Advanced Urine Toxicology Testing

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ABSTRACT. Urine toxicology screening testing is an important standard of care in the addiction and pain treatment setting, offering a reproducible, unbiased, and accurate laboratory test to monitor patients and provide objective support for clinical observations. It has been shown that physicians do not have proficiency in the ordering or interpretation of these tests. This article is an attempt to respond to that need. Current antibody-based enzymatic immunoassays (EIAs) used for urine toxicology screening are useful to detect classes of drugs (ex., opiate) but cannot determine which specific drug (ex., morphine) is present. Gas chromatography and mass spectroscopy can determine exactly which drugs are present, allowing prescribed (or illicit) opiates and benzodiazepines to be identified. This article will discuss principles and details of opiate and benzodiazepine EIA and gas chromatography and mass spectroscopy urine toxicology testing. The approach to detecting patients attributing positive opiate EIAs to prescription opiates who are using heroin or other opioids will be reviewed. Cases of controlled prescription drugs that do not produce the expected positive urine tests (ex., oxycodone producing negative opiate screening tests) will be discussed. How to differentiate codeine from heroin and the role of poppy seeds in toxicology will be examined. The case of an anti-depressant drug that produces false-positive benzodiazepine results and antibiotics that cause positive opiate urine toxicology results will be reviewed. Common benzodiazepines (ex., clonazepam and lorazepam) that do not reliably produce positive benzodiazepine EIAs will be discussed. The approach to detection and management of all these types of toxicology cases will be reviewed, and it is hoped that the analyses presented will impart an adequate information base to medical providers and staff members of drug treatment and pain centers, enabling them to order and interpret these tests in the clinic more effectively as an integrated part of whole patient care.

KEYWORDS. Gas chromatography, mass spectroscopy, urine toxicology, opiate, benzodiazepine, morphine, codeine, EIA, ELISA, EMIT, fluoroquinolone, sertraline, drug screening, lorazepam, clonazepam

INTRODUCTION

Rational for Drug Monitoring

Three basic reasons are used to consider urine drug monitoring in patients who are in drug treatment or who are prescribed opioids or benzodiazepines. The first reason is to reassure medical providers that the patients who are prescribed opioids and benzodiazepines are taking medication as directed, evidenced by positive results in urine drug testing.1–3 The second reason is that urine drug testing is used to detect possible diversion of medication (i.e., stockpiling or selling of controlled prescribed substances to unauthorized others), evidenced by negative urine drug tests. The overdose mortality rate in the United States due to diverted prescribed opioid analgesics as reported on death certificates increased dramatically (by 91.2%) from...
1999 to 2002, indicating that “a national epidemic of drug poisoning deaths began in the 1990’s and prescriptions for opioid analgesics contributed to the increases in drug poisoning deaths.” The most common opioids implicated in overdose deaths were, in order of frequency of occurrence, methadone, fentanyl, oxycodone, hydromorphone, hydrocodone, and morphine and were obtained by overdose victims illegally from patients with legitimate prescriptions; therefore, it is critical for providers to ensure that patients prescribed these medications are taking and not diverting them. In addition to patient history and clinical presentation, random urine drug testing is an essential and objective part of this monitoring process.

The third reason is to detect the presence of illicit non-prescribed drugs, such as heroin, cocaine, methamphetamine, phencyclidine, non-prescribed opioids, and non-prescribed benzodiazepines, according to community prevalences, the presence of which suggests that patients may not be entirely responsible to manage prescribed opioids and benzodiazepines and need further therapeutic intervention.

The discussion in this article will be limited to discussing two common classes of drugs that are widely abused and are widely prescribed by physicians and other medical providers: benzodiazepines and opioids. How urine drug testing can be used to differentiate prescribed versus illicit drugs of these classes and to contribute to the medical and psychosocial care of these patients will be reviewed.

**Enzyme-Immuno-Assay Testing**

The most common drug screening techniques are antibody-based, enzyme-mediated immunoassays. These are inexpensive, automated, rapid, and accurate. Various tests such as Enzymatic Immunoassay (EIA), Enzyme Mediated Immunoassay Technique (EMIT), Enzyme Linked Immunosorbent Assay (ELISA), and Cloned Enzyme Donor Immuno-Assay (CE-DIA) refer to the same basic process: a specific anti-drug antibody is added to the patient’s urine and, if the specific drug is present, the antibody binds to the drug, enabling a measurable indicator reaction to occur that is reported as “positive.” For simplicity, in this article, the term enzymatic immunoassay (EIA) will be used to represent all these antibody-mediated urine toxicology tests.

