

REVIEW

Demystifying Analytical Approaches for Urine Drug Testing to Evaluate Medication Adherence in Chronic Pain Management

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ABSTRACT

This comprehensive review of analytical methods used for urine drug testing for the support of pain management describes the methods, their strengths and limitations, and types of analyses used in clinical laboratories today. Specific applications to analysis of opioid levels are addressed. Qualitative versus quantitative testing, immunoassays, chromatographic methods, and spectrometry are discussed. The importance of proper urine sample collection and processing is addressed. Analytical explanations for unexpected results are described. This article describes the scientific basis for urine drug testing providing information which will allow clinicians to differentiate between valid and questionable claims for urine drug testing to monitor medication adherence among chronic pain patients.

KEYWORDS urine, drug, testing, analytical, medication, chromatography, immunoassays, spectrometry, adherence, pain management

INTRODUCTION

Urine drug testing is a well-accepted tool for detection of recent drug use. Urine is relatively easy to collect, and there are many analytical methods and laboratories available that perform urine drug tests. However, standardization among urine drug tests that are promoted for medication adherence evaluations, such as in the chronic pain management setting, is lacking. There is no such thing as a perfect drug test, and with the wide range of tests available, it may be a challenge to select the best testing approach and an-

alytical methods for a given application. This review article is intended to “demystify” current choices for urine drug testing, with emphasis on analytical methods designed to support adherence testing for chronic pain patients.

Much of the credit for wide-spread availability of urine drug testing comes from the extensively regulated U.S. federal drug testing programs that were initiated in 1986 to assure a drug-free workplace. These programs, now managed by the Substance Abuse and Mental Health Services Administration (SAMHSA), define the specific drugs detected, the analytical methods and performance characteristics thereof, cutoff concentrations, qualification criteria for laboratories that provide testing, and interpretation of results. Today urine drug testing is applied to both forensic and clinical applications, and has deviated substantially from the model set forth by the federal programs. Thus, the drugs of interest, performance characteristics of testing, cutoff concentrations, analytical methods, and interpretation of results for clinical urine drug testing are unique, as compared to the SAMHSA model. In addition, no

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single analytical approach applied to clinical urine drug testing, such as an immunoassay, or mass spectrometric test, can be generalized as more specific, sensitive, or better than another, without considering the details of assay design, including sample preparation and detection methods, as well as methods for data review and acceptance of results.

Urine Drug Testing to Support Pain Management

Pharmacotherapy is integral to the clinical management of most chronic pain patients.¹ When deciding which medications to prescribe, providers must be aware of both prescribed and nonprescribed substances (licit and illicit) that a patient may, or may not, be using. This information helps guide drug and dose selection to maximize efficacy and safety, and minimize toxicity and risk of drug–drug interactions. Due to the use of controlled substance (scheduled) drugs such as opioids and benzodiazepines, both providers and patients are often scrutinized for prescribing and use patterns. Urine drug testing is a tool that benefits both providers and patients by documenting medication adherence to support appropriate behavior. Indeed, random urine drug testing has been shown to reduce the likelihood of diversion and misuse, and has been advocated in guidelines published by professional organizations such as the American Society for Interventional Pain Physicians (ASIPP).^{2,3} Despite this enthusiasm, there are no detailed guidelines for specifically how urine drug testing should be performed to support chronic pain management.

When using urine drug testing to evaluate medication adherence, results should be interpreted relative to clinical expectations and patient admissions. Prescribed or self-reported drugs should be present, and nonprescribed drugs should be absent. The consequences of an unexpected drug testing result could include compromise to the patient–provider relationship through: accusations of inappropriate drug use, increased costs for follow-up care and/or additional drug testing, denial of medications, denial of insurance or reimbursement, or even expulsion from a pain management program. In addition, social consequences such as restriction of parental rights or employment privileges may occur. Unlike traditional workplace drug testing wherein positivity rates are typically less than 1%, the positivity rate of drug testing for medication adherence monitoring should be very high and it is common for multiple drugs to be present. This difference in pre-test expectations is just one reason that SAMHSA testing is not appropriate for adherence testing of chronic pain management patients.⁴

