can bind to the antibody to form the conjugate. If the bound label provides the signal, such as in fluorescence polarization immunoassay (FPIA), the analyte concentration in the specimen is inversely proportional to the signal produced. On the other hand, if the signal is generated by the free label, then the signal is proportional to the concentration of the abused drug in the specimen. The signals are mostly optical, such as absorbance, fluorescence, or chemiluminescence.

There are several variations in this basic format. Although the assays can be homogeneous or heterogeneous, most drugs of abuse assays use the homogeneous format, in which the bound label has different properties than the free label and physical separation between bound and free label is unnecessary before measuring the signal. For example, in FPIA, the free label has different Brownian motion than when the label is complexed to a large antibody (146 kDa). This results in differences in the fluorescence polarization properties of the label, where the bound label is capable of producing the signal [5]. In another type of homogeneous immunoassay, an enzyme is the label, whose activity is modulated differently in the free versus the antibody-bound conditions of the label. This principle is used in the enzyme multiplied immunoassay technique (EMIT) and cloned enzyme donor immunoassay (CEDIA) technologies [6,7]. In the EMIT method, the label enzyme, glucose 6-phosphodehydrogenase (G6PDH), is active unless in the antigen–antibody complex. The active enzyme reduces nicotinamide adenine dinucleotide (NAD) to NADH, and the absorbance is monitored at 340 nm (NAD has no signal at 340 nm, whereas NADH absorbs at 340 nm). To guard against interference from a specimen's native G6PDH, the newer assays use recombinant bacterial enzymes whose activity conditions are different from those of the human enzyme. Similarly, in the CEDIA method, two genetically engineered inactive fragments of the enzyme β-galactosidase are coupled to the antigen and the antibody reagents. When they combine, the active enzyme is produced, and the substrate—a chromogenic galactoside derivative—produces the assay signal. In a third commonly used format of homogeneous immunoassay (turbidimetric immunoassay), analyte (antigen) or its analogs are coupled to colloidal particles of latex, for example [8]. Because antibodies are bivalent, the latex particles agglutinate in the presence of the antibody. However, in the presence of free analytes in the specimen, there is less agglutination. In a spectrophotometer, the resulting turbidity can be monitored as end point or as rate. In the kinetic interaction of microparticles in solution (KIMS) assay method, in the absence of drug molecules, free antibodies bind to drug microparticle conjugate forming particle aggregates, and an increase in absorption is observed. When drug molecules are present in urine specimen, these molecules bind with free antibody molecules and thus prevent formation of particle aggregates and diminish absorbance in proportion to drug concentration. The ONLINE drugs of abuse testings immunoassays marketed by Roche Diagnostics (Indianapolis, IN) are based on the KIMS format.

In the heterogeneous immunoassay format, the bound label is physically separated from the unbound labels before signal is measured. The separation is often done magnetically, where the reagent analyte (or its analog) is provided as coupled to paramagnetic particles (PMPs), and the antibody is labeled. Conversely, the antibody may also be provided as conjugated to the PMPs, and the reagent analyte may carry the label. After separation and wash, the bound label is reacted with other reagents to generate the signal. This is the mechanism in many chemiluminescent immunoassays, in which the label may be a small molecule that generates chemiluminescent signal [9]. The label may also be an enzyme (enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA)) that generates chemiluminescent, fluorometric, or colorimetric signal. Another type of heterogeneous immunoassay uses polystyrene particles. If the particles are micro sizes, this type of assay is called microparticle-enhanced immunoassay [10].

Usually, multiple calibrators (four to six levels) are recommended for accurate measurements of the analyte across the entire assay range in an immunoassay, although 2-point calibrations are also used. Most automated assay systems can store a calibration curve depending on the assay stability of the systems. Therefore, when a sample is analyzed during the period denoted by calibration stability, the assay signal is automatically converted into analyte concentration via the stored calibration curve. Drugs of abuse assays are often used to report "qualitative" results—that is, positive or negative with respect to a certain analyte concentration (the "cutoff" level). Thus, many of the assays are in qualitative or quantitative formats, and in most cases such formats are defined by assay protocol and calibration. In qualitative formats, the calibration can be simplified to only one or two calibrators,

centering on the cutoff point, thus providing the most accuracy around that point. The algorithm compares the signal observed with a sample with that of the cutoff calibrator and reports the result as positive or negative. Semiquantitative results can be reported with a calibration curve containing a minimum of three calibrator samples; often, the combination of the zero-calibrator, together with two or more calibrators at or near cutoff level, is used to generate a calibration curve. Obviously, such assay formats will have increased inaccuracy at analyte concentrations much higher than the cutoff concentration.

Immunoassays used for drugs of abuse testing have high sensitivity, but specificity may vary widely between assays designed for individual drugs. In general, there are certain cutoff concentrations for immunoassays used for drugs of abuse testing. These cutoffs are set by SAMHSA guidelines. Therefore, if the concentration of a target drug or metabolite is below the cutoff concentration, the immunoassay result should be negative, and a negative test result may be indicative of no drug present in the specimen or a drug concentration lower than the cutoff concentration. The GC-MS confirmation cutoff as proposed by SAMHSA guidelines may be the same as the cutoff concentration recommended for immunoassay screening or may be lower than the immunoassay screening threshold. Immunoassay and GC-MS cutoff concentrations of various SAMHSA drugs are summarized in Table 14.1. Immunoassay and GC-MS confirmation cutoffs of other commonly abused drugs (in the workplace drug testing protocols of private employers; non-SAHMSA drugs) are given in Table 14.2. Drugs or metabolites can be detected for a limited time in urine after abuse. Detection windows of various drugs in urine are summarized in Table 14.3.

In legal drug testing, a person may intentionally dilute their urine specimen with water in order to avoid a positive test result. Many investigators have explored the possibility of detecting abused drugs in urine specimens using lower cutoff concentrations than recommended by SAMHSA guidelines in order to identify more subjects who may be abusing drugs. Unfortunately, in the United States, such practice cannot stand legal challenges except for opiates, for which some private employers are still using the 300 ng/mL cutoff concentration that was proposed in the original drug testing guidelines by NIDA. However, the Correctional Services of Canada (CSC) incorporates lower screening and confirmation cutoff for drug/metabolites if needed for diluted urine. These guidelines include the following: all amphetamine screening cutoff, 100 ng/mL; confirmation cutoff, 100 ng/mL; benzodiazepines screening and confirmation cutoff, 50 ng/mL; benzoylecgonine screening and confirmation cutoff, 15 ng/mL; opiates screening and confirmation cutoff, 120 ng/mL; phencyclidine screening and confirmation cutoff, 5 ng/mL; and cannabinoids screening cutoff, 20 ng/mL, but confirmation cutoff of 3 ng/mL. Fraser and Zamecnik [11] reported that between 2000 and 2002, 7912 urine specimens collected by the CSC were dilute, and 26% of these screened positive using SAMHSA cutoff values. When lower values for cutoff and confirmation were adopted, 1100 specimens tested positive for one or more illicit drugs. The drug most often confirmed positive in a diluted specimen was marijuana. Soldin *et al*. [12] reported a more than 100% increase

TABLE 14.1 Original Cutoff Values and New Cutoff Values (Effective October 1, 2010) of SAMHSA-Mandated Drug Testing

Drug or Drug Class	Immunoassay Cutoff (ng/mL)		GC-MS Confirmation (ng/mL)
	Original Value	New Value	New Value
Amphetamine/methamphetamine	1000	500	500
MDMA	Not applicable	500	250
Cannabinoids	50	50	15
Cocaine metabolites	300	150	100
Opiates (codeine/morphine)[a]	2000	2000	2000
6-Acetylmorphine[b]	10	10	10
Phencyclidine	25	25	25

[a]*In the first guidelines, the published cutoff concentration for opiate screening was 300 ng/mL, but the value was increased to 2000 ng/mL in 1998. Some private employers may still use immunoassays of opiate with a cutoff concentration of 300 ng/mL.*
[b]*Testing is recommended if opiate test is positive (>2000 ng/mL).*

TABLE 14.2 Immunoassay Screening and GC-MS Confirmation Cutoffs for Non-SAMHSA Drugs

Drug	Immunoassay Cutoff (ng/mL)	GC-MS Confirmation (ng/mL)
Barbiturates	200 or 300	200
Benzodiazepines	200 or 300	200
Methadone	300	300
Methaqualone	300	300
Propoxyphene	300	300
Oxycodone	100 or 300	100
6-Acetylmorphine	10	10
LSD	0.5	0.5

TABLE 14.3 Typical Window of Detection of Various Drugs in Urine Specimens Using SAMSHA Cutoffs[a]

Drug	Window of Detection
Amphetamine	2 days
Methamphetamine	2 days
3,4-Methylenedioxyamphetamine	1–2 days
Short-acting barbiturates	1–2 days
Long-acting barbiturates	14–21 days
Short-acting benzodiazepine	3 days
Long-acting benzodiazepine	14–21 days
Cocaine	2–3 days
Morphine	2 days
Codeine	2 days
Heroin	>1 day for detecting 6-acetylmorphine
	2 days for detecting morphine
Oxycodone	2–4 days
Methadone	3 days
Propoxyphene	2–14 days
Marijuana	2–3 days after acute use
	5–7 days for moderate use
	30 days or more in chronic users
Methaqualone	14 days
Phencyclidine	8 days
LSD	2–3 days

[a]*Detectability depends on drug amounts, frequency and chronicity of abuse, and individual variables related to drug metabolism and excretion.*

in cocaine-positive specimens when the cutoff was lowered from 300 to 80 ng/mL in a pediatric population because neonates are not capable of concentrating urine to the same extent as adults. Luzzi *et al*. [13] investigated the analytic performance criteria of three immunoassay systems (EMIT, Beckman EIA, and Abbott FPIA) for detecting abused drugs below established cutoff values. The authors concluded that these drugs can be screened at concentrations much lower than established SAMHSA cutoff values. For example, the authors proposed a marijuana metabolite cutoff of 35 ng/mL using EMIT and 14 ng/mL for the Beckman EIA and the Abbott FPIA assays. The proposed cutoff values were based on imprecision studies in which the coefficient of variation was less than 20%. Such lowering of cutoff values increased the number of positive specimens in the screening tests by 15.6%. A 7.8% increase was also observed in the confirmation stage of drugs of abuse testing [13].

TABLE 14.4 Antibody Specificity of Various Immunoassays Used for Drugs of Abuse Testing

Immunoassay for the Drug	Antibody Target
Amphetamine/methamphetamine assays	Methamphetamine or amphetamine
3,4-Methylenedioxymethamphetamine assay	3,4-Methylenedioxymethamphetamine
Cocaine assay	Benzoylecgonine
Opiate assays	Morphine
Oxycodone assay	Oxycodone
Heroin assay	6-Acetylmorphine
Methadone assay	Methadone or EDDP metabolite
Marijuana assay	11-nor-9-carboxy-Δ-9-tetrahydrocannabinol
PCP assay	Phencyclidine
Benzodiazepine assays	Commonly oxazepam, but nordiazepam, nitrazepam, or lormetazepam may be used
Barbiturates	Commonly secobarbital
LSD assay	LSD
Methaqualone assay	Methaqualone

EDDP, 2-ethyldene-1,5-dimethyl-3,3-diphenpyrrolodide.

