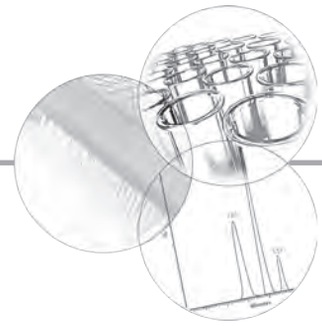

'False-positive' and 'false-negative' test results in clinical urine drug testing

The terms 'false-positive' and 'false-negative' are widely used in discussions of urine drug test (UDT) results. These terms are inadequate because they are used in different ways by physicians and laboratory professionals and they are too narrow to encompass the larger universe of potentially misleading, inappropriate and unexpected drug test results. This larger universe, while not solely comprised of technically 'true' or 'false' positive or negative test results, presents comparable interpretive challenges with corresponding clinical implications. In this review, we propose the terms 'potentially inappropriate' positive or negative test results in reference to UDT results that are ambiguous or unexpected and subject to misinterpretation. Causes of potentially inappropriate positive UDT results include *in vivo* metabolic conversions of a drug, exposure to nonillicit sources of a drug and laboratory error. Causes of potentially inappropriate negative UDT results include limited assay specificity, absence of drug in the urine, presence of drug in the urine, but below established assay cutoff, specimen manipulation and laboratory error. Clinical UDT interpretation is a complicated task requiring knowledge of recent prescription, over-the-counter and herbal drug administration, drug metabolism and analytical sensitivities and specificities.

The terms 'false-positive' and 'false-negative' are widely used in discussions of clinical urine drug test (UDT) results. These terms, however, are inadequate because they are used in very different ways by physicians and laboratory professionals and they convey narrow concepts that do not fully encompass the range of etiologies that lead to potentially misleading drug test results. There is an important difference between forensic and clinical drug testing: the former requires involvement of a medical review officer, whereas no such requirement exists with the latter. In the USA, medical review officers are certified by examination covering all aspects of workplace drug testing, including specific definitions of the terminology used by laboratories when reporting results. In clinical drug testing, clinicians are expected to interpret drug-testing results and surveys have revealed that they are poorly prepared for that task [1]. Certification boards in toxicology also exist for laboratory professionals, including the American Board of Clinical Chemistry (toxicological chemistry) and the American Board of Forensic Toxicology, which qualify doctoral scientists to direct UDT laboratories participating in federal and state drug-free workplace programs, but these certifications are not required of clinical laboratory directors. Therefore, clinicians faced with unexpected UDT results do not always have convenient access to adequate interpretive expertise.

When interpreting the results of an assay for a particular drug of interest, laboratorians are concerned primarily with the question 'is the drug present or not?', while clinicians usually pose the additional question 'what does the result mean in terms of patient behavior?' Consider, for example, opiate-positive urine drug screening immunoassay and subsequent GC–MS confirmation results in an individual not prescribed opioid analgesics, and which, after clinical evaluation, are attributed to poppy seed consumption. Laboratory professionals generally refer to this as a true-positive result, notwithstanding the patient's abstemious behavior, because the analyte(s) in question – morphine and possibly codeine – are actually present [2]. Clinicians, on the other hand, generally describe this as a false-positive result [3], because, despite the presence of morphine and codeine in the urine, the clinical behavior in question – opiate abuse – is absent. Conversely, consider an individual with a history of ongoing phencyclidine (PCP) abuse, whose urine drug screen is negative for PCP at the designated cut-off concentration of 25 µg/l, but whose subsequent GC–MS evaluation at the limit of detection reveals a PCP concentration of 24 µg/l. Laboratorians would describe the screening immunoassay result as a true-negative because the analyte in question – PCP – was not present at or above the screening cut-off of 25 µg/l. Clinicians, however, would generally

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Future science part of fsg

Immunoassay

Economical and often automated analytical method frequently used for detecting drugs and metabolites in biological matrices. All immunoassays involve polyclonal or monoclonal antibodies that react with the drug and/or metabolite

GC–MS

Sophisticated analytical method involving vaporization of the analyte, isolation by GC and measurement by MS, considered to be the most specific method available for identifying organic compounds. GC–MS and related LC–MS^n methods are often used to confirm the presence of drugs or metabolites in biological matrices

consider the screening result to be an example of a false-negative, because the confirmatory analysis of the specimen reveals the use of an illegal drug, even if the concentration is below the screening threshold.

There are many problematic UDT results that defy characterization as 'true' or 'false' positive or negative. These include the detection of nonprescribed opioids, possibly as a result of *in vivo* metabolic conversion of prescribed opioids, the detection of controlled substances, possibly due to nonprescription drug use, positive or negative UDT results attributable to imperfect test specificities or cross-reactivities, low or undetectable drug concentrations caused by metabolic or environmental factors, analytical test method limitations and specimen manipulation.

Clearly, 'true' and 'false' UDT results are a limited subset of a larger universe of potentially misleading, inappropriate and unexpected UDT results. This larger universe, while not solely comprised of technically 'true' or 'false' positive or negative test results, presents comparable interpretive challenges with corresponding clinical implications. In this review, we propose the terms potentially inappropriate positive or negative, in reference to UDT results that are ambiguous and subject to misinterpretation. Causes of potentially inappropriate UDT results include *in vivo* metabolic conversions of a (prescribed) controlled substance to another (nonprescribed) controlled substance, consumption of nonillicit sources of a drug, limited assay specificity, absence of drug in the urine, presence of drug in the urine, but below established assay cut-off, specimen manipulation and laboratory error.

Potentially inappropriate positive UDT results

■ Metabolic 'conversions'

Opiates

Several prescription opioids produce *in vivo* metabolites that are themselves prescription opioids. A well-known example of this is codeine – generally considered to be an analgesic prodrug – which is *O*-demethylated to morphine by the cytochrome P450 (CYP)2D6 enzyme. In most individuals, less than 10% of codeine is metabolized to morphine. Under specific genetic (e.g., *CYP2D6* gene duplication or multiduplication) or environmental (e.g., inhibition of a competing, *CYP3A4*-mediated metabolic pathway) circumstances, a much larger percentage of codeine – perhaps up to 75% – may be metabolized to morphine [4]. Codeine use generally produces detectable levels of morphine, but at a lower concentration than codeine. However, the converse may be observed in individuals with *CYP2D6* polymorphisms (rapid metabolizers).