Definition of specific drug analytes important for adherence testing is evolving, and consensus regarding the specific content of a “comprehensive” or “standard” drug testing panel that meets needs of all pain management clinics does not currently exist. The best approach to drug testing for any application is to employ the test or tests that most closely align with the needs and expectations. As illustrated in Figure 1, drug detection depends on a coordinated understanding of variables associated with the drug, the patient, the specimen, and the analytical method. Drug formulations vary in drug content, efficiency of drug delivery, overall bioavailability, and elimination kinetics. In addition, urine varies in concentration, and may be more likely to be dilute in a sick than a healthy population. The time of urine collection relative to the last drug dose, the pattern and dosing of drug use, the unique pharmacokinetics of the drug in an individual patient, the quality of the specimen provided for testing, and the analytical test characteristics all influence both the likelihood of drug detection and interpretation of results. A highly adherent (compliant) patient with a well preserved and appropriately collected specimen could be faced with an unexpected drug testing result. See Table 1 for a list of possible explanations to consider when investigating an unexpected urine drug test result.

Despite known limitations of the federal drug testing program for clinical drug testing, the most common approach to testing adopts many of the SAMHSA principles. The SAMHSA approach includes an “initial” test and a “confirmation” test. The initial test is also called a drug “screen” which is designed to detect a drug class, and is most commonly performed using an immunoassay. When immunoassay results are positive, meaning that the signal generated by a test (patient’s) urine exceeds that which corresponds to the cutoff concentration, the results are confirmed with a second test. The confirmation test is designed to determine if a specific drug analyte, or list of analytes, is present, and at what concentration(s). Confirmation testing is typically performed by gas chromatography mass spectrometry (GC-MS) or liquid chromatography tandem mass spectrometry (LC-MS/MS). The drugs and drug metabolites detected in a traditional SAMHSA testing environment, and the corresponding cutoff concentrations, are shown in Table 2.

Following the traditional SAMHSA paradigm, only urine samples that are positive in the initial test are confirmed, and only the drug analytes detected in the confirmation test are reported. Only results that are positive by both methods are reported, and essentially all false-positive results are eliminated. This level of concordance in results is extremely important