The opiate EIA uses an anti-morphine antibody to detect opiates; and the Benzodiazepam EIA uses an anti-diazepam antibody to detect benzodiazepines. Because these antibodies are specific to morphine and diazepam, there will be both false-negative and false-positive results with non-morphine opioids (ex., fentanyl) and non-diazepam benzodiazepines (ex., clonazepam), which may confuse the clinical picture and suggest diversion. These issues will be detailed in sections below. Although EIAs are reproducible, inexpensive, and easy to perform, a disadvantage of EIA testing is that only the class of drug is identified (ex., opiate or benzodiazepine) and not the specific drug (ex., codeine or diazepam); further testing, explained in the next section, is required to make a specific identification.

**Gas Chromatography and Mass Spectroscopy Testing**

To determine specific drugs and concentrations, gas chromatography followed by mass spectroscopy is used. These tests are typically used in sequence. Simplistically, the urine specimen is vaporized and injected into a port on the gas chromatograph where the sample is dispersed within a carrier gas that travels upward on a chemically treated column. Lighter molecules in the specimen travel up faster and heavier ones more slowly; hence, separation occurs. As the molecules reach top, an analyzer records concentrations as separate peaks on a graph. Next, each peak is sent through the mass spectroscopy, which bombards the component with electrons, creating a predictable and reproducible microexplosion resulting in an equally reproducible pattern of molecular strikes on a recording plate creating energy peaks viewed as a mass spectrograph. These spectrograph patterns are compared by computer to a database of thousands of mass spectrographs and the specific component is identified (ex., morphine or diazepam).

In short, gas chromatography separates and quantifies drug components and then mass
spectroscopy specifically identifies them. Gas chromatography followed by mass spectroscopy is considered the “gold standard” of urine toxicology testing because it is 99% specific and 99% sensitive. The use of EIA and gas chromatography and mass spectroscopy urine testing to monitor various commonly prescribed opioids and benzodiazepines is the subject of this article and is presented below.

Pain clinic chronic opioid patients have been shown to submit significant numbers of abnormal urine toxicology tests, defined as the presence of illicit substances, excess levels of the prescribed substance, or the absence of the prescribed substance. In one study of 470 pain center patients, 44% abused substances, 20% used illicit non-opioid substances, 14% used additional opioids not prescribed, and 10% did not use the opiate that was prescribed. In another pain center, testing of 196 patients found 32% to be mismanaging drugs; 60% of these were using non-prescribed opiates or diverting opiates and 40% of the 196 patients were primarily abusing cocaine, with 11% of patients not taking the prescribed opiate medication. In a review of several pain clinics, 25% to 44% of patients were found to be abusing various substances or diverting prescribed opioids, and this pattern of drug use was closely correlated with specific behaviors, including repeated loss of prescriptions, excess pill consumption, visits without appointments, frequent telephone calls to the clinic, and multiple drug intolerances or “allergies,” behaviors of which prescribing physicians should be fully aware of and responsive to.

In the primary care setting, a study of 801 daily opioid patients managed by family care physicians showed 24% to be using illicit substances.

Accurate self-report of drug abuse and misuse in these populations can be unreliable. In a study of 110 patients in a methadone clinic, 21% reported illicit substance use in the month under study, yet toxicology demonstrated twice as many (39%) to be using illicit drugs. A study of more than 800 chronic opiate patients found 24% to be using illicit substances, yet 46% of these patients adamantly denied substance abuse, even when guaranteed anonymity. Therefore, urine drug testing has become indispensable as an objective, accurate, and reproducible aid in patient monitoring patients prescribed opioids or benzodiazepines.

**Frequency of Urine Drug Testing**

In pain management settings, it is recommended that urine drug screening be considered at every clinic visit but actually collected on a random basis. Urine collection should be random to eliminate patients’ feelings of being singled out or victimized and to assure collection of a reasonably representative set of toxicology specimens over time, yielding a toxicology profile to assist in evaluating the individual patient. Urine drug tests are not intended to be the sole parameter by which patient management decisions are made; rather, testing is only a small part of the greater whole of patient medical and psychosocial care and should be viewed in that context. For methadone treatment programs, federal government regulations mandate weekly urine drug testing for the first 90 days in treatment; after this period, health care providers may use clinical judgment to determine whether weekly testing should continue or if less frequent testing is warranted as long as 8 urine toxicology specimens are submitted annually; times of heightened patient stress or drug relapse may call for increased frequency of urine testing back to weekly. Ultimately, it is the medical providers’ judgment in conjunction with the patients’ clinical status that should determine the urine testing schedule.

**Physician Awareness**

To be useful, urine toxicology test results must be interpreted properly; however, studies show that primary care physicians are not proficient at this; none of 80 physicians in one study answered all questions correctly on a brief yes-or-no rudimentary toxicology questionnaire, and only 20% answered half or more questions correctly. In a second study, none of 114 physicians answered all questions correctly and only 20% answered half or more questions correctly. In the medical providers’ judgment in conjunction with the patients’ clinical status that should determine the urine testing schedule.
summaries presented later in this article hope to address this deficit.