Cytochrome P450

Family of oxidative enzymes involved in the Phase I metabolism of drugs. Polymorphisms in the genes encoding these enzymes cause interindividual variations in drug metabolism. Pharmacologic induction or inhibition of these enzymes can cause intraindividual variations in drug metabolism

Diacetylmorphine (heroin, diamorphine) is a prescription opioid in several countries including Austria, Canada, Germany, The Netherlands, Switzerland and the UK. This pharmaceutical product is metabolized *in vivo* to morphine via 6-acetylmorphine (6-AM) **(Figure 1)**. The latter has a narrow window of detection in the urine (typically <12 h), but it is a specific marker for heroin administration. On the other hand, nonpharmaceutical heroin is prepared from opium and contains codeine and 6-acetylcodeine (6-AC) as manufacturing impurities [5,6]. 6-AC is rapidly metabolized *in vivo* to codeine. 6-AC has a narrow window of detection (2–8 h), but is a specific marker for nonpharmaceutical heroin administration [5].

The most recently discovered example of an opioid conversion involves morphine, a small percentage of which is converted in some individuals to hydromorphone by an as yet undetermined metabolic pathway. Several recent independent reports support the existence of this metabolic pathway [7–10]. In patients administered high-dose morphine therapy, hydromorphone can often be detected using sensitive and specific techniques such as GC–MS. Based on current knowledge, individuals administered only morphine should produce a urine hydromorphone concentration less than approximately 3% of the urine morphine concentration, consistent with hydromorphone as a metabolic byproduct of morphine; a urine hydromorphone concentration exceeding 5% of the morphine concentration suggests that concurrent hydromorphone administration is likely. As data in this area continue to emerge, considerable caution should be exercised in the interpretation of urinary opioids in the patient whose adherence with a prescribed opioid regimen is being monitored by UDT. Several other opioid metabolic conversions have been described **(Figure 1)**. Of note, buprenorphine, fentanyl, hydromorphone, levorphanol, meperidine, methadone and propoxyphene are not metabolized to other prescription opioids.

Benzodiazepines

Several prescription benzodiazepines, including chlordiazepoxide, clorazepate, diazepam, halazepam, medazepam, prazepam and temazepam are metabolized to other – and sometimes several

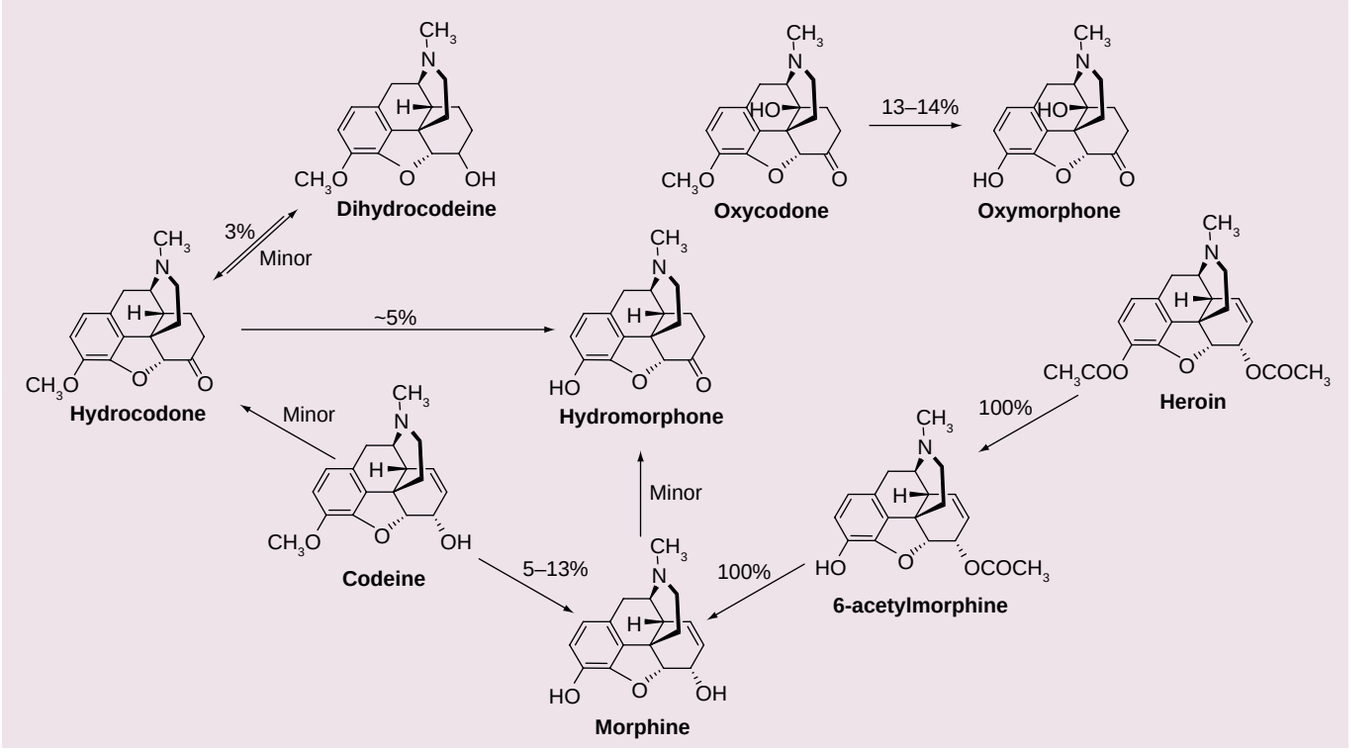

Figure 1. Opioid metabolism. *O*-demethylation of codeine to morphine is influenced by cytochrome P450 (CYP) *2D6* genotypes [4,86], as is conversion of hydrocodone to hydromorphone [87]. The synthetic oxidized derivative of codeine, oxycodone, is similarly *O*-demethylated to oxymorphone [88]. Minor metabolic conversion of codeine [89] and dihydrocodeine [90] to hydrocodone has been described, as well as reduction of hydrocodone to dihydrocodeine [91]. Metabolism of morphine to hydromorphone has been described in several reports [7–10] and appears to be a minor pathway (<3%).
Adapted with permission from [10].

other – prescription benzodiazepines **(Figure 2)**. Reliable human data are not available for the relative urine concentrations of parent drugs and metabolites in this class of drugs. Conversely, alprazolam, clonazepam, estazolam, flunitrazepam, flurazepam, lorazepam, midazolam, triazolam and quazepam are neither metabolites of, nor metabolized to, other prescription benzodiazepines. Thus, the presence in urine of alprazolam (a commonly prescribed and often abused drug in the USA) and flunitrazepam (illegal in the USA but a common drug of abuse and a notorious 'date rape' drug) cannot be explained on the basis of administration of any other benzodiazepine [11].