In general, immunoassays used for drugs of abuse testing target a specific drug or a metabolite. For example, immunoassays designed for detecting cocaine in urine target benzoylecgonine, the active metabolite of cocaine. In contrast, an antibody used in the immunoassay for screening barbiturates may target secobarbital. Antibody targets of various immunoassays used in drugs of abuse testing are listed in Table 14.4. The major limitation of immunoassays is that an antibody may cross-react with a structurally similar drug, causing false-positive test results. Therefore, initial drug screening should be confirmed by GC-MS. Most mass spectrometers used for drugs of abuse testing are operated in electron ionization mode, although mass spectrometers can also be operated in chemical ionization mode. Confirmation methods for drug testing using GC-MS can be found in books devoted to drugs of abuse testing [14]. Liquid chromatography combined with mass spectrometry (LC-MS) and liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) are gaining popularity as alternatives to GC-MS for drug confirmation [15]. Eichhorst *et al*. [16] proposed the use of LC-MS for rapid screening and confirmation of the presence of abused drugs in urine specimen as an alternative to immunoassay screening.

CHALLENGES IN TESTING FOR AMPHETAMINES

In general, immunoassays for amphetamines, in addition to detecting the presence of amphetamine and methamphetamine, can also detect the widely abused designer drugs 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA). However, certain amphetamine immunoassays may have lower capability of detecting MDMA and MDA due to poor cross-reactivity of the methamphetamine-specific antibody. For example, the Abuscreen ONLINE amphetamine assay (Roche Diagnostics) has only 0.1% cross-reactivity with MDMA and 38% cross-reactivity with MDA. The Neogen amphetamine assay has only 2.2% cross-reactivity with MDMA and 0.4% cross-reactivity with MDA. Kunsman *et al*. [17] reported that the typical concentration of MDMA in urine specimens varied widely from 380 to 96,200 ng/mL (mean, 13,400 ng/mL), whereas the typical concentration of MDA varied from 150 to 8600 ng/mL (mean, 1600 ng/mL). The presence of MDA in urine specimens at a concentration of approximately 10−15% of MDMA concentration is consistent with MDMA metabolism that may be indicative of MDMA abuse only [17]. Therefore, an immunoassay for amphetamine with low cross-reactivity with MDMA and MDA may produce false-negative test results if a low concentration of MDMA or MDA is present in the urine specimen. However, specific immunoassays for detecting the presence of MDMA in urine are commercially available. Stout *et al*. [18] compared DRI methamphetamine, DRI ecstasy (MDMA), and Abuscreen ONLINE amphetamine assays at a cutoff of 500 ng/mL and observed that the DRI ecstasy assay performed the best, as expected, with a GC-MS confirmation rate of 90% of the specimens screened positive. In contrast, the DRI amphetamine assay had poor capability of detecting the presence of MDMA in urine because only 6% of specimens were confirmed positive by GC-MS. Only 20% of specimens screened positive by Abuscreen ONLINE amphetamine assay were confirmed by GC-MS. In another study, the authors commented that DRI and CEDIA amphetamine assays do not have good sensitivity in identifying urine specimen containing MDMA [19]. Poklis *et al*. [20] reported that the EMIT d.a.u. monoclonal amphetamine/methamphetamine immunoassay has a cutoff concentration of 3000 ng/mL for racemic MDMA but only 800 ng/mL for MDA. The assay had higher sensitivity for detecting the S(+) isomer of both MDMA and MDA. The authors found EMIT d.a.u. monoclonal amphetamine/methamphetamine assay to be vastly superior to EMIT d.a.u. polyclonal amphetamine/methamphetamine assay for detecting MDMA and MDA.

The FPIA amphetamine/methamphetamine assay for application on the AxSYM analyzer is capable of detecting abuse of paramethoxyamphetamine (PMA) and paramethoxymethamphetamine if the cutoff concentration is set at 300 ng/mL [21]. However, amphetamine/methamphetamine immunoassays are not suitable for detection of the majority of designer drugs structurally related to amphetamine/methamphetamine. Kerrigan *et al*. [22] evaluated cross-reactivities of 11 designer drugs with nine commercially available immunoassays. The designer drugs included in the study were 2,5-dimethoxy-4-bromophenylethylamine (2C-B); 2,5-dimethoxyphenethylamine (2C-H); 2,5-dimethoxy-4-iodophenethylamine (2C-I); 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2); 2,5-dimethoxy-4-isopropylthiophenethylamine (2C-T-4); 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7); 2,5-dimethoxy-4-bromo-amphetamine (DOB); 2,5-dimethoxy-4-ethylamphetamine (DOET); 2,5-dimethoxy-4-iodoamphetamine (DOI); 2,5-dimethoxy-4-methylamphetamine (DOM); and 4-methylthioamphetamine (4-MTA). Cross-reactivities of these designer drugs with immunoassays studied were less than 0.4%, and even at a concentration of 50,000 ng/mL, these designer drugs were insufficient to produce a positive response, indicating that amphetamine/methamphetamine immunoassays are not capable of detecting the presence of most of these drugs in urine. However, 4-MTA was the only drug that demonstrated 5% cross-reactivity with the Neogen amphetamine ELISA assay (Lexington, KY) but a significant 200% cross-reactivity with the Immunalysis amphetamine ELISA assay (Pomona, CA). Apollonio *et al*. [23] reported that 4-MTA had 280% cross-reactivity with the Bio-Quant Direct ELISA assay for amphetamine. However, LC-MS or GC-MS methods are capable of detecting the presence of these drugs or their metabolites in urine specimens. Kerrigan *et al*. [24] described a GC-MS protocol for analysis of 2C-B, 2C-H, 2C-I, 2C-T-2, 2C-T-7, 4-MTA, DOB, DOET, DOI, and DOM in urine specimens. Ewald *et al*. [25] analyzed designer drugs and 2,5-diemethoxy-4-bromo-methamphetamine using GC-MS. Takahashi *et al*. [26] created a psychoactive drug data library by performing analysis using LC with photodiode array spectrophotometry as well as GC-MS. This library has data on 104 drugs with the potential for abuse.

CASE REPORTS Two men experimented with capsules filled with white powder containing an unknown substance in order to experience hallucination. Within 15 min of taking such capsules orally, they experienced intense hallucinations followed by vomiting, and eventually they became unconscious. After an unknown period of time, both men were admitted to the emergency department. The first patient, a 28-year-old, survived but experienced serious convulsion. The second patient, a 29-year-old, died in the hospital 6 days

after admission. The initial drug screens of both patients using CEDIA immunoassays were negative for amphetamines but positive for cannabinoid metabolite for both men. The presence of DOB was confirmed in urine specimens using GC-MS. The concentration of DOB in the serum of the deceased patient was 19 ng/mL, whereas that of the patient who survived was 13 ng/mL. Although DOB is structurally related to amphetamine, the CEDIA amphetamine immunoassay is unable to detect its presence in the urine [27].

Immunoassays used for amphetamine/methamphetamine usually have good sensitivity but poor specificity due to cross-reactivity of structurally related drugs causing false-positive test results. Therefore, GC-MS confirmation is essential for many amphetamine-positive specimens even in a medical drug testing setting, especially if a patient denies any such drug abuse. In one report, the authors investigated the sensitivity and specificity of two immunoassays for detecting amphetamines and reported that the DRI amphetamine assay (Thermo Scientific) identified 1104 presumptive positive urine specimens (out of 27,400 randomly collected specimens), but the presence of amphetamine, methamphetamine, MDA, or MDMA was confirmed by GC-MS in only 1.99% of these presumptive positive specimens. The presence of ephedrine, pseudoephedrine, or phenylpropanolamine was confirmed in 833 urine specimens, which were presumptive positive for amphetamine using the DRI assay. However, the Abuscreen ONLINE assay identified only 317 presumptive positive amphetamine specimens, and the presence of amphetamine, methamphetamine, MDA, or MDMA was confirmed in 7.94% of specimens by GC-MS [28].

Interference of various sympathomimetic amines found in over-the-counter (OTC) cold medications in amphetamine immunoassays is well recognized. However, the GC-MS confirmation test must be able to identify the sympathomimetic amine causing the false-positive test result. Ephedrine or pseudoephedrine, which is present in many OTC cold medications, is responsible for the majority of false-positive results in amphetamine immunoassay screening tests. These sympathomimetic amines may be present in large amounts in urine specimens that are initially tested positive by amphetamine immunoassays. Commonly encountered medications that interfere with amphetamine screening assays are listed in Table 14.5.

Amphetamine and methamphetamine have optical isomers designated *d* (or +) for dextrorotatory and *l* (or −) for levorotatory. The *d* isomers, the more physiologically active compounds, are the intended targets of immunoassays because *d* isomers are abused. Ingestion of medications containing the *l* isomer can cause false-positive results. For example, Vicks inhaler contains the active ingredient *l*-methamphetamine, and extensive use of this product may cause false-positive results for immunoassay screening. Specific isomer resolution procedures must be performed to differentiate the *d* and *l* isomers because routine confirmation by GC-MS does not determine isomer composition. Poklis *et al.* [29] reported relatively high concentrations of *l*-methamphetamine observed in two subjects (1390 and 740 ng/mL, respectively) after extensively inhaling Vicks inhaler every hour for several hours. However, urine specimens tested negative by the EMIT II amphetamine/methamphetamine assay (Dade Behring), even after such extensive use of the Vicks inhaler.

Ranitidine is an H_2 receptor blocking agent (antihistamine) that reduces acid production by the stomach and is available OTC without any prescription. Dietzen *et al.* [30] reported that ranitidine, if present in urine at a concentration greater than 43 μg/mL, may cause a false-positive amphetamine screen test result using Beckman Synchron immunoassay reagents (Beckman Diagnostics, Brea, CA). These concentrations of ranitidine are expected in patients taking ranitidine at the recommended dosage. Trazodone interferes with both amphetamine and MDMA assay. A series of patients who tested positive for MDMA (using

TABLE 14.5 Drugs that Interfere with Amphetamine Immunoassays

Structurally similar amines	Brompheniramine, benzphetamine, ephedrine, isometheptene, mephentermine, methylphenidate, pseudoephedrine, phentermine, propylhexedrine, phenylephrine, phenmetrazine, tyramine
Antidepressant/antipsychotic	Chlorpromazine, bupropion, desipramine, doxepin, fluoxetine, perazine, thioridazine, trimipramine, trazodone
Antihistamine	Ranitidine
Antimalarial	Chloroquine
Antispasmodic	Mebeverine
β-Blocker	Labetalol
Cardioactive	*N*-acetyl procainamide (metabolite of procainamide), Mexiletine
Narcotic analgesic	Fentanyl
Nonsteroidal anti-inflammatory	Tolmetin
Tocolytic agent	Ritodrine
Antiviral	Amantadine
Herbal supplement	Dimethylamine

Ecstasy EMIT II assay) did not show any presence of MDMA in urine when confirmed by a specific LC-MS/MS method. However, all specimens showed the presence of trazodone and its metabolite meta-chlorophenylpiperazine [31]. Baron *et al*. [32] demonstrated that the trazodone metabolite meta-chlorophenylpiperazine is responsible for the interference.