**DRUGS OF INTEREST**

**Oxycodone and Opiate EIA Testing**

In a study of 52 gas chromatography and mass spectroscopy-confirmed oxycodone positive urine specimens, only 30% were determined to be positive by opiate EIA (i.e., the standard opiate EIA does not detect oxycodone in the majority of oxycodone specimens). In an review of 8 commercially available opiate EIAs, the rates of detection of codeine, morphine, and semi-synthetic opiates were as follows: morphine (100%), codeine (100%), oxymorphone (≤15%), meperidine (≤3%), hydromorphone (≤60%), levorphanol (≤29%), and oxycodone (≤12%). This demonstrates that the standard opiate EIA is not designed to and will not detect 100% of semi-synthetic opiates, including oxycodone.

Thus, oxycodone produces mostly negative opiate EIAs. In a survey of 359 primary care physicians, only 12% knew that oxycodone is usually not detected by most opiate EIA screening immunoassays. Oxycodone is a “semi-synthetic” opioid molecule, synthesized by the chemical addition of varying side-chains to a morphine molecule. The side-chains alter the morphine structure to the extent that the morphine antibody of the opiate EIA is unable to reliably bind to or “react” with oxycodone, resulting in frequent negative opiate EIAs.

Patients taking oxycodone submitting the expected negative opiate EIA urine in approximately 90% of specimens may, because of physician unawareness, stand accused of medication diversion and undergo sanctions, denial of medication, or clinic discharge. In one report, the authors state:

We recently encountered a patient we suspected of abusing or misusing prescribed OxyContin® (oxycodone). In order to determine whether the patient was taking the MEDICATION as prescribed, we ordered a urine-based immunoassay drug screen (Opiate EIA). The results were negative; the patient appeared to not have oxycodone in his system. Based on these results, we dismissed the patient from our practice.

If these providers were aware that oxycodone and other semi-synthetics produced a significant amount of negative standard opiate EIAs, as seen in this case, and that an EIA specific for oxycodone was available, the patient would not have experienced sanctioning.

The oxycodone EIA is an extremely reliable test: in a study of 435 urine samples containing oxycodone, the oxycodone EIA demonstrated a sensitivity of 99.1% and specificity of 99.8%. Medical providers can evaluate patients taking oxycodone who submit negative urine opiate EIAs with the specific oxycodone EIA; if oxycodone is demonstrated, false accusations and unwarranted sanctions may be averted.

However, if the oxycodone EIA does not find oxycodone in an patient taking oxycodone, there are three major considerations. First, there is the possibility that the patient is diverting or stockpiling medication, indicating a need for further urine testing and clinical intervention. Second, some compliant patients have very low urine oxycodone levels (under the 300 ng/mL level of detection) and may innocently produce false-negative oxycodone EIAs. This can be seen in rapid metabolizers or in very low dose cases; in these instances, oxycodone can be detected by gas chromatography and mass spectroscopy. Third, oxycodone patients submitting oxycodone negative specimens may be substituting another individual’s urine to avoid detection of drug abuse or diversion. If urine substitution is suspected, a supervised specimen may be collected in a respectful manner or an oral fluid test that can be administered by a staff member observing the oral swab in the patients’ oral cavity can be done, ensuring that the specimen belongs to the patient.

**Semi-Synthetic Opiates**

As in the case of oxycodone, other semi-synthetic opiates, including buprenorphine, oxymorphone, oxycodone, hydromorphone, and
levorphanol, all produce a significant number of negative standard urine opiate EIAs.³,⁶ Many physicians are unaware of this fact.¹⁷ Because, as with oxycodone, the molecular shapes of semi-synthetic morphine-based opiates differ from morphine, with side-chain substitutions they become poorly reactive with standard opiate EIA morphine antibodies and frequently produce negative results.⁶,⁸,¹⁹ Therefore, negative opiate EIAs in patients taking levorphanol, hydromorphone, buprenorphine, or other semi-synthetics do not necessarily indicate diversion. To prevent unfounded allegations, practitioners can monitor semi-synthetic patients with the specific EIAs available for oxycodone, buprenorphine, or hydromorphone.¹⁸ These EIAs will verify semi-synthetic presence in those taking medication properly and will reveal absence of semi-synthetics in those not taking medication regularly or diverting it.²⁷ If semi-synthetic specific EIAs are unavailable, gas chromatography and mass spectroscopy testing may also be used to identify semi-synthetic opiates, including oxycodone.³,⁸,⁹ Providers should familiarize themselves with which tests their toxicology laboratory uses.