■ Exposure to nonillicit sources of the drug

Opiates

Exposure to various food products, as well as prescription and over-the-counter medications and their metabolites, can yield potentially inappropriate positive drug screening and confirmatory test results for substance(s) of abuse. For example, it has been well-documented that poppy seed consumption can produce positive screening and confirmatory test results for morphine and codeine [12]. Positive UDT results for opiates due to poppy seed consumption are more likely in clinical drug testing, where the threshold for positive results is ordinarily 300 µg/l, as opposed to workplace (forensic) drug testing, where the positive threshold was raised to 2000 µg/l specifically to minimize this concern. For federal workplace drug testing programs, the US Department of Health and Human Services has set a threshold of 15,000 µg/l, above which poppy seed administration cannot be accepted as a valid explanation for an opiate-positive drug test [13]. There is evidence, however, that poppy seed-related total urine morphine concentrations can exceed this threshold [6].

Cocaine

Coca tea – no longer sold in the USA, but available elsewhere and via the internet – contains appreciable quantities of cocaine. A cup of coca tea contains approximately 2.0–2.5 mg of cocaine [14,15], compared with a typical 'line' of cocaine,

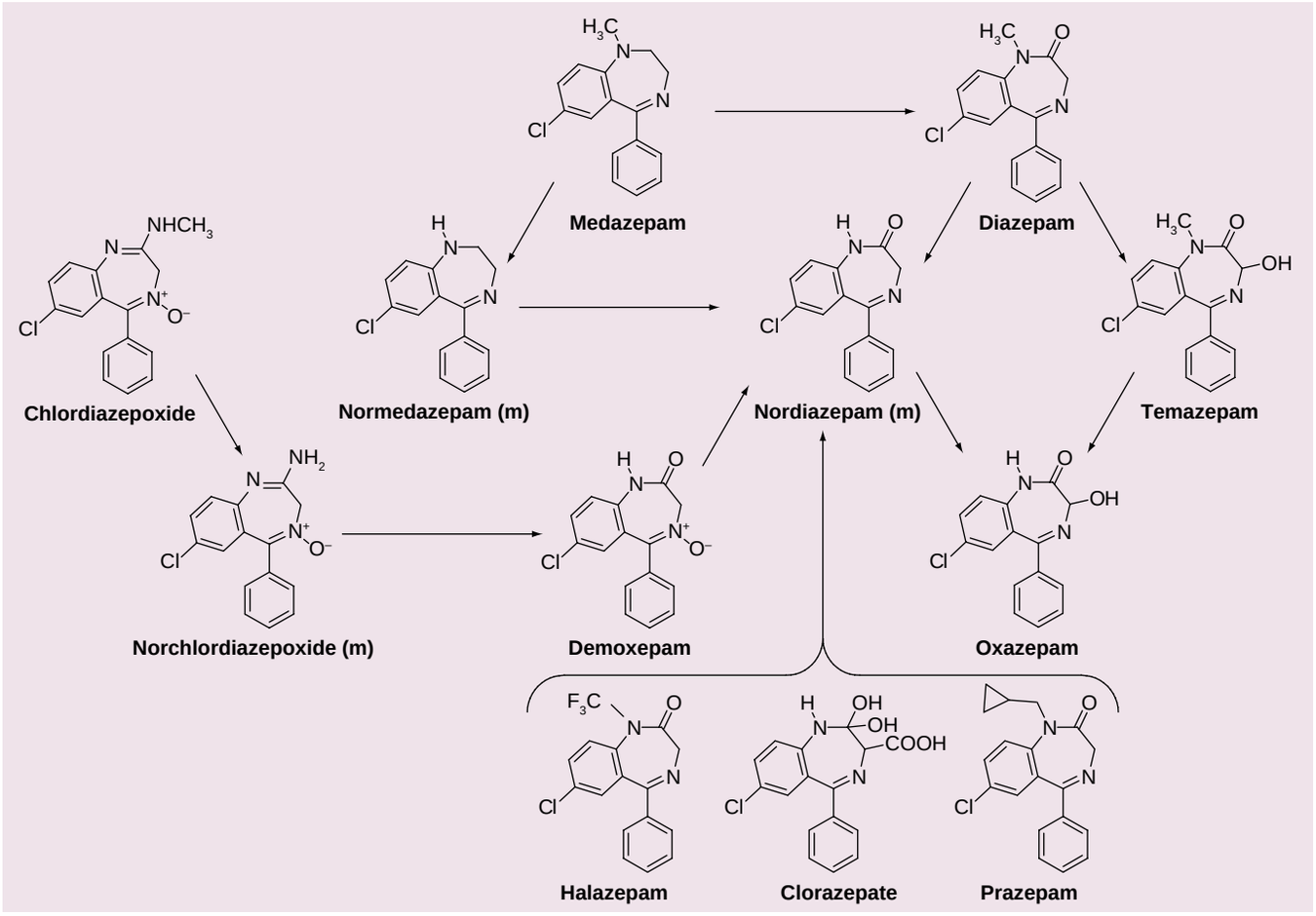

Figure 2. Metabolism of the 1,4-benzodiazepin-2-one and 2-amino-benzodiazepin-*N*-oxide derivatives of pharmaceutical benzodiazepines. All of these drugs are metabolized to nordiazepine and/or oxazepam. Most immunoassays for benzodiazepines are calibrated to 300 ng of oxazepam per milliliter, but cross-react broadly among the derivatives in these two groups. Demoxepam is a prescription drug not available in the USA, *N*-trifluoroethyl-2-thionyl- (quazepam), triazolo- (estazepam, triazolam, alprazolam) and imidazo- (midazolam) benzodiazepine derivatives are converted to hydroxylated metabolites that may have limited reactivity with benzodiazepine-screening immunoassays.
m: Metabolite that is not used pharmaceutically.

which contains approximately 20–30 mg of the drug [16]. Consumption of coca tea has been reported to produce positive screening and confirmatory test results for the cocaine metabolite benzoylecgonine at a cut-off of 300 µg/l for at least 24 h following consumption of a single cup of tea [14] and for at least 36 h following the consumption of several cups of tea [17].