Labetalol, a β-blocker commonly used for control of hypertension in pregnancy, can cause false-positive amphetamine screen results using an immunoassay. A labetalol metabolite is structurally similar to amphetamine and methamphetamine, thus causing interference in the assay [33]. Casey *et al*. [34] reported that bupropion, a monocyclic antidepressant and an aid for smoking cessation, may cause false-positive screen results using the EMIT II amphetamine immunoassay. Vidal and Skripuletz [35] reported the case of a 50-year-old male who showed positive amphetamine and lysergic acid diethylamide (LSD) in his urine specimen analyzed by the CEDIA immunoassay. However, GC-MS confirmation failed to confirm either drug. The authors identified bupropion as the cause of false-positive immunoassay screening results for both amphetamine and LSD. The antidepressant desipramine and the antiviral agent amantadine also interfere with amphetamine immunoassays [36].

Mebeverine, an N-substituted ethylamphetamine, is an antispasmodic drug that is metabolized to mebeverine-alcohol, veratric acid, methoxyethylamphetamine, hydroxyethylamphetamine, and PMA. Kraemer *et al*. [37] reported that an FPIA amphetamine assay showed positive response in urine specimens following oral ingestion of a 405-mg mebeverine tablet by volunteers. The authors concluded that positive amphetamine immunoassay test results were due to the presence of methoxyethylamphetamine, hydroxyethylamphetamine, and PMA in the urine specimens. Confirmation of these compounds can be achieved by using GC-MS [37]. Vorce *et al*. [38] showed that dimethylamylamine (DMAA) may cause false-positive test results with a KIMS amphetamine assay and the EMIT II Plus amphetamine assay if present at a concentration of 6900 ng/mL due to structural similarity of DMAA with amphetamine. DMMA is an aliphatic amine naturally found in geranium flowers but also used in body building natural supplements such as Jack3d and OxyELITE Pro. It has been promoted as a safe alternative to ephedrine. The authors further analyzed 134 urine specimens that were tested false positive for amphetamine but confirmed negative by GC-MS and did not contain any known drugs that may cause false-positive amphetamine test results. They observed the presence of DMAA in 92.3% of specimens, with concentrations varying from 2500 to 67,000 ng/mL [38].

CHALLENGES IN TESTING OF COCAINE METABOLITE

Cocaine abuse is usually confirmed by detecting the presence of benzoylecgonine, an inactive metabolite of cocaine, in the urine. In general, the antibodies used in cocaine immunoassays are specific for benzoylecgonine, and these assays, unlike amphetamine immunoassays, demonstrate good specificity as well as sensitivity. Armbruster *et al*. [39] reported that EMIT II and Abuscreen ONLINE cocaine immunoassays had good sensitivity and specificity because 99% of specimens screened positive by these assays were confirmed for the presence of benzoylecgonine by GC-MS. However, life-threatening acute cocaine overdose may not be identified by urine toxicological screen because sufficient concentration of benzoylecgonine may not be present in the urine due to the fact that not enough time has passed after cocaine ingestion for benzoylecgonine to accumulate in the urine. In one case report, in which the person died from cocaine overdose, the urine drug test was negative for cocaine using the EMIT assay. Later GC-MS analysis confirmed that the concentration of benzoylecgonine was only 75 ng/mL, whereas the concentration of cocaine in the urine was only 55 ng/mL in the urine specimen. The immunoassay cutoff concentration of 300 ng/mL cannot detect such a low level of benzoylecgonine. Moreover, cocaine has poor cross-reactivity with the antibody specific for benzoylecgonine (reported cross-reactivity of cocaine with the EMIT assay was 25,000 ng/mL). However, the heart blood concentration of cocaine was 18,330 ng/mL, thus explaining the cause of death as cocaine overdose [40].

Although fluconazole, an antifungal agent, does not produce false-negative test results with cocaine immunoassays, it may cause false-negative results in the GC-MS confirmation step using trimethylsilyl derivative because derivatized fluconazole elutes with derivatized benzoylecgonine. However, such interference can be eliminated by using a pentafluoropropionyl derivative of benzoylecgonine because derivatized benzoylecgonine elutes before derivatized fluconazole [41].

Importantly, positive cocaine test results, both by immunoassay screening and by GC-MS confirmation method, may occur after drinking coca tea, Mazor *et al*. [42] reported that when five healthy subjects drank a cup of coca tea, all urine specimens tested positive for cocaine (as benzoylecgonine) 2 hr after ingestion, and three of five participants' urine specimens remained positive up to 36 hr postingestion. The reason is that coca tea may still contain cocaine. Mean urinary benzoylecgonine concentration in all specimens was 1777 ng/mL.

CHALLENGES IN TESTING FOR OPIATES

In order to circumvent false-positive test results due to ingestion of poppy seed-containing food in opiate assays, the current SAMHSA guideline recommends a cutoff of 2000 ng/mL for opiate screening tests. However, private employers may still use the old cutoff concentration of 300 ng/mL for opiate, and consumption of poppy seed-containing food can easily results in a positive screening as well as confirmation of codeine and morphine by GC-MS. In contrast, 6-monoacetylmorphine (also known as 6-acetylmorphine), which is a specific metabolite of heroin, is not consistent with ingestion of poppy seed-containing food. Antibodies used in most immunoassays target morphine because it is the common metabolite of both codeine and heroin. In general, antibodies specific for opiates have poor cross-reactivity with oxycodone and other 6-keto opioids such as oxymorphone and hydrocodone. Smith *et al*. [43] commented that, in general, immunoassays for opiates displayed substantially lower sensitivity for detecting 6-keto opioids, and urine specimens containing low to moderate amounts of hydromorphone, hydrocodone, oxymorphone, and oxycodone will likely be undetected by opiate immunoassays. Detecting oxycodone is a practical challenge for opiate immunoassays due to the low cross-reactivity of oxycodone with opiate immunoassays. For example, based on information provided in package inserts, the Abuscreen ONLINE opiate assay has only 0.3% cross-reactivity with oxycodone, whereas the CEDIA opiate assay has only 3.1% cross-reactivity with oxycodone. To circumvent such problems, several diagnostic companies have marketed immunoassays that are specifically capable of detecting oxycodone. In one report, the authors analyzed 17,069 urine specimens for the presence of oxycodone using the DRI oxycodone immunoassay during a 4-month period. The DRI oxycodone assay demonstrated 97.7% sensitivity and 100% specificity at an oxycodone cutoff concentration of 300 ng/mL [44]. Gingras *et al*. [45] evaluated the performance of CEDIA and DRI immunoassays for oxycodone (both from Microgenics) using the Hitachi 917 analyzer and concluded that a combination of both assays provided the best performance (98% sensitivity and specificity) when results were compared with those of the GC-MS confirmation method, in contrast to using only one assay. Opiate immunoassays are also not capable of detecting fentanyl and methadone, which also belong to the opioid class. Immunoassays are commercially available for screening for the presence of methadone or its metabolites in urine. A fentanyl homogeneous enzyme immunoassay is now commercially available from Immunalysis Corporation. Snyder *et al*. [46] reported that this assay has 97% sensitivity and 99% specificity in comparison to LC-MS/MS assay for fentanyl. The authors concluded that this assay is a rapid way of accurately detecting fentanyl in urine.

CASE REPORT A 2-year-old female was brought to the hospital after exhibiting signs of rubbing mouth and staggering. A urine toxicology screen performed in the hospital laboratory was negative. She was eventually discharged, but she was brought to the hospital the next morning because she was unresponsive. She experienced severe cardiopulmonary arrest and was pronounced dead on arrival to the hospital. Toxicological analysis of postmortem specimens by GC-MS showed the presence of oxycodone at a concentration of 1360 ng/mL in heart blood, 47,230 ng/mL in urine, and 222,340 ng/mL in the gastric content. The cause of death was oxycodone poisoning. This case report indicates the limitation of hospital urine toxicology screening tests using opiate immunoassays that do not significantly detect oxycodone [47].

Heroin is first metabolized to 6-acetylmorphine and then further transformed into morphine by a liver enzyme. The presence of 6-monoacetylmorphine is considered as confirmation of heroin abuse. Opiate immunoassays may not be capable of detecting the presence of 6-monoacetylmorphine in the urine as effectively as morphine. A specific immunoassay for detecting 6-acetylmorphine is available from Microgenics. Holler *et al*. [48] compared the performance of this CEDIA 6-acetylmorphine assay and the Roche Abuscreen ONLINE opiate assay for detecting the presence of 6-monoacetylmorphine in the urine. They observed that out of 37,713 urine specimens analyzed, 3 specimens screened positive for 6-monoacetylmorphine at a cutoff concentration of 10 ng/mL using the CEDIA assay, whereas the presence of 6-acetylmorphine was confirmed in only 1 specimen using the GC-MS confirmation method. However, when 87 urine specimens in which the presence of 6-acetylmorphine was previously confirmed by GC-MS were re-analyzed using CEDIA assay, all specimens screened positive. However, 12 specimens containing 6-monoacetylmorphine were screened negative by the Abuscreen ONLINE opiate assay. The authors concluded that urine specimens containing predominately 6-monoacetylmorphine may screen negative (false negative) using the opiate immunoassay. In 2010, the new SAMHSA guidelines implemented for drugs of abuse testing recommended screening 6-monoacetylmorphine in urine at the cutoff concentration of 10 ng/mL.

CASE REPORT A 3-year-old female was brought to the medical center after being found shivering and unattended outside a public shopping center in winter. An intravenous bolus of naloxone was administered in the emergency room. However, toxicological analysis of her urine upon arrival to the emergency room was negative. In contrast, when a urine toxicology screen was ordered the next day, it was positive for opiates at the 300 ng/mL cutoff concentration. However, GC-MS confirmation failed to identify any opioids, including codeine, hydrocodone, oxycodone, morphine, hydromorphone, and oxymorphone. The CEDIA opiate assay used in the hospital laboratory cross-reacts with naloxone at a concentration of 6000 ng/mL, and the cause of the opiate-positive second specimen was established to be due to the presence of naloxone in the specimen because the naloxone was administered minutes before the first urine specimen was collected. It was absent in the first specimen, which was negative for opiate and other illicit drugs [49].