Hydrocodone

Hydrocodone is a semi-synthetic and will usually not be detected by most standard opiate EIAs, as discussed in the previous section.¹⁸ However, there is a caveat to this statement; in 2 of 8 commercially available opiate EIAs reviewed, the EMIT II Plus Opiate Assay and the Archetict/Aeroset Opiate Assay-hydrocodone was readily identified as an opiate, making it the exception to the rule that semi-synthetic opiates typically present as opiate-negative with standard opiate EIAs.⁶,¹⁸ Thus, it is critical that practitioners communicate with the laboratory and learn which EIAs are in use. If the opiate EIA in use is one that readily identifies the semi-synthetic hydrocodone, expect positive opiate EIAs in hydrocodone patients; if the laboratory does not use one of the 2 hydrocodone-sensitive EIAs, most opiate EIAs in patients taking hydrocodone will be negative. Gas chromatography and mass spectroscopy may be used to identify this substance.⁶,¹⁸ Given that diverted hydrocodone has been implicated in more opioid analgesic overdose deaths than morphine, monitoring patients taking hydrocodone with ongoing urine drug testing and the appropriate hydrocodone-sensitive EIA is an important initiative.⁴

Heroin

Approximately 30% of the weight of opium poppy fluid is comprised of opiate alkaloids consisting mostly of morphine with lesser amounts of codeine and other non-narcotic compounds.²⁴,²⁹ By the chemical addition of 2 acetyl groups to opium morphine, heroin (diacetyl-morphine) is synthesized.³⁰

Heroin is rapidly metabolized from diacetyl-morphine to 6-mono-acetyl morphine (6-MAM), then to morphine, and then to morphine-6-glucuronide, all with short half-lives measured in minutes such that that heroin becomes undetectable in 40 minutes, 6-MAM in 4 to 12 hours, morphine in 8 hours, and morphine-6-glucuronide in 48 hours. Because 70% to 80% of the heroin dose is recovered in the urine and all of these metabolites are readily detected by the opiate EIA’s morphine antibody, heroin and its morphine-moiety metabolites are easily detected with standard opiate EIAs producing a positive opiate result at the 300 ng/mL level of detection for up to 2 days after a single intravenous or inhaled heroin dose.³¹,³²

6-MAM and Heroin Detection

Heroin (3,6-diacetyl-morphine) is rapidly metabolized by liver cytochromes directly into the molecule 6-MAM, making 6-MAM an ideal candidate for specific identification of heroin use.⁵ In a landmark 1988 study of heroin metabolites, investigators measured morphine levels and, for the first time, 6-MAM levels in 12 heroin users to quantify urine morphine levels in these cases, and to determine whether 6-MAM was useful to identify heroin use. Gas chromatography and mass spectroscopy detected 6-MAM levels of 53 to 3,390 ng/mL (≥10 ng/mL = “positive”) in 11 of 12 cases. Only 1 case with a low morphine level (1,414 ng/mL) was 6-MAM negative.³³ In another study of heroin users, 73% of 100 specimens were found to be positive for
6-MAM. Because 6-MAM is the first product of heroin metabolism and no other compound with the exception of heroin yields 6-MAM, the presence of 6-MAM in the urine is unequivocal evidence of heroin use and, thus, an exceedingly important forensic urine monitoring tool.33,34

Although evidence of 6-MAM proves heroin use, a limitation of 6-MAM testing is its short half-life, rendering it detectable for only up to 12 hours after heroin use and it can be missed if heroin use occurred prior to that.32 Thus, repeated testing for 6-MAM may be necessary to ultimately identify heroin use in patients with positive opiate EIAs who are suspected of but deny heroin use.34,35

Several commercial 6-MAM EIA tests are available and, in a study of 525 6-MAM positive specimens, these immunoassays proved to be 98% sensitive and 98% specific, making the 6-MAM EIA an important tool to assist patient management.5,36

**Morphine Levels in Heroin Use**

After 6-MAM, heroin rapidly metabolizes to morphine and, depending when samples are collected in relation to heroin use, morphine levels vary. In one study of 12 heroin users, randomly collected urine morphine levels ranged from 1,400 to 87,350 ng/mL, with half of the cases having morphine levels of 8,000 ng/mL or higher.33 In a larger study of 63 heroin users, the mean urine morphine level was 62,370 ng/mL (range: 7,100 to 476,000 ng/mL) and 80% of participants demonstrated morphine levels > 15,000 ng/mL. Thus, a morphine level greater than 15,000 ng/mL is highly suggestive of heroin use but is certainly not absolute proof because morphine use or abuse and codeine use or abuse may also produce morphine at these levels.33,34 Because the presence of morphine in the urine specimen can indicate heroin use but can also indicate the use of morphine, codeine, or poppy seeds, to better identify heroin use, two other modalities independent of the absolute morphine level can link morphine positivity to heroin use. The first is the 6-MAM EIA test, which when present proves heroin use, but if absent does not exclude heroin use and repeat testing may be needed.36 The second modality is the morphine:codeine ratio, which can be helpful in the setting of a negative 6-MAM in a heroin user (discussed in the next section).34