Cannabinoids

Results of screening and confirmatory assays for prescription cannabinoids will vary according to the specific product and metabolites that are tested. Dronabinol (Δ^9-THC; Marinol®) and nabilone (Cesamet®) are synthetic cannabinoids. The former will yield positive screening immunoassays for cannabinoids and positive confirmatory assays for the most commonly tested metabolite, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (Δ^9-THC-COOH), whereas the latter will yield neither positive screening nor positive confirmatory assays [18]. A natural pharmaceutical product, Δ^9-THC/cannabidiol (Sativex®), is an extract of genetically and chemically characterized *Cannabis sativa*, and will thus yield positive screening and confirmatory results for cannabinoids and Δ^9-THC-COOH, respectively. Δ^9-tetrahydrocannabivarin (THCV) is a constituent of the cannabis plant and its detection in urine will distinguish between consumption of synthetic and natural cannabis products. The product monograph for Sativex includes no mention of whether, or in what quantities, THCV is present [101].

Amphetamines

For drug-testing laboratories, methods for detecting amphetamine and methamphetamine are particularly troublesome. Some OTC cold medications contain phenylethylamine-related sympathomimetics such as ephedrine, pseudoephedrine and phenylpropanolamine (no longer available OTC drug in the USA), which cross-react with amphetamine/methamphetamine immunoassay methods. Positive screening test results due to these medications are common. Other OTC preparations contain the *R*(-)-stereoisomer of methamphetamine (e.g., Vicks Vapor Inhaler and generics), with which immunoassays for *S*(+)-methamphetamine may cross-react, yielding positive methamphetamine screening and confirmatory test results [13]. The potential for positive confirmatory test results due to *R*(-)-methamphetamine (also known as levomethamphetamine or desoxyephedrine) provide an example in UDT where the screening immunoassay may, in fact, be more specific than the confirmatory method. Antibodies, upon which immunoassays depend for detection of the target molecule, are stereoselective by virtue of their 3D antigen-binding site. Some amphetamine/methamphetamine immunoassays have minimal cross-reactivity with *R*(-)-methamphetamine [19,20].

MS is incapable of distinguishing between enantiomers, since stereochemical information is lost when the molecule is fragmented in the ion source; hence, *S*(+)- and *R*(-)-methamphetamine produce identical mass spectra. However, chromatographic methods in combination with chiral derivatizing reagents have the potential to resolve stereoisomeric pairs, including the *R* and *S* stereoisomers of methamphetamine [21]. For confirmation of methamphetamine-positive specimens, US Substance Abuse and Mental Health Services Administration (SAMHSA)-certified laboratories use a strategy based on minimal (<2%) metabolic demethylation of *R*(-)-methamphetamine, compared with 4–7% (and a sometimes greater percentage) of *S*(+)-methamphetamine that is metabolized to amphetamine [13]. Therefore, the absence of confirmed amphetamine at a concentration threshold of 200 µg/l in a methamphetamine-positive specimen is taken as evidence that the positive result is not due to *S*(+)-methamphetamine. There have also been reports suggesting that methamphetamine may be produced as an artifact in specimens with high ephedrine or pseudoephedrine concentrations, purportedly due to chemical reactions that take place in the GC injector, where the molecule is exposed to high temperatures [22]. In such cases, there was no analytical evidence of amphetamine.

In the USA, several prescription medications contain *S*(+)-amphetamine (Dexedrine®), *S*(+)-methamphetamine (Desoxyn®) or a racemic mixture of the two (Adderall®), and will, of course, screen and confirm positive for methamphetamine and/or amphetamine. Furthermore, several prescription phenylethylamines, while not themselves amphetamines, are metabolized to amphetamines. Benzphetamine (Didrex®), an anorexiant, is metabolized to *S*(+)-methamphetamine and *S*(+)-amphetamine; selegiline (Eldepryl®, Zelapar® and Emsam®), a selective monoamine oxidase type B inhibitor used for the treatment of Parkinson's disease and depression, is metabolized in part to *R*(-)-methamphetamine and *R*(-)-amphetamine,and famprofazone (not available in the USA) is metabolized to both the *S*(+) and *R*(-) isomers of amphetamine and methamphetamine [22]. Each of these drugs will yield positive methamphetamine/amphetamine screening and confirmatory tests results.

Laboratory error

Forensic drug-testing laboratories are highly regulated, yet the analytical scope of their services is quite limited. Clinical laboratories in the USA are required to maintain accreditation – and in some states, licensure – but the specifications for, and surveillance of, drug testing in clinical laboratories are not as comprehensive as in forensic drug-testing laboratories (e.g., chain of custody documentation is not required). Hence, drug tests performed in clinical laboratories are vulnerable to the same types of errors as most other laboratory tests. Laboratory errors can be divided into three categories: pre-analytical, analytical and postanalytical. A large number of discrete actions are involved from the time a laboratory test is ordered until the result is reported, and each of those actions has the potential to compromise the integrity of the test result. Potentially inappropriate positive and negative test results can occur due to errors in the clinic [24] or the laboratory [25]. The majority of laboratory errors involve the pre-analytical phase. Specimen misidentification is the leading source of pre-analytic error [26], occurring at a rate that has been estimated to be 0.1–5% [27].

Table 1. Examples of pre-analytical errors that affect urine drug test results.

Error	Examples	Potential effect on test result
Incorrectly labeled specimen	Wrong patient name and/or patient identifier (medical record number)	Result is not posted to correct record; result is posted to the wrong patient's record
	Incorrect collection date and/or time	Test may be cancelled by the laboratory if the time between collection and arrival at the laboratory exceeds stability requirements; pre- or post-dose measurements may be misleading if the collection time is incorrect
Incorrect test ordered	Written order misinterpreted by laboratory	An inappropriate test is performed and the results may be useless, misleading or misinterpreted
	Inappropriate test ordered by clinician (e.g., ordering 'opiate' screen for oxycodone or methadone)	The result may be misinterpreted if the test is not designed to detect the drug suspected by the clinician
Incorrect container	Urine collected in container with acid added (acidified urine is recommended to stabilize certain analytes, such as catecholamines)	Antibody reactivity and enzyme activity can be affected by extreme pH; some drugs are not stable in urine of extreme pH
Incorrect specimen	A specimen has been purposefully substituted by the patient	The result will not accurately reflect the patient's urine drug status
Adulterated specimen	A chemical has been added to interfere with the assay	The results may be negative when drug is present above the threshold concentration
	The specimen has been diluted	The results may be negative when the drug is present above the threshold concentration in the undiluted specimen
	A prescribed – but nonadministered – drug has been added directly to the voided urine specimen	The result may be positive when the patient has consumed all of the drug or diverted the drug

■ Pre-analytical errors

Table 1 summarizes several pre-analytical errors that will affect the accuracy of UDT results. Included among these pre-analytical errors are mistakes in ordering the proper test. A clinician may order a 'urine drug screen,' which may include innumerable combinations of immunoassays, depending on the laboratory, when the clinical question is focused on a specific drug. There are many circumstances, discussed below, in which nonspecific screening tests do not detect the drug(s) of interest, and clinical laboratory personnel are, in general, unaware of the intent of the ordering physician. A system to ensure that laboratory personnel – or, more appropriately, a toxicologist – are aware of the physician's intent would prevent many inappropriate orders and alleviate this common source of pre-analytical error.