Buprenorphine is a morphine-based semisynthetic opioid that is a partial antagonist of mu-opioid receptors in the brain. This drug is used in addiction treatment and also has analgesic properties. However, the morphine antibody used in opiate immunoassay does not detect the presence of buprenorphine. In order to monitor compliance of buprenorphine therapy, a specific immunoassay that is designed for detecting buprenorphine in urine must be used. Hull *et al*. [50] reported that using a 5 ng/mL cutoff concentration, there was 97.9% agreement between the results obtained using the CEDIA buprenorphine immunoassay and those obtained using LC-MS/MS.

Opiate immunoassays, like other immunoassays, suffer from providing false-positive test results due to the presence of various cross-reacting substances other than opioid in urine specimens. Certain quinolone antibiotics may cause false-positive test results with opiate immunoassay screening. Baden *et al*. [51] evaluated potential interference of 13 commonly used quinolones (levofloxacin, ofloxacin, pefloxacin, enoxacin, moxifloxacin, gatifloxacin, trovafloxacin, sparfloxacin, lomefloxacin, ciprofloxacin, clinafloxacin, norfloxacin, and nalidixic acid) with various opiate immunoassays (at 300 ng/mL cutoff concentration) and observed that levofloxacin and ofloxacin may cause false-positive opiate test results with Abbot Laboratories assays manufactured for application on the AxSYM analyzer, as well as with CEDIA, EMIT II, and Abuscreen ONLINE assays. In addition, pefloxacin administration may cause false positives with CEDIA, EMIT II, and Abuscreen ONLINE assays; gatifloxacin administration may cause false positives with CEDIA and EMIT II assays; and lomefloxacin, moxifloxacin, ciprofloxacin, and norfloxacin administration may cause false positives with the Abuscreen ONLINE assay [51]. Straley *et al*. [52] reported a case of a 48-year-old male participating in a residential treatment program who tested positive for opiate during a routine urine drug screen but the GC-MS confirmation was negative for any opiate. The patient received gatifloxacin for treating a urinary tract infection. The authors concluded that the presence of gatifloxacin in the urine specimen was responsible for the false-positive opiate test result. Rifampicin is used in treating tuberculosis and may cause false-positive test results with opiate immunoassays such as the KIMS assay on the Cobas Integra analyzer (Roche Diagnostics). A false-positive result may be observed even after 18 hr of administration of a single oral dose of 600 mg of rifampicin [53].

False-positive test results with methadone immunoassays due to the presence of interfering substance in the urine have also been reported. In one report, the authors observed false-positive methadone test results using the Cobas Integra Methadone II test kit (Roche Diagnostics) in three schizophrenia patients treated with quetiapine monotherapy. The authors used a 300 ng/mL cutoff concentration of methadone in urine specimens for their screening of urine specimens. However, no methadone was detected in the plasma specimen of any patient using LC-MS [54]. Rogers *et al*. [55] reported positive methadone urine drug test results in a patient using the One Step Multi-Drug, Multi-Line Screen Test Device (ACON Laboratories, San Diego, CA), a point-of-care device for urine drug screen. The patient had no history of methadone exposure but ingested diphenhydramine. The GC-MS confirmatory test failed to detect any methadone in the urine specimen, confirming that the presumptive methadone test result was a false positive. When drug-free urine specimens were supplemented with diphenhydramine, false-positive methadone tests were also observed using the point-of-care device. Doxylamine intoxication may cause false-positive results with both EMIT d.a.u. opiate and methadone assays. The urine doxylamine concentration needed to cause a positive test result was 50 μg/mL for methadone and 800 μg/mL for opiate [56].

CHALLENGES IN TESTING FOR MARIJUANA METABOLITES

Immunoassays for screening of marijuana in urine usually target 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol, the major metabolite of marijuana. In general, marijuana immunoassays show good sensitivity and specificity. For example, only 2 or 3% of urine specimens that test

positive for marijuana metabolite by EMIT d.a.u. marijuana assay cannot be confirmed by GC-MS [57]. Passive inhalation of marijuana, however, cannot be detected by marijuana immunoassays because the concentration of the metabolite is substantially lower than the cutoff concentration of the assay. In one study, after eight volunteers were exposed to passive inhalation of marijuana in a coffee shop for 3 hr, none of the urine specimens collected from the volunteers tested positive by marijuana immunoassays even at a cut-off concentration of 25 ng/mL (usual cutoff concentration, 50 ng/mL) because the concentration of the metabolite was up to 7.8 ng/mL after hydrolysis of the conjugated metabolite as determined by GC-MS [58]. Similarly, use of hemp oil should not produce positive marijuana test results because hemp seeds are washed with water prior to extraction of oil, a procedure that removes traces of marijuana from the seed hull. However, prescription use of synthetic marijuana (Marinol) should cause positive marijuana test results.

A number of new designer drugs have emerged in the market known as "legal highs" or "herbal highs." These drugs include both herbal substances and synthetic designer drugs that can be purchased through Internet sites and include synthetic cannabinoids such as JWH-018, JWH-073, JWH-250, HU-210, and CP-47,497 and its homologs. These compounds are lipid soluble, typically containing 20−26 carbon atoms, are more potent than marijuana, and are called "spice." JWH-018 was the first compound of this class reported in 2008, and it is an effective cannabinoid receptor (CB1) agonist [59]. HU-20 is a synthetic agonist analog of marijuana [60]. Unfortunately, the presence of these compounds in urine cannot be determined using marijuana immunoassays because these compounds do not cross-react with the antibody. Therefore, GC-MS or LC-MS/MS must be used for detecting the presence of these compounds in urine.

Although uncommon, false-positive marijuana test results may occur during the screening step due to cross-reactivity from other compounds that are not illicit drugs. Boucher *et al*. [61] described a case of a 3-year-old female who was hospitalized because of behavioral disturbance of unknown cause. The only remarkable finding in her medication history was suppositories of neflumic acid, which was initiated 5 days before hospitalization. After admission, her urinary toxicology screen was positive for the presence of marijuana metabolite, but her parents strongly denied such exposure. Further analysis of the specimen using chromatography failed to confirm the presence of marijuana metabolite, but niflumic acid was detected in the specimen. The authors concluded that the false-positive marijuana test result was due to the presence of niflumic acid in the urine specimen. The antiviral agent efavirenz is known to cross-react with marijuana immunoassays. In one study, the authors analyzed 30 urine specimens collected from patients receiving efavirenz using the Rapid Response Drugs of Abuse Test Strips, the Synchron marijuana immunoassay (Beckman Coulter, Brea, CA), and the Cannabinoid II assay (Roche Diagnostics). Only the Rapid Response test strips demonstrated positive marijuana test results in 28 of 30 specimens, whereas the two other immunoassays did not show any interference from efavirenz. As expected, GC-MS confirmation failed to demonstrate the presence of marijuana metabolite in any of the 30 specimens analyzed [62].

CHALLENGES IN TESTING FOR PHENCYCLIDINE

False-positive test results may occur in phencyclidine (PCP) immunoassays due to cross-reactivity of several drugs with various commercially available immunoassays. Dextromethorphan is an antitussive agent found in many over-the-counter cough and cold medications. Ingesting large amounts of dextromethorphan (>30 mg) may result in positive false-positive test results with opiate and PCP immunoassays. In one report, the authors observed three false-positive PCP tests in pediatric urine specimens using an on-site testing device (Instant-View multi-test drugs of abuse panel; Alka Scientific Designs, Poway, CA). The authors concluded that false-positive PCP tests were due to the cross-reactivities of ibuprofen, metamizol, dextromethorphan, and their metabolites with the PCP assay [63]. Thioridazine is known to cause false-positive PCP test with both EMIT d.a.u. and EMIT II phencyclidine immunoassays [64]. In our experience, most screened positive PCP urine specimens are false positive because the prevalence of PCP abuse is currently low in the United States.

CASE REPORT A 13-year-old female taking venlafaxine regularly for depression was overdosed with 48 tablets of 150 mg venlafaxine. Her other medications were topical Benzamycin and pyridoxine 50 mg daily for acne. On admission, her urine drug screen using Abbott assays on an AxSYM analyzer was positive for PCP, but GC-MS failed to confirm the presence of PCP in urine. A serum specimen obtained 3 hr after overdose showed a venlafaxine concentration of 3930 ng/mL as measured by GC-MS. The therapeutic concentration of venlafaxine along with its *O*-desmethylvenlafaxine metabolite is 250−750 ng/mL. When a urine specimen was supplemented with venlafaxine and its metabolite, the

authors observed a positive phencyclidine test using the same immunoassay, thus confirming the positive test result due to interference of venlafaxine [65].

CHALLENGES IN TESTING FOR BENZODIAZEPINES

Currently, more than 14 various benzodiazepines are approved for use in the United States, and usually antibodies used in benzodiazepine immunoassays target oxazepam and have variable cross-reactivity to other benzodiazepines. However, less commonly, antibodies may target other drugs in the benzodiazepine class (see Table 14.4). Unfortunately, benzodiazepine immunoassays suffer from producing false-negative results for two reasons:

1. The antibody may have poor cross-reactivity with a particular drug, such as lorazepam.
2. The drug may be present in concentrations lower than 200 ng/mL, the usual cutoff for benzodiazepine immunoassays. A drug such as clonazepam may be present at a concentration much lower than 200 ng/mL after therapeutic use.

Therefore, benzodiazepine immunoassay may not be appropriate for monitoring compliance of a patient with a drug in the benzodiazepine class. Clonazepam is a common benzodiazepine used in treating panic disorder and also in controlling seizure. However, detecting clonazepam in urine after therapeutic use using benzodiazepine immunoassays is a challenge due to the low concentration of the drug in urine specimens. Clonazepam is metabolized to 7-aminoclonazepam. West *et al*. [66] reported that when urine specimens collected from subjects taking clonazepam were tested using the DRI benzodiazepine immunoassay at 200 ng/mL cutoff, 38 specimens out of 180 tested positive by the immunoassay (21% positive). However, using LC-MS/MS, 126 out of 180 specimens tested positive (70% positive) when the detection limit of the LC-MS/MS assay was set at 200 ng/mL, the same cutoff used by the DRI benzodiazepine assay, indicating poor detection capability of the benzodiazepine immunoassay for clonazepam and its metabolite in urine. When the authors used a lower cutoff (40 ng/mL), 157 out of 180 specimens tested positive, indicating that the 200 ng/mL cutoff is too high to monitor compliance of patients with clonazepam therapy.