**The Morphine/Codeine Ratio**

Urine samples from heroin users typically contain substantial amounts of morphine with lesser amounts of codeine (reflecting poppy fluid contents) and dividing the morphine level by the codeine level yields the valuable morphine:codeine ratio. A morphine:codeine ratio greater than 2:1 as determined by gas chromatography and mass spectroscopy has been shown to be a reliable indicator of heroin use. In a study of 100 heroin users, morphine:codeine ratios were greater than 20:1 in the majority (84%) of cases, greater than 10:1 in 90% of cases, and 2:1 or greater in 94% of cases, demonstrating that a morphine:codeine ratio greater than 2:1 is corroborative evidence of heroin use.34 The only non-heroin drug combination that could produce a morphine:codeine ratio greater than 2:1 would be a patient using or abusing morphine in larger amounts and using or abusing codeine in smaller amounts simultaneously. This combination would conceivable produce a morphine:codeine ratio mimicking that of heroin (i.e., greater than 2:1) and a careful drug history becomes critical in this case.

**Codeine**

Codeine is nearly identical to morphine in structure24 so that standard urine opiate EIAs will easily detect codeine at the 300 ng/mL level of detection.6,8 Maximum therapeutic codeine level by urine gas chromatography and mass spectroscopy should not exceed 15,000 ng/mL; levels higher than 15,000 ng/mL are indicative of codeine abuse.37,38

Codeine is metabolized by cytochrome 2D6 to morphine;39 thus, codeine patients will have both codeine and morphine detectable in gas chromatography and mass spectroscopy urine samples for up to 48 hours after a single dose.24,37 Although both codeine and morphine are easily identified by opiate EIAs and this information is readily available, only 29% of physicians who used urine drug testing in their practices
were aware that codeine metabolizes to morphine, placing legitimate patients taking codeine at risk for accusations of morphine abuse.21 Because codeine metabolizes to morphine, a morphine:codeine ratio can also be determined in codeine users; although heroin produces a morphine:codeine ratio of 2:1 or greater and is often much greater (ex., > 10:1), codeine produces a morphine:codeine ratio of less than 2:1. If a patient taking codeine demonstrates a morphine:codeine ratio of 2:1 or greater, this is too much morphine to be explained by codeine and other morphine sources, including heroin, poppy seeds, and morphine itself, should be considered.34,37,38

**Codeine without Morphine**

Rare patients lack the liver cytochrome 2D6 enzyme needed to convert codeine to morphine, meaning that they cannot metabolize codeine to morphine. These urine specimens will show codeine only and no morphine, rendering the morphine:codeine ratio ineffective in differentiating drug use; supervised urine specimens or oral fluid specimens (administered directly observed by staff members) will show only codeine.40

Another source of codeine-only specimens could be patients who are not taking their prescribed codeine (stockpiling or diverting) and who may spike drug-free urine samples with crushed codeine tablets to maintain the appearance of a positive opiate EIA. These urine samples will have codeine only without morphine but examination of urine specimens will reveal a characteristic white precipitate along the bottom of the urine container.28

Codeine normally produces positive opiate EIAs. If confirmation is desired in adherent cases, gas chromatography and mass spectroscopy testing will reveal codeine or morphine levels of < 15,000 ng/mL and a morphine/codeine ratio of less than 2:1. The heroin metabolite 6-MAM EIA will be negative. In patients taking codeine, codeine or morphine levels of > 15,000 ng/mL indicate codeine abuse37,38 and a morphine/codeine ratio greater than 2:1 indicate heroin use.34,41

**Poppy Seed**

As a product of the opium poppy, poppy seeds contain small amounts of morphine and codeine and, under proper conditions (ex., consumption of 1 poppy Danish streusel pastry), can readily produce positive opiate EIAs.42 Many physicians are not aware of this poppy seed effect. In a recent survey of 359 emergency room physicians, the majority (60%) were unaware that poppy seeds could produce a positive opiate EIA.23

Goods baked with poppy seeds are widely available. Typically, poppy seed bagels, muffins, Danish, and Streusel contain morphine in amounts of 1, 2, 6, and 6 mg per piece, respectively, so that consumption of a single muffin, Danish, Streusel, or 2 bagels can produce a positive opiate EIA at the 300 ng/mL morphine level of detection and the test can remain positive for 24 hours after ingestion.42