■ Analytical errors

Analytical methods approved by the US FDA for *in vitro* diagnostic use have been subjected to extensive validation studies to ensure that their performance meets the high standards set for medical diagnosis and treatment. In the case of urine drug screening immunoassays, which are used both in forensic and clinical laboratories, there are additional performance standards that are required by agencies that license and certify forensic drug-testing services. Part of the validation requirement involves testing the analytical method for potential interferences.

Immunochemical methods for detecting drugs in urine may produce unexpected results due to several analytical interferences:

- The detection antibody may cross-react with a pharmacologically and/or structurally unrelated molecule that possesses a similar antigenic determinant to the target drug;
- The chemical environment may inhibit antigen (i.e., drug or metabolite) binding to the antibody component of the immunoassay;
- A compound may be present that interferes with the chemical label (e.g., enzyme or fluorophore) used to measure drug- or drug metabolite–antibody complex;
- A chemical may be present that interferes directly with the drug or metabolite, either binding to it or destroying it.

■ Cross-reactivity

Cross-reactivity is a phenomenon of immunoassay-based screening tests, in which antibodies directed toward a drug of interest have varying degrees of reactivity toward drugs and/or metabolites with similar chemical structures (discussed earlier), and sometimes with unrelated

chemical structures. There are numerous examples of positive immunoassay screening results due to assay cross-reactivity involving opiates, amphetamines, benzodiazepines, barbiturates, THC and PCP. These constitute genuine false-positive screening test results and, hence, will not be confirmed as positive if analyzed by more specific techniques such as GC–MS. For example, a 2002 report described two cases of unexpected amphetamine-positive urine drug screens (using the cloned enzyme donor immunoassay method) in patients administered the antimalarial drug chloroquine [28]. Confirmatory testing of the specimens using GC with a nitrogen–phosphorous detector and GC–MS identified chloroquine and its major metabolite, desethylchloroquine, but failed to confirm the presence of amphetamine. Subsequent analysis of chloroquine-fortified urine (again, using CEDIA) confirmed that the amphetamine screening method cross-reacted with this drug. An extensive list of reported positive interferences in UDT is presented in **Table 2**.

Confirmatory methods are also subject to analytical interferences. Interferences have been reported with the more recently introduced LC–MS/MS confirmatory methods. Venlafaxine, a commonly prescribed selective serotonin and norepinephrine-reuptake inhibitor, produces a metabolite that is structurally similar to tramadol and co-eluted from the LC column. Furthermore, the venlafaxine metabolite produced a parent/daughter ion pair identical to tramadol [30]. Other LC–MS/MS interferences that have been described include zolpidem mistaken for lysergic acid diethylamide (LSD), benzoylecgonine mistaken for atropine and clomipramine mistaken for several phenothiazines [31].

■ Postanalytical errors

Postanalytical errors can occur in two ways: the information provided by the laboratory is incorrect or the interpretation of valid laboratory results by the clinician is incorrect.

Technology has minimized the potential for erroneous transmission of laboratory results that are not otherwise compromised by pre-analytical or analytical failures. In most laboratories, results pass from the analytical platform to the patient's medical record electronically, minimizing the possibility of a transcription error. There are examples, however, of erroneous laboratory results that have eluded the surveillance mechanisms designed to preclude that possibility [33].

Misinterpretation of valid laboratory results is a postanalytical variable that demands some attention, particularly in UDT. Accrediting agencies require clinical laboratories to report results with relevant reference ranges to aid clinicians in their interpretation of laboratory data. For most laboratory measurements, this requirement involves validation of the appropriate reference interval for the specific test in healthy individuals. However, UDT screening results, which are qualitative rather than quantitative, involve complexities that make their interpretation difficult, and this is a significant source of postanalytical error. These postanalytical errors can occur when the laboratory result is transmitted in a way that does not provide sufficient interpretive information, or when a laboratory result is interpreted incorrectly even when the limitations of the analytical method should be apparent [35].

Potentially inappropriate negative UDT results

■ Limited test specificity

Opiate screening tests, the vast majority of which are immunoassays, are typically designed to detect the presence of the natural opiates morphine and codeine. Due to limited antigenic diversity among the semisynthetic opioids, opiate screening assays predictably cross-react with many of these drugs, with the important exceptions of oxycodone and oxymorphone, which will generally yield opiate-negative screening results [35]. One report described an individual who was dismissed from a medical practice due to suspicion of diverting his prescribed oxycodone. Subsequent confirmatory testing of the purportedly 'negative' urine specimen by GC–MS revealed the presence of oxycodone and a metabolite. The screening immunoassay had minimal cross-reactivity with oxycodone [34].

Unexpected negative screening test results are common for immunoassays designed to detect broad classes of drugs, such as benzodiazepines and opioids, which include many congeneric chemical derivatives designed to modify the pharmacokinetic or pharmacodynamic profile of the drug. The 'benzodiazepine' and 'opiate' screens, therefore, are misnomers since the specificity of immunochemical UDT methods limits the ability of these assays to detecting only certain members of these classes of drugs. Moreover, even when an immunoassay is configured to detect one of the drugs of a certain class, its reactivity for that particular drug may be substantially lower (or higher) than for other drugs

of the same class. Therefore, an important, and possibly common, source of potentially inappropriately negative test results occurs when a drug is administered at therapeutic doses, but fails to produce a positive screening result due to limited reactivity of the immunoassay for the drug or metabolite. Clinicians should understand that most opiate screening assays are designed to detect the natural opiates morphine and codeine. Detection of semisynthetic opioids is broad, although, as noted above, most screens will not detect oxycodone and oxymorphone

Table 2. Selected list of urine drug screen interferences.