Flunitrazepam (Rohypnol) is used in date rape and is not approved for clinical use in the United States. However, despite the fact that it is not available in the United States, patients may have access to the drug, particularly those living in southern states bordering Mexico. Although flunitrazepam is a benzodiazepine drug, some commercial immunoassays for benzodiazepines using an antibody-targeting oxazepam may have relatively low cross-reactivity with flunitrazepam. In addition, urine concentration of flunitrazepam may not be adequate, and it is a challenge for routinely used benzodiazepine assays to detect the presence of flunitrazepam in urine. Forsman *et al*. [67], using a CEDIA benzodiazepine assay at a cutoff of 300 ng/mL, failed to obtain a positive result in the urine of volunteers after they received a single dose of 0.5 mg flunitrazepam. In addition, only 22 of 102 urine specimens collected from volunteers after receiving the highest dose of flunitrazepam (2 mg) showed positive screening test results using the CEDIA benzodiazepine assay. Kurisaki *et al*. [68] reported that the Triage benzodiazepine assay has low sensitivity in detecting estazolam, brotizolam, and clotiazepam. Therefore, a negative result in a Triage test may not mean the absence of these drugs in the urine specimen. In another report, the authors demonstrated that the Abuscreen ONLINE benzodiazepine assay has 96% specificity but only 36% sensitivity because all urine specimens containing lorazepam and lormetazepam (as confirmed by GC-MS) tested negative by the immunoassays due to poor cross-reactivities of these drugs with the antibody used in the assay [69].

CASE REPORT A 58-year-old divorced white female with familial manic–depressive disorder who was socially isolated and unable to cope with her problem ingested her prescription medication lorazepam along with alcohol despite a physician's warning that such practice may cause severe respiratory depression. She was brought to the hospital by her sister, and it was determined that she had ingested 12 lorazepam tablets (2 mg) in the past 24 hr. Surprisingly, her urinary toxicology screen was negative for benzodiazepine using the Cobas Integra benzodiazepine assay (KIMS assay). Other drugs of abuse tests were also negative. Another urine specimen collected 4 hr after admission was also negative. However, GC-MS analysis of both urine specimens showed concentrations of lorazepam greater than 20,000 ng/mL. The author further investigated why the urine screen using the KIMS benzodiazepine assay was negative despite such a high concentration of lorazepam. On dilution of the original urine specimen (20 and 40×), positive benzodiazepine test results by the immunoassay were observed. The author concluded that the false-negative benzodiazepine test using immunoassay was either due to antigen excess (lorazepam level too high) causing immune complex formation or due to the presence of an inhibitor in the specimen that may change the expected microparticle aggregation-generating signal [70].

In general, benzodiazepine assays have good sensitivity, but specificity may vary widely among various assays. Lum *et al*. [71] reported that with the Multigent benzodiazepine assay for application on the Architect chemistry analyzer (Abbott Laboratories), 615 urine specimens out of 2447 screened between June and July 2007 tested positive for the benzodiazepines. However, the presence of benzodiazepines was confirmed in 457 specimens using high-performance liquid chromatography (HPLC), indicating that 25.1% of the specimens screened positive by the immunoassays were false positives. The authors randomly selected 50 false-positive urine specimens (which were tested negative by HPLC) and determined after reviewing medical records that 16 false-positive specimens were obtained from patients receiving sertraline (Zoloft). Of these 50 specimens, 47 screened negative by the Syva EMIT assay.

Interference of oxaprozin, a nonsteroidal anti-inflammatory drug (NSAID), in immunoassays for benzodiazepines has been reported. In one study, the authors investigated potential interference of oxaprozin with FPIA, CEDIA, and EMIT d.a.u. benzodiazepine immunoassays. In their study, 36 urine specimens collected from 12 subjects after each subject received a single dose of 1200 mg of oxaprozin were analyzed by all three immunoassays of benzodiazepines using a cutoff concentration of 200 ng/mL. All 36 urine specimens showed positive results using both CEDIA and EMIT d.a.u. assays, but the FPIA assay showed 35 of 36 specimens positive for benzodiazepines. The authors concluded that a single dose of oxaprozin may cause false-positive benzodiazepine test results using immunoassays [72].

CHALLENGES IN DETERMINING OTHER DRUGS BY IMMUNOASSAYS

Barbiturates that are abused are usually short or intermediate acting, such as amobarbital, pentobarbital, secobarbital, and butalbital. Long-acting barbiturates such as phenobarbital, which is also an anticonvulsant, are rarely abused. In general, the specificity for detecting individual barbiturates varies with the immunoassays. Propoxyphene is used for treating mild to severe pain. Although both propoxyphene and its metabolites are found in urine, in general, antibodies used in propoxyphene immunoassays target the parent drug but may have variable cross-reactivity with norpropoxyphene, a metabolite of propoxyphene. McNally *et al*. [73] concluded that the ONLINE propoxyphene assay (Roche Diagnostics) has better sensitivity that the EMIT propoxyphene assay for detecting the presence of propoxyphene in urine because the antibody used in the ONLINE assay has 77% cross-reactivity with norpropoxyphene, whereas the EMIT assay showed only 7% cross-reactivity with norpropoxyphene. Another report indicates that diphenhydramine (Benadryl) interferes with the EMIT propoxyphene immunoassay [74].

Methaqualone is metabolized to 2′-hydroxy and 3′-hydroxy metabolites, which are then conjugated and excreted in urine as glucuronide. Brenner *et al*. [75] reported that both the Roche ONLINE methaqualone immunoassay and the EMIT II methaqualone immunoassay have high cross-reactivity toward both 2- and 3-hydroxy metabolites of methaqualone as well as their conjugated form and are useful for screening of methaqualone in urine specimens. When volunteers received 200 mg of methaqualone, all urine specimens tested highly positive (300 ng/mL cutoff) for 72 hr. When the specimens were analyzed by GC-MS without hydrolysis of glucuronide conjugates, low levels of methaqualone and metabolites were detected. However, when urine specimens were hydrolyzed with β-glucuronidase and then analyzed again by GC-MS, high concentrations of metabolites were found. Therefore, authors recommend hydrolysis of the urine specimen prior to GC-MS analysis.

Studies indicate that NSAIDs may interfere with results in multiple immunoassays screening for the presence of drugs of abuse in urine specimens. Joseph *et al*. [76] studied 14 NSAIDs for potential interference with EMIT and FPIA assays for various drugs of abuse and observed that tolmetin interferes with EMIT immunoassays at high concentrations (1800 μg/mL and higher) because of high molar absorptivity at 340 nm, the wavelength used for detection in the EMIT technology. Samples containing cannabinoid and benzoylecgonine tested negative in the presence of tolmetin, but there was no effect on the FPIA assay because the detection wavelength was 525 nm. Rollins *et al*. [77] commented that although the frequency of false-positive test results with immunoassays is low with acute or chronic ibuprofen use, chronic use of naproxen even at therapeutic dosage may cause false-positive test results using the EMIT d.a.u. marijuana assay or the FPIA barbiturate assay (Abbott Laboratories).

ADULTERANTS AND DRUGS OF ABUSE TESTING

Adulteration of a specimen is not an issue in medical drug testing because the specimen is collected by a heath care professional in an overdosed patient admitted to the emergency department. However, adulteration of a urine specimen is possible in workplace drug testing, in which a drug abuser may want

to cheat. Many detoxifying agents for beating drug tests are available through the Internet. However, contrary to claims, such agents usually contain high amounts of caffeine or a diuretic such as hydrochlorothiazide and cannot flush out a drug from the system. The manufacturers usually recommend a subject drink excess water along with these detoxifying agents, and the end result is production of diluted urine in which the drug concentration may be reduced. However, in workplace drug testing, diluted urine can be easily identified with observation of low creatinine and specific gravity, and further analysis may not be conducted. In this case, a person may be denied a job, or an employee may be fired.

Common household chemicals such as laundry bleach, table salt, toilet bowl cleaner, hand soap, and vinegar have been used for many years as adulterants of urine specimens in an attempt to avoid a positive drug test. Common adulterants used to invalidate drug testing include table salt, vinegar, liquid laundry bleach, lemon juice, and Visine eye drops [78,79]. Household vinegar and concentrated lemon juice make urine acidic and can be easily detected by checking the pH of the specimen. Table salt increases specific gravity of urine. However, the presence of Visine eye drops in urine cannot be detected by the usual specimen integrity tests. Both the collection site and the laboratory have a number of mechanisms to detect potentially invalid specimens. For example, the temperature should be 90.5-98.9°F. The specific gravity should be between 1.005 and 1.030, and pH should be between 4.0 and 10.0. The creatinine concentration should be 20−400 mg/dL. However, some drug testing laboratories consider a creatinine concentration of 15 mg/dL as the lower end of the cutoff concentration. Adulteration with sodium chloride at a concentration necessary to produce a false-negative result always produces a specific gravity greater than 1.035.

However, there are urinary adulterants available through the Internet that are effective in producing false-negative test results during immunoassay screening tests. Because GC-MS confirmation may not be performed if the screening immunoassay test is negative, a person might effectively beat drug testing by adulteration of their urine specimen using these agents. Unfortunately, the presence of these compounds in the specimen cannot be determined using routine specimen integrity testing, and special tests must be performed during the pre-analytical stage to determine the presence of these adulterants in the specimen. Because adulterating a specimen is equivalent to refusal to test, the person may be denied employment. In addition, in certain states, adulteration of a urine specimen submitted for legal drug testing is a violation of the state law and the person can be prosecuted. One such adulterant to mask drug screening, Stealth, is available through the Internet. The reagent pack contains a powdered catalyst, which should be added to a urine sample cup before voiding. Then a liquid activator reagent should be added to the specimen. The combination of reagents successfully masks urine drug screening by both EMIT and FPIA. Unfortunately, the color of the urine does not change after adding these reagents. However, these reagents contain some powerful reducing substances and show a strong positive glucose urine dipstick result. In addition, Stealth can be detected in urine by using a simple spot test utilizing a stock solution containing 2% potassium dichromate in distilled water and 2N hydrochloric acid. When a few drops of potassium dichromate solution were added to five drops of urine in a test tube followed by addition of two drops of 2N hydrochloric acid, an intense blue color developed that became colorless after approximately 2 min (Dasgupta, unpublished data). No color change was observed if no Stealth was present in the urine specimen.

Wu *et al*. [80] reported that the active ingredient of another Internet-based urinary adulterant, Urine Luck, is 200 mmol/L of pyridinium chlorochromate (PCC). The authors reported a decrease in the response rate for all EMIT II drug screens and for the Abuscreen morphine and marijuana assays. In contrast, the Abuscreen amphetamine assay produced a higher response rate, whereas no effect was observed on the results of benzoylecgonine and PCP. This adulteration of urine did not alter GC-MS confirmation of methamphetamine, benzoylecgonine, and phencyclidine. However, apparent concentrations of opiates and marijuana metabolite were reduced. Wu *et al*. also described the protocol for detection of PCC in urine using spot tests. The indicator solution contains 10 g/L of 1,5-diphenylcarbazide in methanol. The indicator detects the presence of chromium ions and is colorless when prepared. Two drops of indicator solution are added to 1.0 mL of urine. If a reddish-purple color develops, the test is positive. In this author's experience, addition of a few drops of 3% hydrogen peroxide causes a dark brown precipitation if PCC is present. In the absence of PCC, the light yellow color of urine is bleached to almost colorless.