In a classic 1987 study, consumption of 3 poppy seed bagels produced positive opiate EIAs with gas chromatography and mass spectroscopy morphine levels of 2,797 ng/mL at 3 hours, and 676 ng/mL at 22 hours (codeine levels were 214 and 16 ng/mL, respectively).43 In another investigation of 10 participants who consumed measured amounts of whole poppy seeds reflecting amounts in baked goods, and who underwent multiple urine sampling over 24 hours, morphine was found in all cases at 24 hours. Two morphine levels were more than 2,000 ng/mL (2,635 and 2,199 ng/mL), but all other samples were less than 1,700 ng/mL, with most poppy seed morphine concentrations less than 1,000 ng/mL. The authors concluded that a morphine level of 3,000 ng/mL or less can be explained by poppy seed ingestion (but this can also be due to heroin or morphine use, see discussion below) and that most cases of poppy seed ingestion will have morphine levels less than 1,000 ng/mL.44

**Poppy Seed Morphine:Codeine Ratio**

The morphine:codeine ratio in poppy seed ingestion, like heroin ingestion, is also 2:1 or greater (ex., 13:1, 42:1), consistent with opium poppy fluid as the opiate source for both heroin and poppy seeds. In one study, the average
morphine:codeine ratio at 22 hours post-papaya seed ingestion was 45:1. Other poppy seed studies also show morphine:codeine ratios of more than 2:1 (i.e., 19:1 to 295:1); thus, high morphine:codeine ratios with low morphine levels (<3,000 ng/mL, usually <2,000 ng/mL) are consistent with poppy seed consumption and do not automatically indicate heroin abuse.

**Poppy Seed Ingestion versus Heroin Use**

It can be difficult to determine whether positive opiate EIAs are the result of poppy seed ingestion or heroin use, both being derived from the same source (opium poppy). Gas chromatography and mass spectroscopy will show that poppy seeds contain morphine and codeine indistinguishable from that of heroin and in the same ratio found in heroin (2:1 or greater), but absolute levels of morphine and codeine are typically lower with poppy seeds, with morphine < 3,000 ng/mL and codeine < 300 ng/mL; most poppy seed cases produce urinary morphine levels less than 1,000 ng/mL.

High morphine levels (>3,000 ng/mL with a morphine:codeine ratio of 2:1 or greater) are most likely due to heroin use but any morphine level, even ones as low as 1,000 ng/mL with a morphine:codeine ratio of 2:1 or greater, can be caused by heroin use. Because poppy seed ingestion and heroin use can both produce lower morphine levels, further testing for 6-MAM may be needed to identify heroin use with certainty in these low level morphine cases.

In some cases, the only way to prove that morphine is due to poppy seed ingestion is to have patients discontinue ingesting poppy seed products and observe opiate EIAs reverting to negative levels in 48 hours. All patients are advised to avoid poppy seed products to avoid unnecessary positive opiate EIAs and possible unfounded sanctions from providers.

If 6-MAM is repeatedly negative and morphine is less than 3000 ng/mL with a morphine:codeine ratio of 2:1 or greater, it is impossible to differentiate poppy seeds from heroin. The clinical impression of the medical provider and patient history of poppy seed ingestion in conjunction with repeated gas chromatography and mass spectroscopy and 6-MAM testing is needed to finally identify the true source of opiate. Nonetheless, morphine levels greater than 3,000 ng/mL, with a morphine:codeine ratio of 2:1 or greater, usually indicate heroin use and the 6-MAM EIA proves it if positive.

**Fluoroquinolones**

The only substances that normally produce 100% positive opiate EIAs are the morphine-based opiates such as morphine, heroin, codeine, and poppy seeds and the semi-synthetic hydrocodone (if a hydrocodone-sensitive opiate EIA is in use). Fluoroquinolone antibiotics, such as ofloxacin or ciprofloxacin, can also produce positive opiate EIAs, potentially leading to unfounded accusations of drug abuse and dismissal or other disciplinary action for patients enrolled in pain clinics or drug treatment programs.

Fluoroquinolone opiate EIA positivity is closely correlated to peak quinolone urine levels. In one investigation, urine collected 6 to 8 hours (peak levels) after ofloxacin or levofloxacin ingestion produced 100% positive (false-positive) opiate EIAs in all 6 cases, with morphine levels registering just above the 300 ng/mL cutoff, even though there was no morphine present in the specimens. Why opiate EIAs identify fluoroquinolones as morphine itself is unknown since chemical structures are completely dissimilar. Individuals with positive opiate EIAs should be questioned regarding fluoroquinolone use and can be evaluated with gas chromatography and mass spectroscopy testing, which will demonstrate no opiates present in these specimens in the absence of opiate use or abuse.

