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.

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. Clinicians, on the other hand, generally describe this as a false-positive result, 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
LC–MSn methods are often used for identifying organic compounds. GC–MS and related methods are considered the most specific method of measurement by MS, and involve vaporization of the analyte, isolation by GC and GC–MS methods are 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 methods are often used to confirm the presence of drugs or metabolites in biological matrices.

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.

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 non-prescribed 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 nonlicit 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 (CYP2D6) 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).

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-acetylcodine (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 it 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...
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,
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 (Δ9-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-Δ9-tetrahydrocannabinol (Δ9-THC-COOH), whereas the latter will yield neither positive screening nor positive confirmatory assays [18]. A natural pharmaceutical product, Δ9-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 Δ9-THC-COOH, respectively. Δ9-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 stereochanical 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].
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.

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

- 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
Postanalytical errors of reported positive interferences in UDT is cross-reacted with this drug. An extensive list confirmed that the amphetamine screening method for lysergic acid diethylamide (LSD), benzoylecgonine mistaken for atropine and clomipramine mistaken for several phenothiazines that possibility surveillance mechanisms designed to preclude error. There are examples, however, of erroneous laboratory results that have eluded the 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.

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. 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.

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 inappropriate 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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Method</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opiates</td>
<td>EMIT II, AxSYM FPIA, CEDIA, Roche Abuscreen OnLine reagents, Beckman opiate reagents</td>
<td>[-] Quinolones</td>
</tr>
<tr>
<td></td>
<td>EMIT</td>
<td>[-] Tolmetin</td>
</tr>
<tr>
<td></td>
<td>Syva RapidTest Genix RapidTech</td>
<td>[+} Rifampin</td>
</tr>
<tr>
<td></td>
<td>EMIT II</td>
<td>[+} Ofloxacin</td>
</tr>
<tr>
<td>THC</td>
<td>EMIT</td>
<td>[+} Elavirenz</td>
</tr>
<tr>
<td></td>
<td>EMIT</td>
<td>[+} Ibuprofen, naproxyn</td>
</tr>
<tr>
<td></td>
<td>GC–MS</td>
<td>[-] Ibuprofen</td>
</tr>
<tr>
<td></td>
<td>EMIT</td>
<td>[-] Tolmetin</td>
</tr>
<tr>
<td></td>
<td>EMIT</td>
<td>[+} Pantoprazole</td>
</tr>
<tr>
<td>Cocaine</td>
<td>EMIT, EMIT II</td>
<td>[-] Salicylates</td>
</tr>
<tr>
<td></td>
<td>GC–MS</td>
<td>[-] Fluconazole</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>EMIT II</td>
<td>[+} Benzphetamine, phenmetrazine, phentermine, ephedrine, mephentermine</td>
</tr>
<tr>
<td></td>
<td>FPIA</td>
<td>[+} Mephentermine, phenmetrazine, phentermine, phenylpropanolamine, tyramine</td>
</tr>
<tr>
<td></td>
<td>EMIT</td>
<td>[-] Tolmetin</td>
</tr>
<tr>
<td></td>
<td>FPIA, GC–MS</td>
<td>[+} Selegiline</td>
</tr>
<tr>
<td></td>
<td>EMIT</td>
<td>[+} Phentermine</td>
</tr>
<tr>
<td></td>
<td>EMIT II</td>
<td>[+} Trazodone</td>
</tr>
<tr>
<td></td>
<td>CEDIA, EMIT II</td>
<td>[+} Bupropion</td>
</tr>
<tr>
<td></td>
<td>FPIA</td>
<td>[+} Fluorescein</td>
</tr>
<tr>
<td></td>
<td>EMIT</td>
<td>[+} Ciprofloxacin, mefanamic acid, metronidazole, tolmetin</td>
</tr>
<tr>
<td></td>
<td>EMIT II Plus</td>
<td>[+} Phenothiazines</td>
</tr>
<tr>
<td></td>
<td>Bio-Quant amphetamine ELISA</td>
<td>[+} Phentermine, phenylethylamine</td>
</tr>
<tr>
<td></td>
<td>Bio-Quant methamphetamine ELISA</td>
<td>[+} Ephedrine, pseudoephrine</td>
</tr>
<tr>
<td></td>
<td>EMIT II Plus</td>
<td>[+} Pseudoephrine</td>
</tr>
<tr>
<td></td>
<td>Biosite Triage</td>
<td>[-] Chlorpromazine metabolites</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>EMIT d.a.u.</td>
<td>[+} Oxaprozin</td>
</tr>
<tr>
<td></td>
<td>FPIA</td>
<td>[+} Fenoprofen, flurbiprofen, indomethacin, ketoprofen, tolmetin</td>
</tr>
<tr>
<td>Methadone</td>
<td>Integra Methadone II</td>
<td>[+} Quetiapine</td>
</tr>
<tr>
<td></td>
<td>Integra Methadone II</td>
<td>[+} Cyamemazine, levomepromazine, possible olanzapine</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>CEDIA</td>
<td>[+} Morphine, [+} methadone, [+} codeine, [+} dihydrocodeine</td>
</tr>
</tbody>
</table>