Glutaraldehyde has also been used as an adulterant to mask urine drug test. This product is available under the trade name UrinAid. A 10% solution of glutaraldehyde is available from pharmacies as over-the-counter medication for the treatment of warts. Glutaraldehyde at a concentration of 0.75−2% by volume can lead to false-negative screening results on EMIT II drugs of abuse screening assays. The assay for cocaine (as benzoylecgonine) was mostly affected [81].

Klear is available through various Internet sites, and the manufacturer claims that it can mask all positive drug test results. The Klear product consists of two microtubes of white crystalline material, with each tube containing approximately 500 mg. This product readily dissolves in urine with no change of color or temperature of urine, and it may cause false-negative GC-MS confirmation of marijuana metabolite. ElSohly *et al*. [82] first reported this product as potassium nitrite, and they provided evidence that nitrite leads to decomposition of ions of marijuana metabolite. The authors further reported that using a bisulfite treatment step at the beginning of sample preparation could eliminate this interference. Nitrite in urine may arise *in vivo* and is found in urine in low concentrations. Patients receiving medications such as nitroglycerine, isosorbide dinitrate, nitroprusside, and ranitidine may increase nitrite levels in blood. However, concentrations of nitrite were below 36 μg/mL in specimens cultured positive for microorganisms, and nitrite concentrations were below 6 μg/mL in patients receiving medications that are metabolized to nitrite. On the other hand, nitrite concentrations were 1910−12,200 μg/mL in urine specimens adulterated with nitrite [83]. In our experience, if present in a urine specimen as an adulterant, nitrite can be detected by adding a few drops of 2% potassium permanganate solution to five drops of urine followed by addition of two drops of 2N hydrochloric acid. The pink color of the solution immediately becomes colorless if nitrite is present in the urine [84].

AdultaCheck 4 and AdultaCheck 6 test strips can be used to detect common adulterants in urine. AdultaCheck 4 consists of four individual tests, whereas AdultaCheck 6 detects creatinine, oxidants, nitrite, glutaraldehyde, pH, and chromate. The Intect7Check test strip for checking adulteration in urine is composed of seven different pads to test for creatinine, nitrite, glutaraldehyde, pH, specific gravity, bleach, and PCC [85]. SAMSHA guidelines require additional tests for urine specimens with abnormal physical characteristics or ones that show characteristics of an adulterated specimen during initial screening or confirmatory tests (nonrecovery of internal standard, unusual response, etc.) [86]. A pH less than 3 or greater than 11 and nitrite concentrations greater than 500 mg/mL indicate the presence of adulterants. A nitrite colorimetric test or a general oxidant colorimetric test can be performed to identify nitrite. The presence of chromium in a urine specimen can be confirmed by a chromium colorimetric test or a general test for the presence of oxidant. A confirmatory test can be performed using multi-wavelength spectrophotometry, ion chromatography, atomic absorption spectrophotometry, capillary electrophoresis, or inductively coupled plasma mass spectrometry. Halogens such as fluorine, chlorine, bromine, and iodine are found in nature, and these halide salts (e.g., sodium chloride) are also found in urine. However, elemental halogens (e.g., pure bromine or iodine) can be used as adulterants. The presence of these elemental halogens should be confirmed by a halogen colorimetric test or a general test for the presence of oxidants. The presence of glutaraldehyde should be detected by a general aldehyde test or the characteristic immunoassay response in one or more drug immunoassay tests for initial screening. The presence of PCC should be confirmed by using a general test for the presence of oxidant and a GC-MS confirmatory test. The presence of a surfactant should be verified by using a surfactant colorimetric test with a dodecylbenzene sulfonate equivalent cutoff of 100 mg/mL or greater.

OTHER DRUGS NOT DETECTED BY ROUTINE TOXICOLOGY SCREENS

Ketamine is used at rave parties, but this drug is not tested routinely in toxicological screen. As mentioned previously, designer drugs related to the structure of amphetamine and marijuana may not be detected by routine toxicology screens performed in most hospital laboratories for diagnosis of drug overdoses. In addition, drugs such as LSD and methaqualone are infrequently abused; therefore, routine testing may be unnecessary [87]. Nevertheless, immunoassays are available for both drugs. Wiegand *et al*. [88] compared EMIT II, CEDIA, and DPC RIA assays for detecting LSD in forensic urine specimens and commented that at 500 pg/mL LSD cutoff, of 221 forensic urine specimens that screened positive by the EMIT II assay, only 11 tested positive by the CEDIA assay and 3 with the RIA assay, indicating a high false-positive rate with the EMIT II assay for LSD. However, each assay correctly identified 23 of 24 urine specimens that had previously been found to contain LSD by GC-MS at a cutoff of 200 pg/mL. The authors concluded that the CEDIA assay demonstrated superior precision, accuracy, and decreased cross-reactivity to compounds other than LSD compared with the EMIT II assay and does not require handling of radioactive compounds. The chemical structure of ketamine is given in Figure 14.2.

CASE REPORT A 31-year-old male with severe end-stage cardiomyopathy secondary to rheumatic heart disease and crack cocaine use called emergency medical services for shortness of breath. He was diagnosed with cardiogenic shock secondary to sepsis and was admitted to the hospital. His initial urine drug

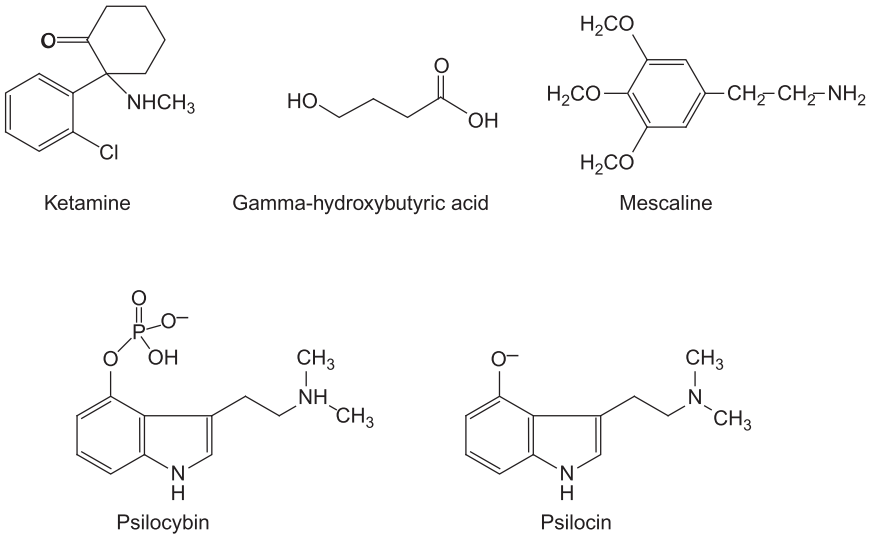

FIGURE 14.2 Chemical structures of ketamine, GHB, mescaline, psilocybin, and psilocin.

screen was negative. However, his girlfriend, who visited him regularly, had suspicious behavior, and the patient became incoherent and began hallucinating. One urine specimen collected at that time was positive for LSD using both CEDIA and EMIT assays. Another specimen collected 3.5 hr later was also positive for LSD, but LC-MS failed to show the presence of LSD or its metabolite, 2-oxo-3-hydroxy LSD, in both specimens. Examination of the medical record by the authors showed that the patient received fentanyl 24 hr prior to each false-positive LSD specimen. GC-MS analysis revealed the presence of fentanyl in both urine specimens (0.67 μg/mL in the first specimen and 0.7 μg/mL in the second specimen). The authors concluded that fentanyl may cause false-positive test results with LSD immunoassays [89].

γ-Hydroxybutyric acid (GHB) is often used at rave parties, and especially in date rape situations, because this compound is tasteless and colorless and can be easily mixed with a drink to make the victim unconscious. Currently, there is no immunoassay for routine screening of GHB in urine or any other biological matrix. Unfortunately, GHB cannot be detected by routine drugs of abuse testing protocols. In the case of suspected overdose of GHB, a more sophisticated analytical technique such as GC-MS should be employed for confirming the presence of GHB in blood or urine. GHB in blood can be determined using GC-MS after liquid–liquid extraction and di-trimethylsilyl derivatization [90]. The chemical structure of GHB is given in Figure 14.2.

The active component of peyote cactus is mescaline. The chemical structure of mescaline is given in Figure 14.2. Native Americans sometimes use peyote cactus for religious ceremonies. There is no commercially available immunoassay for determining the presence of mescaline in urine, and only chromatographic methods are available for determination of mescaline concentration in biological fluids after suspected overdose. Although uncommonly encountered, abuse of peyote cactus may cause clinically significant symptoms requiring hospitalization. In one study, the authors identified 31 cases of peyote cactus abuse in the California Poison Control System database between 1997 and 2008 [91]. Severe toxicity and even death from mescaline overdose have been reported. One person who died under the influence of mescaline showed 9.7 μg/mL of drug in serum and 1163 μg/mL of drug in urine [92].

Magic mushrooms (psychoactive fungi), which grow in the United States, Mexico, South America, and many other areas of the world, contain the hallucinogenic compounds psilocybin and psilocin. Psilocybin and psilocin, along with other compounds in the "tryptamine" class of drugs, are classified as Class I controlled substances with no known medical use but have a high abuse potential. Chemical structures of psilocybin and psilocin are given in Figure 14.2. Unlawful possession of a Class I controlled substance is a felony by law in the United States. Although not commonly abused, and not routinely tested due to lack of availability of immunoassays, magic mushroom abuse may cause serious medical complications and even death. After ingestion of magic mushroom, psilocybin, often the major component of magic mushroom, is rapidly converted by dephosphorylation into psilocin, which has psychoactive effects similar to those of LSD. Although the presence of psilocybin and psilocin in biological fluids can only be determined by chromatographic methods, Tiscione and Miller [93] identified psilocin in a urine specimen during a routine investigation for driving under the influence of drugs using FPIA for screening for

TABLE 14.6 Drugs Not Usually Detected by Routine Toxicology Screen

Drug	Comments
Designer drugs related to amphetamine	Other than MDMA and MDA, most drugs structurally related to amphetamine or methamphetamine cannot be detected by amphetamine/methamphetamine assays
Flunitrazepam	Date rape drug flunitrazepam (Rohypnol) may not be detected by benzodiazepine assays due to low concentration in urine
Clonazepam and lorazepam	May not be detected due to low levels
Oxycodone, methadone, fentanyl	Opiate assay does not detect these drugs. Specific assays must be used
Hydrocodone, oxymorphone, hydromorphone	May have low cross-reactivity with certain opiate immunoassays
Designer drugs such as "spice"	These designer drugs related to structure of THC may not cross-react with marijuana immunoassays
Ketamine	No immunoassay available
Magic mushroom abuse (psilocybin)	No immunoassay available
Peyote cactus abuse (mescaline)	No immunoassay available

THC, tetrahydrocannabinol, the active component of marijuana.

amphetamine/methamphetamine in urine. The authors determined that at a concentration of 50 μg/mL, the cross-reactivity of psilocin with the amphetamine immunoassay is 1.3%. In contrast, McClintock *et al*. [94] reported a case of a 28-year-old male with a history of alcohol and drug abuse who had three emergency room visits and three admissions to the hospital, including one in the intensive care unit, in the past 2 months of the study. All laboratory toxicology studies, including GC-MS analysis of urine specimens, were negative. The patient admitted using magic mushroom to a nurse, and the authors concluded that his symptoms were consistent with magic mushroom abuse. This case illustrates the difficulty of diagnosing magic mushroom poisoning using routine toxicological analysis. Drugs that are not detected by routine toxicology screen are listed in Table 14.6.