**Buprenorphine**

Buprenorphine is a morphine-based, semi-synthetic molecule, which is a partial agonist at brain opiate mu-receptors. Due to the addition of extensive side chains to the base morphine molecule to create buprenorphine, altering the appearance extremely from that of morphine; the morphine core is nearly unrecognizable in 2-dimensional depictions of buprenorphine. Due to this configurational alteration, the morphine antibody in standard opiate EIAs will not
react with buprenorphine, yielding negative opiate EIAs in these individuals.\textsuperscript{8,51}

However, fortunately highly sensitive and highly specific buprenorphine EIA tests are available and should be used to monitor patients taking buprenorphine. One buprenorphine EIA showed a specificity of 97\% and a sensitivity of 99.5\%\textsuperscript{52} and another “demonstrated a sensitivity and specificity of 100\%,”\textsuperscript{54} making these tests essential to the management of the buprenorphine patient.\textsuperscript{53,55}

However, conversely if testing in patients taking buprenorphine reveals the absence of buprenorphine, then diversion, stockpiling, or selling of medication becomes a concern. In a recent study of widespread buprenorphine diversion, medical personnel were alerted by the absence of buprenorphine in monitoring urine specimens with the buprenorphine EIA.\textsuperscript{54} On questioning, only 43\% of 71 diversion cases admitted to diversion, reinforcing the importance of ongoing urine drug monitoring as a necessary objective tool to improve patient monitoring and enhance patient management.\textsuperscript{1,3,53}

\textbf{Fentanyl}

Fentanyl is a wholly synthetic (as opposed to semi-synthetic) opiate and, not being morphine-based, produces negative opiate EIAs.\textsuperscript{55} Other common synthetic opiates include methadone, propoxyphene, meperidine, tramadol, and pentaizocine. These synthetic opioids do not react with the opiate EIA morphine antibody and will yield negative opiate results. Gas chromatography and mass spectroscopy and more specific EIA testing (discussed below) can be used to confirm the presence of these synthetics.\textsuperscript{19,25}

Because fentanyl diversion is a growing problem in the United States,\textsuperscript{56} prescribers need to be aware of fentanyl urine testing characteristics; fentanyl does not produce positive standard opiate EIAs.\textsuperscript{25} The absence of positive opiate EIAs in patients taking fentanyl, although a normal occurrence that is consistent with fentanyl, should not obviate the need for further confirmatory testing to explicitly demonstrate the presence of fentanyl. A study of fentanyl patch patients showed a sensitivity and specificity of 98\% for the fentanyl urine EIA.\textsuperscript{57} Several fentanyl-specific EIA tests available are capable of detecting minute concentrations of fentanyl in urine specimens and can be used to monitor patients taking fentanyl to assure this compound is present in patient urine specimens.\textsuperscript{57,58} Providers should communicate with their toxicology laboratories to determine whether a fentanyl EIA is available and apply it to patients taking fentanyl in accordance with clinical needs.

If patients taking fentanyl are submitting positive standard opiate EIAs and these positives were attributed to fentanyl (which is not possible), some morphine-based drug is being used or abused in addition to or in lieu of fentanyl, and gas chromatography and mass spectroscopy can be used for identification.\textsuperscript{6}

\textbf{Methadone}

Methadone is a wholly synthetic opioid molecule, which is not structurally related to morphine; therefore, methadone does not react with the standard opiate EIA morphine antibody and will yield negative opiate results in screening toxicology tests.\textsuperscript{8} Providers who wish to monitor patients taking methadone can use one of the commercially available methadone EIAs that have been shown to be 98\% sensitive and 98\% specific.\textsuperscript{59}

\textbf{Sertraline}

Patients taking the anti-depressant sertraline can produce sporadic false-positive benzodiazepine EIA urine toxicology specimens.\textsuperscript{60} Unfounded accusations of benzodiazepine abuse in these patients may arise. In one study, 16 of 50 positive benzodiazepine EIAs were submitted by patients taking sertraline who were not ingesting benzodiazepines. In a review phase of the same study, false-positive benzodiazepine EIAs caused by sertraline were found in 26\% of 2,447 patients taking sertraline urine specimens.\textsuperscript{61} Recently, the benzodiazepine EIA test manufacturer released the following statement:

\begin{quote}
patients taking therapeutic doses of \textit{Sertraline (Zoloft\textsuperscript{R})} of 100–200 mg per day \textbf{can test positive for benzodiazepines.}
\end{quote}
An unidentified metabolite of Sertraline in urine is responsible for the cross-reactivity as unmetabolized Sertraline is not present in sufficient quantities to cause a positive test.62

The chemical structures of sertraline and its urinary metabolite, des-methyl-sertraline, bear a striking similarity to diazepam,24 which likely enables the benzodiazepine EIA to react causing a false-positive benzodiazepine result.62 Sertraline is now listed as one of the compounds, along with diazepam and alprazolam, which can cause a positive benzodiazepine EIA result in the 2008 EIA cross-reactivity list.8