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%) [38]. The immunoanalysis (ELISA) benzodiazepines assay displays poor cross-reactivities with clonazepam (8.3%), prazepam (8.3%) midazolam (9%), triazolam (10.5%) and lorazepam (13.8%) [38]. 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>
<thead>
<tr>
<th>Table 3. List of analytes commonly detected by commercial immunoassays*.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay</td>
<td>Analytes</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>Amphetamine, ephedrine, methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, phentermine, phenylpropanolamine, pseudoephedrine</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital, secobarbital, thiopental</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Alprazolam, α-hydroxyalprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, flunitrazepam, flurazepam, lorazepam, midazolam, nordiazepam, oxazepam, temazepam, triazolam, α-hydroxytriazolam</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>Δʻ-tetrahydrocannabinol, 11-hydroxy-Δʻ-tetrahydrocannabinol, 11-nor-Δʻ-tetrahydrocannabinol-9-carboxylic acid</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Benzylecgonine, cocaine, cocaethylene, ecgonine, ecgonine methyl ester</td>
</tr>
<tr>
<td>Methadone</td>
<td>α-acetyl-methadol, methadone</td>
</tr>
<tr>
<td>Methaqualone</td>
<td>Hydroxymethaqualone, methaqualone</td>
</tr>
<tr>
<td>Opiates</td>
<td>Codeine, dihydrocodeine, hydrocodone, hydromorphone, morphine, morphine-glucuronide</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>Phencyclidine, phencyclidine analogs</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>Norpropoxyphene, propoxyphene</td>
</tr>
</tbody>
</table>

*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 fentanyl, 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 fentanyl \[46\] and it is speculated that all CYP3A4 substrates, including buprenorphine, codeine, hydrocodone and meperidine, may be affected.

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 radiommunoassay (Roche Abuscreen), despite the verified administration of 60 mg/day of methadone. Genetic testing revealed that the patient was heterozygous for the \textit{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 \textit{in vivo} and \textit{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 \textit{in vivo} by the oral or parenteral administration of large volumes of fluid with or without the co-administration of diuretics or by the administration of commercial ‘body cleansers’ (e.g., XXTra Clean, Green Clean or Liquid Stuff). Dilution can be performed \textit{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 \textit{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 at least 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 available that assess pH, creatinine concentration and specific gravity, as well as the presence of adulterants such as glutaraldehyde, nitrites and pyridinium chlorochromate. 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-Δ⁹-THC-9-carboxylic acid (THCA) and, to a lesser degree, morphine, breaks down within hours to days and becomes undetectable. 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. 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. Fluconazole has been reported to produce a derivative that co-elutes with the derivatized cocaine metabolite trimethylsilylbenzoylecgonine, prohibiting confirmation of a positive cocaine immunoassay screening result.

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 metabolites 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).

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patients received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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.

Bibliography
Papers of special note have been highlighted as:
• of interest
•• of considerable interest

• Recent and comprehensive review of the field of benzodiazepine metabolism, will be of interest to those tasked with interpreting the presence of the (sometimes) multiple benzodiazepine metabolites present in the urine of individuals prescribed benzodiazepine therapy.
•• Reference text for medical review officers in the context of US federally mandated workplace drug-testing programs, it offers a wealth of information on all aspects of urine drug testing, most of which is relevant to the clinical arena.
False-positive & false-negative test results in clinical urine drug testing

- Current and comprehensive review of urine-tampering techniques.
- US government document addressing every aspect of urine drug testing in the context of federally mandated workplace drug-testing programs. Much of this information will be of interest to clinicians and laboratorians involved in clinical urine drug testing.
- Most recent and comprehensive review of adulterants and their implications for urine drug-test interpretation.
- Gottesman L. Sustiva may cause false positive on marijuana test. WORLD 96, 7 (1999).


**Websites**

101 Sativex monograph
- [www.omco.pd.it/news/12–06/sativex.pdf](http://www.omco.pd.it/news/12–06/sativex.pdf)
- Accessed 4 May 2009

102 Tainted weight loss pills flagged as health risks. US FDA
- [www.fda.gov/consumer/updates/weightloss_pills122908.html](http://www.fda.gov/consumer/updates/weightloss_pills122908.html)
- Accessed 19 February 2009