Drug	Method	Interference	Ref.
Opiates	EMIT II, AxSYM FPIA, CEDIA, Roche Abuscreen OnLine reagents, Beckman opiate reagents	[+] Quinolones	[58]
	EMIT	[-] Tolmetin	[59]
	Syva RapidTest Genix RapidTech	[+] Rifampin	[59]
	EMIT II	[+] Ofloxacin	[60]
THC	EMIT	[+] Efavirenz	[61]
	EMIT	[+] Ibuprofen, naproxyn	[62]
	GC–MS	[-] Ibuprofen	[63]
	EMIT	[-] Tolmetin	[61]
	EMIT	[+] Pantoprazole	[92]
Cocaine	EMIT, EMIT II	[-] Salicylates	[6.2,65]
	GC–MS	[-] Fluconazole	[29,30]
Amphetamines	EMIT II	[+] Benzphetamine, phenmetrazine, phentermine, ephedrine, mephentermine	[66]
	FPIA	[+] Mephentermine, phenmetrazine, phentermine, phenylpropanolamine, tyramine	[66]
	EMIT	[-] Tolmetin	[59]
	FPIA, GC–MS	[+] Selegiline	[67]
	EMIT	[+] Phentermine	[68]
	EMIT II	[+] Trazodone	[69]
	CEDIA, EMIT II	[+] Bupropion	[70,71]
	FPIA	[+] Fluorescein	[13]
	EMIT	[+] Ciprofloxacin, mefanamic acid, metronidazole, tolmetin	[13]
	EMIT II Plus	[+] Phenothiazines	[72,73]
	Bio-Quant amphetamine ELISA	[+] Phentermine, phenylethylamine	[74]
	Bio-Quant methamphetamine ELISA	[+] Ephedrine, pseudoephedrine	[74]
	EMIT II Plus	[+] Pseudoephedrine	[75]
	Biosite Triage	[-] Chlorpromazine metabolites	[76]
Benzodiazepines	EMIT d.a.u. FPIA CEDIA	[+] Oxaprozin	[77]
	FPIA	[+] Fenoprofen, flurbiprofen, indomethacin, ketoprofen, tolmetin	[57,78]
Methadone	Integra Methadone II	[+] Quetiapine	[79,80]
	Integra Methadone II	[+] Cyamemazine, levomepromazine, possible olanzapine	[81]
Buprenorphine	CEDIA	[+] Morphine, [+] methadone, [+] codeine, [+] dihydrocodeine	[82–84]

CEDIA: Cloned Enzyme Donor Immunoassay (Microgenics Corporation); EMIT: Enzyme-Multiplied Immunoassay Technique (Dade Behring Incorporated); FPIA: Fluorescence polarization immunoassay; Positive [+] indicates an interference resulting in false positive results; negative [-] indicates interference resulting in false-negative results.

due to the presence of a 14-hydroxyl group [13]. The synthetic opioids (e.g., meperidine, methadone, propoxyphene and fentanyl) will not yield positive opiate screening assays.

Most benzodiazepine immunoassays cross-react with the spectrum of prescription benzodiazepines, but there are notable exceptions. The Neogen benzodiazepines assay is poorly cross-reactive with lorazepam (0.16%), triazolam (1.1%) and oxazepam (2.1%) [35]. The immunalysis (ELISA) benzodiazepines assay displays poor cross-reactivities with clonazepam (8.3%), prazepam (8.3%) midazolam (9%), triazolam (10.5%) and lorazepam (13.8%) [35]. Further complicating interpretation of benzodiazepine UDTs, some assays are poorly reactive with certain benzodiazepines, but exquisitely reactive to their metabolites. As an example, the Immunalysis benzodiazepine UDT is poorly cross-reactive both with clonazepam and prazepam. Clonazepam has no metabolites that significantly cross-react with this assay, but prazepam is metabolized to nordiazepam, which displays exquisite (150%) cross-reactivity with the Immunalysis assay. Thus, correct interpretation of a potentially inappropriate negative benzodiazepine UDT requires knowledge of benzodiazepine metabolism, in addition to the specifications of the particular assay **(Figure 2)**.

Potentially inappropriate negative test results in an individual who has been prescribed a drug that belongs to a large class of similar drugs may be due to limited reactivity of the immunoassay for that particular drug. **Table 3** summarizes typical cross-reactivity data obtained from the product information supplied by the manufacturers of several common UDT immunoassays. Complete cross-reactivity data is published in UDT assay package inserts, but the format is not uniform. Some product inserts list the concentrations of cross-reactive compounds necessary to produce positive results, whereas other assays present cross-reactivity data as a percentage of the threshold concentration of the compound to which the assay is calibrated. Laboratories offering UDT services to clinicians should make this information readily available, either electronically or in the interpretive comments included with laboratory reports.

■ Drug is absent from the urine

Both screening and confirmatory UDTs should yield negative test results if the drug of interest is not being administered. The differential diagnosis for a negative test result includes: lack of recent administration due to symptom abatement (or resolution), unacceptable side effects, inability to afford the medication and hoarding of the prescribed drug in order to be assured of a future supply for medical or nonmedical (e.g., abuse, addiction or diversion) purposes. Each of these scenarios will prompt a clinical decision whether or not to continue prescribing a drug that was, and perhaps remains, intended to

Table 3. List of analytes commonly detected by commercial immunoassays*.

Assay	Analytes
Amphetamines	Amphetamine, ephedrine, methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, phentermine, phenylpropanolamine, pseudoephedrine
Barbiturates	Amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital, secobarbital, thiopental
Benzodiazepines	Alprazolam, α-hydroxyalprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, flunitrazepam, flurazepam, lorazepam, midazolam, nordiazepam, oxazepam, temazepam, triazolam, α-hydroxytriazolam
Cannabinoids	Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid
Cocaine	Benzoylecgonine, cocaine, cocaethylene, ecgonine, ecgonine methyl ester
Methadone	L-α-acetyl-methadol, methadone
Methaqualone	Hydroxymethaqualone, methaqualone
Opiates	Codeine, dihydrocodeine, hydrocodone, hydromorphone, morphine, morphine-glucuronide
Phencyclidine	Phencyclidine, phencyclidine analogs
Propoxyphene	Norpropoxyphene, propoxyphene

**This list is not all-inclusive. Refer to assay package inserts for a complete list of analytes commonly detected by commercial immunoassays.*
Data from [85].

alleviate the patient's symptoms. Nonadherence with a prescribed therapeutic regimen may be benign, or it may indicate aberrant drug-related behavior that jeopardizes the patient's health as well as the physician–patient relationship. Potentially inappropriate negative test results have a significant impact on patient care, because physicians expect that patients will adhere to the treatment they offer. When a prescribed drug appears not to have been administered, the clinician's expectation is violated and the reason for the negative test result should be pursued.