CONCLUSIONS

Drug of abuse testing in urine specimens is most common, although for legal drug testing, alternative specimens such as hair and oral fluids are gaining popularity. Usually, for both medical and legal drug testing, initial screening of urine specimens is conducted using commercially available immunoassays. If the initial screening is positive, then the individual drug or drug class must be confirmed by an alternative method, most commonly GC-MS for all legal drug testing. For medical drug testing, GC-MS confirmation may or may not be performed depending on the physician's request. Although the initial screening of specimens using immunoassays is a fast and effective way for determining the presence of a drug or drug class in the specimens, immunoassays suffer from cross-reactivity to structurally related compounds and false-positive drug testing is common with immunoassays. Moreover, due to poor cross-reactivity with the morphine antibody used in opiate immunoassays, opioids such as oxycodone, methadone, fentanyl, propoxyphene, and, to a certain extent, oxymorphone, hydrocodone, and hydromorphone may not be detected during routine toxicological screen. Therefore, specific immunoassays must be used for detecting oxycodone, methadone, propoxyphene, and fentanyl. Brahm *et al*. [95] reviewed the effects of commonly prescribed drugs causing false-positive test results with immunoassays, and Tenore [96] reviewed challenges in urine toxicology screening. Interested readers should refer to these two articles for more in-depth information on this topic.

References

[1] 2010 National Survey on Drug Use and Health. Substance Abuse and Mental Health Services Administration, Rockville, MD. Available at: http://www.oas.samhsa.gov/nhsda.htm.

[2] Montoya ID, Elwood WN. Fostering a drug free workplace. Health Care Superv 1995;14:1–3.

[3] Carpenter CS. Workplace drug testing and worker drug use. Health Serv Res 2007;42L:795–810.

[4] Jaffee WB, Truccp E, Teter C, Levy S, et al. Focus on alcohol and drug abuse: ensuring validity in urine drug testing. Psychiatr Serv 2008;59:140–2.

[5] Jolley ME, Stroupe SD, Schwenzer KS, et al. Fluorescence polarization immunoassay III: an automated system for therapeutic drug determination. Clin Chem 1981;27:1575–9.

[6] Hawks RL, Chian CN, editors. Urine testing for drugs of abuse. Rockville, MD: National Institute of Drug Abuse. Department of Health and Human Services; 1986 [NIDA research monograph 73].

[7] Jeon SI, Yang X, Andrade JD. Modeling of homogeneous cloned enzyme donor immunoassay. Anal Biochem 2004;333:136–47.

[8] Datta P, Dasgupta A. A new turbidimetric digoxin immunoassay on the ADVIA 1650 Analyzer is free from interference by spironolactone, potassium canrenoate, and their common metabolite canrenone. Ther Drug Monit 2003;25:478–82.

[9] Dai JL, Sokoll LJ, Chan DW. Automated chemiluminescent immunoassay analyzers. J Clin Ligand Assay 1998;21:377–85.

[10] Montagne P, Varcin P, Cuilliere ML, Duheille J. Microparticle-enhanced nephelometric immunoassay with microsphere-antigen conjugate. Bioconjugate Chem 1992;3:187–93.

[11] Fraser AD, Zamecnik J. Impact of lowering the screening and confirmation cutoff values for urine drug testing based on dilution indicators. Ther Drug Monit 2003;25:723–7.
[12] Soldin SJ, Morales AJ, D'Angelo LJ, Bogema SC, Hicks JC. The importance of lowering the cut-off concentrations of urine screening and confirmatory tests for benzoylecgonine/cocaine [Abstract]. Clin Chem 1991;37:993.
[13] Luzzi VI, Saunders AN, Koenig JW, Turk J, et al. Analytical performance of immunoassays for drugs of abuse below established cutoff values. Clin Chem 2004;50:717–22.
[14] Paul B, Past MR. Confirmation methods in drug testing: an overview. In: Dasgupta A, editor. Critical issues in alcohol and drugs of abuse testing. Washington, DC: AACC Press; 2009. p. 125–61.
[15] Maralikova B, Weinmann W. Confirmatory analysis for drugs of abuse in plasma and urine by high performance liquid chromatography-tandem mass spectrometry with respect to criteria for compound identification. J Chromatogr B Analyt Technol Biomed Life Sci 2004;811:21–30.
[16] Eichhorst JC, Etter ML, Rousseaux N, Lehotay DC. Drugs of abuse testing by tandem mass spectrometry: a rapid simple method to replace immunoassay. Clin Biochem 2009;42:1531–42.
[17] Kunsman GW, Levine B, Kuhlman JJ, Jones RL, et al. MDA-MDMA concentrations in urine specimens. J Anal Toxicol 1996;20:517–21.
[18] Stout PR, Klette KL, Wiegand R. Comparison and evaluation of DRI methamphetamine, DRI ecstasy, Abuscreen ONLINE amphetamine, and a modified Abuscreen ONLINE amphetamine screening immunoassays for the detection of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) in human urine. J Anal Toxicol 2003;27:265–9.
[19] Huang MK, Dai YS, Lee CH, Liu C, et al. Performance characteristics of DRI, CEDIA and REMEDi system for preliminary tests for amphetamines and opiates in urine. J Anal Toxicol 2006;30:61–4.
[20] Poklis A, Fitzgerald RL, Hall KV, Saddy JJ. EMIT-d.a.u monoclonal amphetamine/methamphetamine assay II: detection of methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA). Forensic Sci Int 1993;59:63–70.
[21] Lin DL, Liu HC, Tin HL. Recent paramethoxymethamphetamine (PMMA) deaths in Taiwan. J Anal Toxicol 2007;31:109–13.
[22] Kerrigan S, Mellon MB, Banuelos S, Arndt C. Evaluation of commercial enzyme-linked immunosorbent assays to identify psychedelic phenethylamine. J Anal Toxicol 2011;25:444–51.
[23] Apollonio LG, Whittall IR, Pianca DJ, Kyd JM, et al. Matrix effect and cross-reactivity of select amphetamine-type substances, designer analogues, and putrefactive amines using the Bio-Quant direct ELISA presumptive assays for amphetamines and methamphetamines. J Anal Toxicol 2007;31:208–13.
[24] Kerrigan S, Banuelos S, Perrella L, Hardy B. Simultaneous detection of ten psychedelic phenethylamine in urine by gas chromatography-mass spectrometry. J Anal Toxicol 2011;35:459–69.
[25] Ewald AH, Fritschi G, Bork WR, Maurer HH. Designer drugs 2,5-dimethoxy-4-bromo-amphetamine (DOB) and 2,5-diemthoxy-4-bromo-methamaphetamine (MDOB): studies of their metabolite and toxicological detection in rat urine using gas chromatography/mass spectrometric techniques. J Mass Spectrom 2006;41:487–98.
[26] Takahashi M, Nagashima M, Suzuki J, Seto T, et al. Creation and application of psychoactive designer drugs data library using liquid chromatography with photodiode array spectrometry detector and gas chromatography-mass spectrometry. Talanta 2009;77:1245–72.
[27] Balikova M. Nonfatal and fatal DOB (2,5-dimethoxy-4-bromoamphetmaine) overdose. Forensic Sci Int 2005;153:85–91.
[28] Stout PR, Klette KL, Horn CK. Evaluation of ephedrine, pseudoephedrine, and phenylpropanolamine concentrations in human urine specimens and a comparison of the specificity of DRI amphetamines and Abuscreen online (KIMS) amphetamines screening immunoassays. J Foresnic Sci 2004;49:160–4.
[29] Poklis A, Jortani WSA, Brown CS, Crooks CR. Response of the EMIT II amphetamine/methamphetamine assay to specimens collected following use of Vicks inhalers. J Anal Toxicol 1993;17:284–6.
[30] Dietzen DJ, Ecos K, Friedman D, Beason S. Positive predictive values of abused drug immunoassays on the Beckman SYNCHRON in a Veteran population. J Anal Toxicol 2001;25:174–8.
[31] Logan BK, Costantino AG, Rieders EF, Sanders D. Trazodone, meta-chlorophenylpiperazine (an hallucinogenic drug and trazodone metabolite) and the hallucinogen trifluoromethylphenylpiperazine cross-react with EMITII ecstasy immunoassay in urine. J Anal Toxicol 2010;34:587–9.
[32] Baron JM, Griggs DA, Nixon AL, Long WH, et al. The trazodone metabolite meta-chlorophenylpiperazine can cause false positive urine amphetamine immunoassay result. J Anal Toxicol 2011;35:364–8.
[33] Yee LM, Wu D. False positive amphetamine toxicology screen results in three pregnant women using labetalol. Obstet Gynecol 2011;117(2 Pt 2):503–6.
[34] Casey ER, Scott MG, Tang S, Mullins ME. Frequency of false positive amphetamine screens due to bupropion using the Syva EMIT II immunoassay. J Med Toxicol 2011;7:105–8.
[35] Vidal C, Skripuletz T. Bupropion interference with immunoassays for amphetamines and LSD. Ther Drug Monit 2007;29:373–5.
[36] Merigian KS, Beowning RG. Desipramine and amantadine causing false positive urine test for amphetamine. Ann Emerg Med 1993;22:1927–8.
[37] Kraemer T, Wenning R, Maurer HH. The antispasmodic drug mebeverine leads to positive amphetamine results by fluorescence polarization immunoassays (FPIA) studies on the toxicological analysis of urine by FPIA and GC-MS. J Anal Toxicol 2001;25:333–8.
[38] Vorce SP, Holler JM, Cawrse BM, Magluilo J. Dimethylamine: a drug causing positive immunoassay results for amphetamines. J Anal Toxicol 2011;35:183–7.
[39] Armbruster DA, Schwarzhoff RH, Hubster EC, Liserio MK. Enzyme immunoassay, kinetic microparticle immunoassay, radioimmunoassay and fluorescence polarization immunoassay compared for drugs of abuse screening. Clin Chem 1993;39:2137–46.
[40] Baker JE, Jenkins AJ. Screening for cocaine metabolite fails to detect an intoxication. Am J Forensic Med Pathol 2008;29:141–4.
[41] Dasgupta A, Mahle C, McLemore J. Elimination of fluconazole interference in gas chromatography/mass spectrometric confirmation of benzoylecgonine, the major metabolite of cocaine using pentafluoropropionyl derivative. J Forensic Sci 1996;41:511–13.
[42] Mazor SS, Mycyk MB, Wills BK, Brace LD, et al. Coca tea consumption causes positive urine cocaine assay. Eur J Emerg Med 2006;13:340–1.
[43] Smith ML, Hughes RO, Levine B, Dickerson S, et al. Forensic drug testing for opiates: VI. Urine testing for hydromorphone, hydrocodone, oxymorphone, and oxycodone with commercial opiate immunoassays and gas chromatography-mass spectrometry. J Anal Toxicol 1995;19:18–26.