Patients taking Sertraline who submit positive benzodiazepine EIAs and deny benzodiazepine use should not automatically be accused of benzodiazepine abuse; they may undergo gas chromatography and mass spectroscopy testing, which can confirm the absence of benzodiazepines, indicating a false-positive EIA due to sertraline. In cases of patients taking sertraline who are using or abusing benzodiazepines, gas chromatography and mass spectroscopy can be used to demonstrate the presence and concentration of the specific benzodiazepine.61

Clonazepam and Lorazepam

Patients taking clonazepam have been shown to produce a significant number of negative benzodiazepine EIAs due to lower reactivity of clonazepam with the EIA’s diazepam antibody.63 Similarly, lorazepam has also been shown to produce mostly or all negative benzodiazepine EIAs for the same reason. In a study of 4 different manufacturers’ benzodiazepine EIAs, 38 different benzodiazepines and their metabolites were evaluated to determine the rate of benzodiazepine detection for each benzodiazepine EIA. Results showed that all immuno-assays tested detected diazepam and alprazolam; however, none detected clonazepam or lorazepam.63 In an analysis of urine levels in 53 patients taking benzodiazepine, 10% of whom were taking lorazepam, none of three commercially available standard benzodiazepine EIAs were able to identify any lorazepam in any patient.64 Finally, a study of one popular commercially available benzodiazepine EIA’s ability to react with 15 different benzodiazepines demonstrated “good” reactivity (i.e., antibody binding) to diazepam, only “moderate” reactivity to clonazepam, and “low” reactivity to lorazepam, indicating that negative benzodiazepine EIAs with clonazepam and lorazepam are expected, normal results.65 Because these negative benzodiazepine EIAs may lead to accusations of benzodiazepine diversion, providers should consider gas chromatography and mass spectroscopy when needed to demonstrate clonazepam or lorazepam in these specimens.9 However, if gas chromatography and mass spectroscopy fail to demonstrate clonazepam or lorazepam in patients who say they are taking these medications, then diversion or missed doses should be considered.

Conversely, if patients taking clonazepam or lorazepam are submitting mostly or all positive benzodiazepine EIAs (not a clonazepam or lorazepam pattern) the use of other non-clonazepam or non-lorazepam benzodiazepines, such as diazepam and alprazolam, is suggested and gas chromatography and mass spectroscopy can reliably determine their types and concentrations.9

CONCLUSION

We have reviewed the use of screening urine EIAs and gas chromatography and mass spectroscopy to verify legitimate prescriptions of specific drugs and to identify abused substances in the urine in those claiming legitimate prescriptions. Standard opiate EIAs identify only morphine, heroin, hydrocodone, and codeine as “positive” opiates; if the exact drug (i.e., morphine) and the concentration is desired, then gas chromatography and mass spectroscopy must be used to supply this information.

Heroin use typically yields morphine levels greater than 3,000 ng/mL and poppy seed ingestion yields less than 3,000 ng/mL. Most heroin samples yield morphine levels in excess of 15,000 ng/mL, with morphine:codeine ratios greater than 2:1, usually 10:1 and greater. 6-MAM, a heroin metabolite, is definitive evidence of heroin use, although short lived under 12 hours. A morphine:codeine ratio greater than
2:1 indicates heroin or poppy seed use; a ratio of less than 2:1 is consistent with codeine use.

Synthetic opiates (non-morphine based), such as fentanyl, methadone, and meperidine, will be opiate EIA negative. Semi-synthetic opiates, such as oxycodone and hydromorphone, usually yield negative opiate EIAs but, being distant morphine congeners, will also produce sporadic positive opiate EIAs (i.e., mixed positive and negative results). Specific EIAs and gas chromatography and mass spectroscopy can identify synthetic and semi-synthetic opioids. Providers should consult with the toxicology laboratory to determine which tests are available.

Fluoroquinolones such as ciprofloxacin can produce false-positive opiate EIAs. Gas chromatography and mass spectroscopy can be used for clarification in all of these instances. Sertraline can cause sporadic false-positive benzodiazepine EIAs via sertraline metabolites resembling diazepam. Clonazepam and lorazepam with structures dissimilar to diazepam will frequently produce false-negative benzodiazepine EIAs and gas chromatography and mass spectroscopy may be used to confirm their presence. Urine toxicology testing is an important part of comprehensive care for individuals attending drug treatment programs or receiving chronic opioid or benzodiazepine therapy and should be considered as an objective test within a greater integrated psychosocial and medical format and toxicology results should never be interpreted in a vacuum, but rather always within the broader clinical patient context.

REFERENCES


18. Smith ML, Hughes RO, Levine B, Dickerson S, Darwin WD, Cone EJ. Forensic drug testing for opiates. VI. Urine testing for hydromorphone, hydrocodone, oxymorphone, and oxycodone with commercial opiate