■ Drug is present in the urine but at a concentration below the established assay cut-off

Both screening and confirmatory assays may yield negative test results if the drug in question is present, but at a concentration below the designated cut-off. Low concentrations of a drug or metabolite in the urine may result from a variety of causes, both behavioral and metabolic. Behavioral explanations include some of the causes listed in the preceding section as well as specimen manipulation (discussed below), which can effectively reduce the concentration of a drug or metabolite to a concentration below the reporting threshold [36]. Metabolic factors may also account for the presence of drug below the designated cut-off, due either to pharmacologic induction or, less commonly, genetic polymorphisms. The CYP450 system plays a significant role in the metabolism of most opioids. One of the most important isoenzymes involved in the metabolism of opioids is CYP3A4, which acts on several of these drugs, including the fentanils, hydrocodone, meperidine and oxycodone.

The concentration thresholds above which UDT results are reported as 'positive' vary by assay, and are based primarily on considerations relevant to forensic drug testing applications. In high-throughput forensic drug testing laboratories, false-positive results waste resources, because confirmatory testing by GC–MS is expensive and time consuming. Therefore, an incentive exists to establish positive thresholds high enough to minimize false-positive results. However, the principal aim of workplace drug testing programs is to identify illicit drug use, and higher thresholds will classify as negative many urine specimens containing drug or drug metabolite. Balancing these two objectives requires establishing a concentration threshold that will minimize false-positive screening results, while ensuring an acceptable likelihood that a drug user will test positive. As an example, the SAMHSA concentration threshold for cannabinoids (marijuana metabolites) was originally established at 100 µg/l, but was lowered to 50 µg/l when studies showed a 23–53% increase in the number of true (confirmed) positive screening results at the lower (50 µg/l) threshold [37]. Other studies have demonstrated similar increases in true-positive results at lower screening thresholds for cocaine metabolite [38] and opiates [39]. Conversely, the SAMHSA-specified threshold for opiates was increased from its original 300 µg/l to 2000 µg/l to minimize positive results due to poppy seed ingestion [40].

There is evidence that UDT screening immunoassays have sufficient sensitivity to detect subthreshold concentrations of drugs and/or metabolites [40]. However, commercially available UDT methods are typically calibrated to the SAMHSA-specified concentration thresholds (there are exceptions: screening methods for barbiturates, benzodiazepines, cannabinoids and opiates are available at multiple cut-off concentrations) and changing those thresholds requires recalibration of the immunoassay with calibrators prepared specifically for that purpose. For many laboratories, making this type of modification may be impractical.

■ Pharmacologic induction

Several drug classes, including antiretrovirals, anticonvulsants and antibiotics (specifically rifampin), are capable of CYP450 enzyme induction, causing rapid metabolism of opioids and sometimes resulting in negative screening and confirmatory assays, unless specific metabolites are sought and detected. Methadone has recently been recognized as an important CYP2B6 substrate [41] and phenobarbital is a strong CYP2B6 inducer [42]. Consequently, patients co-administered methadone and phenobarbital may experience accelerated methadone metabolism and opioid withdrawal [43] and may yield methadone-negative urine drug screens. In these cases, administration of the drug can be verified with an immunoassay screen for the methadone metabolite 2-ethylidene-1,5-dimethyl 3,3-diphenylpyrrolidine (EDDP) [44].

Oxycodone is a CYP3A4 substrate and rifampin, an antibiotic, is a potent CYP3A4 inducer. A recent case report described an individual administered oxycodone (40–60 mg/day) and rifampin, who repeatedly produced negative test results for oxycodone by GC–MS. Detection of the metabolites noroxycodone

and oxymorphone in the urine confirmed the patient's adherence with the opioid regimen [45]. Rifampin-induced CYP3A4 induction may also result in the ultrarapid metabolism of fentanyl [46] and it is speculated that all CYP3A4 substrates, including buprenorphine, codeine, hydrocodone and meperidine, may be affected.

Nonprescription drugs can also induce the CYP system. St John's Wort, a popular herbal preparation for the treatment of depression, is a potent CYP3A4 inducer and has been reported to speed up the metabolism of methadone [47]. In addition, the FDA recently released a consumer report warning about tainted OTC weight loss products with active pharmaceutical ingredients, including the CYP3A4 inducer, phenytoin [102].

■ Genetic polymorphism

Genetic variations in CYP oxidase enzymes can have dramatic effects on drug metabolism and have given rise to pharmacogenomic approaches to therapy, most notably involving the vitamin K antagonist coumadin (warfarin). A recent case report described an individual whose urine (by witnessed collection) screened negative for methadone by radioimmunoassay (Roche Abuscreen), despite the verified administration of 60 mg/day of methadone. Genetic testing revealed that the patient was heterozygous for the *CYP3A5(*)1* allele, which has been associated with very high levels of CYP3A4, an enzyme believed to play a role in methadone metabolism. Subsequent evaluation of urine samples by high-performance liquid chromatography revealed very low concentrations of methadone, but high concentrations of its primary metabolite EDDP [48].

■ Specimen manipulation

There are a variety of *in vivo* and *in vitro* techniques designed to defeat drug screens by means of specimen dilution, substitution or adulteration. Dilution, intended to reduce the drug and/or drug metabolite concentrations to below the specified positive threshold, can be accomplished *in vivo* by the oral or parenteral administration of large volumes of fluid with or without the coadministration of diuretics or by the administration of commercial 'body cleansers' (e.g., XXTra Clean, Green Clean or Liquid Stuff). Dilution can be performed *in vitro* by addition of water or another fluid with a urine-like appearance to the urine specimen. A 'clean' urine specimen from human, animal, or synthetic (e.g., Dr John's Famous Pee Pee, Quick Fix or Sub-Solution) sources can be substituted for a legitimate urine specimen. In witnessed urine collections, substitution can occur by a variety of methods, including the use of prosthetic devices (e.g., the Whizzinator 5000), or by retro-catheterization, in which the urinary bladder is filled with 'clean' urine via a transurethral catheter. A recent systematic review of tampering methods, including a comprehensive review of adulterants, their substrates and specific vulnerable assays, was published by Jaffee *et al.* [49].

Adulteration of UDT specimens includes a variety of methods designed to:

- Degrade the drug and/or metabolite of interest, for example oxidizing agents such as peroxide/peroxidase (Stealth), pyridinium chlorochromate (Urine Luck) and nitrite (Klear);
- Bind with the drug or metabolite of interest (papain);
- Interfere with the assay, such as glutaraldehyde (Instant Clean ADD-IT-ive).