[44] Abadie JM, Allison KH, Black DA, Garbin J, et al. Can an immunoassay become a standard technique in detecting oxycodone and its metabolites? J Anal Toxicol 2005;29:825–9.
[45] Gingras M, Laberge MH, Lafebvre M. Evaluation of the usefulness of an oxycodone immunoassay in combination with a traditional opiate immunoassay for the screening of opiates in urine. J Anal Toxicol 2010;34:78–83.
[46] Snyder ML, Jarolim P, Melanson SE. A new automated urine fentanyl immunoassay: technical performance and clinical utility for monitoring fentanyl compliance. Clin Chim Acta 2011;412:946–51.
[47] Armstrong EJ, Jenkins AJ, Sebrosky GF, Balraj EK. An unusual fatality in a child due to oxycodone. Am J Forensic Med Pathol 2004;25:338–41.
[48] Holler JM, Bosy TZ, Klette KL, Wiegand R, et al. Comparison of the microgenics CEDIA heroin metabolite (6-AM) and the Roche Abuscreen ONLINE opiate immunoassays for detection of heroin use in forensic urine samples. J Anal Toxicol 2004;28:489–93.
[49] Straseski JA, Stolbach A, Clarke W. Opiate positive immunoassay screen in a pediatric patient. Clin Chem 2010;56:1220–5.
[50] Hull MJ, Bierer MF, Griggs DA, Long WH, et al. Urinary buprenorphine concentration in patients treated with Suboxone as determined by liquid chromatography-mass spectrometry and CEDIA immunoassay. J Anal Toxicol 2008;32:516–21.
[51] Baden LR, Horowitz G, Jacoby H, Eliopoulos GM. Quinolones and false positive urine screening for opiates by immunoassay technology. JAMA 2001;286:3115–19.
[52] Straley CM, Cecil EJ, Herriman MP. Gatifloxacin interfere with opiate urine drug screen. Pharmacotherapy 2006;26:435–9.
[53] De Paula M, Saiz LC, Gonzalez-Revalderia J, Pascual T, et al. Rifampicin causes false positive immunoassay results for opiates. Clin Chem Lab Med 1998;36:241–3.
[54] Widschwendter CG, Zernig G, Hofer A. Quetiapine cross-reactivity with urine methadone immunoassays [Letter to the Editor]. Am J Psychiatry 2007;164:172.
[55] Rogers SC, Pruitt CW, Crouch DJ, Caravati EM. Rapid urine drug screens: diphenhydramine and methadone cross-reactivity. Pediatr Emerg Care 2010;26:665–6.
[56] Hausmann E, Kohl B, von Boehmer H, Wellhoner HH. False positive EMIT indication for opiates and methadone in doxylamine intoxication. J Clin Chem Clin Biochem 1983;21:599–600.
[57] Frederick DL, Green J, Fowler MW. Comparison of six cannabinoid metabolite assays. J Anal Toxicol 1985;9:116–20.
[58] Rohrich J, Schimmel I, Zorntlein S, Becker J, et al. Concentrations of delta-9-tetrahydrocannabinol and 11-nor 9-carboxytetrahydrocannabinol in blood and urine after passive exposure to cannabis smoke in a coffee shop. J Anal Toxicol 2010;34:196–203.
[59] Zawilska JB. "Legal highs"—New players in the old drama. Curr Drug Abuse Rev 2011;4:122–30.
[60] Vardakou I, Pistos C, Spiliopoulou CH. Spice drugs as a new trend: mode of action, identification and legislation. Toxicol Lett 2010;197:157–62.
[61] Boucher A, Vilette P, Crassard N, Bernard N, et al. Urinary toxicological screening: analytical interference between niflumic acid and cannabis. Arch Pediatr 2009;16:1457–60 [In French].
[62] Oosthuizen NM, Laurens JB. Efavirenz interference in urine screening immunoassays for tetrahydrocannabinol. Ann Clin Biochem 2011;49:194–6.
[63] Marchei E, Pellegrini M, Pichini S, Martin I, et al. Are false positive phencyclidine immunoassay instant-view multi test results caused by overdose concentrations of ibuprofen, metamizol and dextromethorphan?. Ther Drug Monit 2007;29:671–3.
[64] Long C, Crifasi J, Maginn D. Interference of thioridazine (Mellaril) in identification of phencyclidine. Clin Chem 1996;42:1885–6 [Letter to the Editor].
[65] Bond GR, Steele PE, Uges DR. Massive venlafaxine overdose resulted in a false positive Abbott AxSYM urine immunoassay for phencyclidine. J Toxicol Clin Toxicol 2003;41:999–1002.
[66] West R, Pesce A, West C, Crews B, et al. Comparison of clonazepam compliance by measurement of urinary concentration by immunoassay and LC-MS/MS in patient management. Pain Physician 2010;13:71–8.
[67] Forsman M, Nystrom I, Roman M, Berglund L, et al. Urinary detection times and excretion patterns of flunitrazepam and its metabolites after a single oral dose. J Anal Toxicol 2009;33:491–501.
[68] Kurisaki E, Hayashida M, Nihira M, Ohno Y, et al. Diagnosis performance of Triage for benzodiazepines: urine analysis of the dose of therapeutic cases. J Anal Toxicol 2005;29:539–43.
[69] Augsburger M, Rivier L, Mangin P. Comparison of different immunoassays and GC-MS screening of benzodiazepines in urine. J Pharm Biomed Anal 1998;18:681–7.
[70] Wenk RE. False negative urine immunoassay after lorazepam overdose. Arch Pathol Lab Med 2006;130:1600–1 [Letter to the Editor].
[71] Lum G, Mushlin B, Farney L. False positive rates for the qualitative analysis of urine benzodiazepines and metabolites with the reformulated Abbott Multigent reagents. Clin Chem 2008;54:220–1 [Letter to the Editor].
[72] Fraser AD, Howell P. Oxaprozin cross-reactivity in three commercial immunoassays for benzodiazepines in urine. J Anal Toxicol 1998;22:50–4.
[73] McNally AJ, Pilcher I, Wu R, Salamone SJ, et al. Evaluation of the online immunoassay for propoxyphene: comparison to EMIT II and GC-MS. J Anal Toxicol 1996;20:537–40.
[74] Schneider S, Wennig R. Interference of diphenhydramine with the EMIT II immunoassay for propoxyphene. J Anal Toxicol 1999;23:637–8.
[75] Brenner C, Hui R, Passarelli J, Wu R, et al. Comparison of methaqualone excretion patterns using Abuscreen ONLINE and EMIT II immunoassay and GC/MS. Forensic Sci Int 1996;79:31–41.
[76] Joseph R, Dickerson S, Willis R, Frankenfield D, et al. Interference by nonsteroidal antiinflammatory drugs in EMIT and TDx assays for drugs of abuse. J Anal Toxicol 1995;19:1–7.
[77] Rollins DE, Jennison TA, Jones G. Investigation of interference by non-steroidal antiinflammatory drugs in urine tests for abused drugs. Clin Chem 1990;36:602–6.
[78] Mikkelsen SL, Ash O. Adulterants causing false negative in illicit drug test. Clin Chem 1988;34:2333–6.
[79] Warner A. Interference of household chemicals in immunoassay methods for drugs of abuse. Clin Chem 1989;35:648–51.
[80] Wu A, Bristol B, Sexton K, Cassella-McLane G, Holtman V, Hill DW. Adulteration of urine by Urine Luck. Clin Chem 1999;45:1051–7.
[81] George S, Braithwaite RA. The effect of glutaraldehyde adulteration of urine specimens on Syva EMIT II drugs of abuse assay. J Anal Toxicol 1996;20:195–6.
[82] ElSohly MA, Feng S, Kopycki WJ, Murphy TP, Jones AB, Davis A, et al. A procedure to overcome interference's caused by adulterant "Klear" in the GC-MS analysis of 11-nor-Δ9-THC-9-COOH. J Anal Toxicol 1997;20:240–2.
[83] Urry F, Komaromy-Hiller G, Staley B, Crockett D, Kushnir M, Nelson G, et al. Nitrite adulteration of workplace drug testing specimens: sources and associated concentrations of nitrite and distinction between natural sources and adulteration. J Anal Toxicol 1998;22:89–95.
[84] Dasgupta A, Wahed A, Wells A. Rapid spot tests for detecting adulterants in urine specimens submitted for drug testing. Am J Clin Pathol 2002;117:325–9.

[85] Dasgupta A, Chughtai O, Hannah C, Davis B, Wells A. Comparison of spot test and AdultaCheck 6 and Intect 7 urine test strips for detecting the presence of adulterants in urine specimens. Clin Chim Acta 2004;348:19–25.
[86] Bush DM. The US mandatory guidelines for federal workplace drug testing programs: current status and future considerations. Forensic Sci Int 2008;174:111–19.
[87] Melanson SE, Baskin L, Magnani B, Kwong TC, et al. Interpretation and utility of abuse immunoassays: lessons from laboratory drug testing surveys. Arch Pathol Lab Med 2010;134:735–9.
[88] Wiegand RF, Klette KL, Stout PR, Gehlhausen JM. Comparison of EMIT II, CEDIA and DPC RIA assays for the detection of lysergic acid diethylamide in forensic urine samples. J Anal Toxicol 2002;26:519–23.
[89] Gagajewski A, David GK, Poch GK, Anderson CJ, et al. False positive lysergic acid diethylamide immunoassay screen associated with fentanyl medication. Clin Chem 2002;48:205–6 [Letter to the Editor].
[90] Elian AA. GC-MS determination of gamma-hydroxybutyric acid (GHB) in blood. Forensic Sci Int 2001;122:43–7.
[91] Carstairs SD, Cantrill FL. Peyote and mescaline exposures: a 12 year review of a statewide poison center database. Clin Toxicol 2010;48:350–3.
[92] Reynolds PC, Jindrich EJ. A mescaline associated fatality. J Anal Toxicol 1985;9:183–4.
[93] Tiscione NB, Miller MI. Psilocin identified in a DUID investigation. J Anal Toxicol 2006;30:342–5.
[94] McClintock RL, Watts DJ, Melanson S. Unrecognized magic mushroom abuse in a 28 year old man. Am J Emerg Med 2008;26(972):e3–4.
[95] Brahm NC, Yeager LL, Fox MD, Farmer KC, et al. Commonly prescribed medications and potential false-positive urine drug screens. Am J Health Syst Pharm 2010;67:1344–50.
[96] Tenore PL. Advanced urine toxicology testing. J Addictive Dis 2010;29:436–48.