Manipulation of a urine specimen can be detected by several methods of specimen validity testing. Typical freshly-voided human urine has the following characteristics:

- Temperature: 90–100°F (32–38°C) within 4 min of voiding;
- pH: 4–9 (<9.5 if stored at room temperature or higher for 1-2 days) [50];
- Creatinine concentration: 20–250 mg/dl;
- Specific gravity: 1.003–1.020.

Manipulated urine specimens can be classified in the following ways [51] in regulated forensic urine drug testing programs:

- Dilute: creatinine concentration is between 2 and 20 mg/dl and specific gravity is between 1.001 and 1.003;
- Adulterated: the pH is less than 3 or at least 11; the nitrite concentration is more than 500 µg/ml and/or the chromium(VI) concentration is at least 50 µg/ml; the halogen (e.g., iodine, fluoride or bleach) at aforementioned nitrite or chromium(VI)-equivalent cutoff or pyridinium chlorochromate at aforementioned nitrite or chromium(VI) equivalent cutoff; the surfactant concentration is 100 µg/ml dodecylbenzene sulfonate-equivalent cutoff or greater; glutaraldehyde, or any other adulterant if detected at any concentration;

- Substituted: creatinine concentration is 2 mg/dl or less and the specific gravity is 1.0010 or less or at least 1.0200;
- Invalid: creatinine concentration is 2 mg/dl or less and the specific gravity is at least 1.0010 and 1.0200 or less, or creatinine concentration is at least 2 mg/dl and the specific gravity is 1.0010 or less.

In addition to laboratory specimen validity and adulterant testing, there are a number of point-of-care devices [52] available that assess pH, creatinine concentration and specific gravity, as well as the presence of adulterants such as glutaraldehyde [53], nitrites [54] and pyridinium chlorochromate [55]. In a perpetual game of cat-and-mouse, the manufacturers of adulterants are continually reformulating their products as the proprietary ingredients are discovered and adulterant tests are developed for their detection. Thus, for example, Klear (nitrite) has been replaced by NuKlear, the constituents of which are thus far undetermined. Peroxidase, which oxidizes 11-nor-Δ^9-THC-9-carboxylic acid (THCA) and, to a lesser degree, morphine, breaks down within hours to days and becomes undetectable [13]. Papain is capable of reducing urine concentrations of THCA (and perhaps nordiazepam), but does not generally render specimens invalid and is not routinely detected in clinical or forensic laboratories; papain may be capable of defeating both immunoassays (enzyme multiplied immunoassay and fluorescence polarization immunoassay) and GC–MS [55]. Unlike forensic drug-testing laboratories, clinical laboratories performing UDT do not routinely assess specimen validity by testing for adulterants, so adulterated samples are likely to go undetected.

Laboratory error

A variety of pre-, intra- and post-analytical errors can lead to potentially inappropriate negative UDT results (please see the subsection on laboratory error in the section on potentially inappropriate positive UDT results). Negative interference is a less common cause of potentially inappropriate negative UDT results.

■ Cross-reactivity

Rare examples exist of negative interference in immunoassay screens **(Table 2)**. For example, tolmetin, a nonsteroidal anti-inflammatory drug, has a high molar absorptivity at 340 nm, the wavelength used in EMIT assays. Analysis of the urine of individuals administered tolmetin results in depressed milliabsorbance (δ A) relative to calibrators. When tolmetin samples are mixed with samples containing opiates or cannabinoids, negative screens have been reported [56]. Fluconazole has been reported to produce a derivative that co-elutes with with the derivatized cocaine metabolite trimethylsilyl-benzoylecgonine, prohibiting confirmation of a positive cocaine immunoassay screening result [29,30].

Conclusion

Clinical UDT, particularly for the purpose of monitoring patients who are prescribed controlled substances, is an unfamiliar endeavor for most physicians. Physicians are typically uninformed about this area of clinical laboratory practice and often assume that interpretation is as simple as accepting the positive or negative test result of a laboratory report. UDT, however, poses significant challenges in laboratory test interpretation. Clinical UDT is a relatively new field and is fraught with uncertainties. Competent interpretation of UDT results requires knowledge of patient behaviors, including the dose, frequency and pattern of drug use for several days prior to the test, as well as an awareness of all prescription, OTC and herbal drugs and nutritional supplements that may influence test results. Drug metabolism, including genetic and environmental influences, interconversions between drug metabolitess and the characteristics and limitations of the analytical methods designed to detect drugs are all important considerations when interpreting UDT results. Responsible clinical use of UDT requires synthesis of this information, which can be achieved only through close communication between physicians and qualified laboratory professionals.

Future perspective

Urine drug testing is a relatively new aspect of clinical medicine, with heretofore limited penetration. It will play an increasing role as physicians attempt to satisfy the dual imperatives of treating chronic pain, which often requires the prescription of opioid analgesics, while minimizing the abuse and diversion of these medications.

Urine as a testing matrix has several advantages: simplicity, relative low cost, noninvasive specimen collection, plentiful supply of a concentrated ultrafiltrate of plasma and decades of

accumulated knowledge with regard to drug–metabolite excretion and assay interpretation. It also has important limitations, such as a relatively narrow temporal window for drug detection (generally hours to days) and vulnerability to tampering. To mitigate these limitations, urine will be complemented, although not replaced, by other testing matrices such as hair, oral fluid and sweat.

As clinical urine drug testing becomes more common, challenges – legal and other – to 'unfavorable' test results will prompt a shift toward the handling of specimens in a forensically defensible manner (e.g., standardization in collection and testing and chain of custody for specimen handling).

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Executive summary

- Potentially inappropriate positive urine drug test results can result from:
  - Metabolic conversions of prescription-controlled substances, including amphetamines, benzodiazepines and opioids to other prescription controlled substances.
  - Certain foods and beverages or prescription, over-the-counter and herbal medications.
  - Pre-, intra- and post-analytical laboratory error.
- Potentially inappropriate negative urine drug test results can result from:
  - Limited specificities (cross-reactivities) of certain members of drug classes with screening immunoassays.
  - Absence of drug in the urine secondary to lack of administration due to benign (e.g., symptom abatement) or malignant (e.g., drug diversion) reasons.
  - Presence of drug in the urine but below reporting threshold, due to lack of recent drug administration or rapid metabolism secondary to induction of cytochrome P450 metabolizing enzymes or genetic polymorphisms.
  - Urine specimen manipulation through *in vivo* (e.g., aggressive hydration) or *in vitro* (adulteration) techniques.
  - Pre-, intra- and post-analytical laboratory error.

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