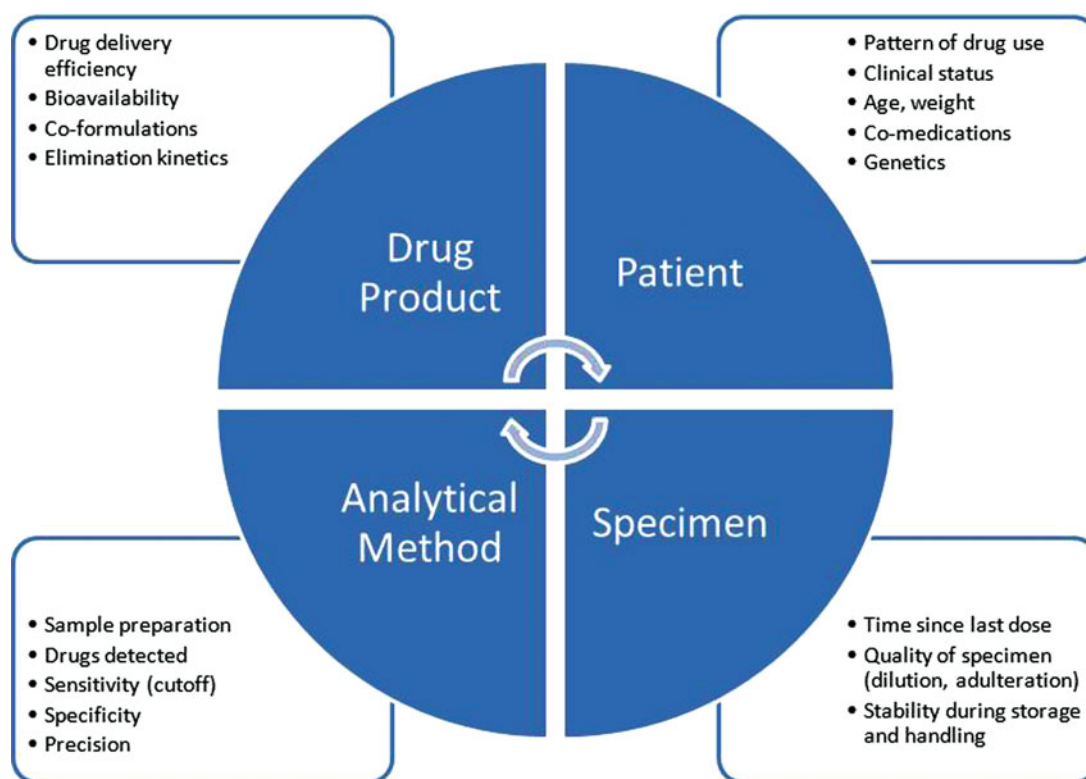


FIGURE 1. Factors that influence detection of drugs in urine.

for establishing credibility and confidence when urine drug testing results are applied to a forensic scenario, such as determining the involvement of a drug in a crime, or when qualifying a person to work in a safety-sensitive position. This two-step process is not typically required for clinical applications of drug testing.

Application of the SAMHSA model to medication adherence monitoring may contribute harm to the provider and patient, specifically by generating unexpected negative results. A commonly cited paper representing nearly one million urine samples collected from chronic pain management patients, that were tested using the traditional two-step model, reported that 38% of the samples did not contain detectable concentrations of the expected prescribed drug. The authors suggest that 75% of patients were unlikely to be compliant with the therapeutic plan.⁵ Other studies suggest that the incidence of inappropriate urine drug tests ranges from 9% to 50%.⁶ One could argue that the patients with unexpected results are noncompliant, yet one could also argue that the tests employed were inappropriate for the application. Indeed, the ASIPP evidence assessment for urine drug testing was “fair” regarding the use of traditional urine drug testing to identify patients who are noncompliant.³

Apparent false negatives are particularly common for semi-synthetic or synthetic opioids and benzodiazepines. A study that considered approximately 8000 urine samples collected from chronic pain management patients failed to identify 69.3% of hydromorphone-positive urine samples and 53.5% of alprazolam-positive urine samples when the traditional approach was applied.⁷ Yet another study demonstrated that 66.1% of clonazepam-positive urine samples were missed when utilizing the traditional two-step (screen with reflex to confirmation) approach. It is now recognized that such unexpected immunoassay results are not false, but rather, represent incongruence between the test and the pre-test expectations, and should stimulate an investigation of contributing factors such as those illustrated in Table 1. Due to the high incidence of false negatives associated with immunoassays designed to detect benzodiazepines and opioids, one laboratory group described how they optimized medication adherence monitoring for those drug classes by eliminating the screen component in favor of a targeted testing using LC-MS/MS.⁸

The cutoff concentrations used for interpretation of urine drug testing results are another potential source of apparent false-negative results. While cutoff

TABLE 1. Examples of Explanations for Unexpected Urine Drug Testing Results

Source	Unexpected positive	Unexpected negative
Patient	Prescription from another provider was taken/administered Unexpected non-prescribed drug was taken/administered Drug detected is an unfamiliar metabolite of the expected drug Non-disclosed use of historical prescription	Prescription not filled Drug was taken/administered incorrectly Drug was not taken/administered Drug was not absorbed Rapid drug metabolism/elimination Other unique pharmacokinetic variable
Specimen	Substituted urine Drug added to urine after voiding Clinic or laboratory mix-up	Substituted urine Dilute urine Adulterated urine Urine collected too long after last dose Inappropriate storage/handling of urine before testing Clinic or laboratory mix-up
Drug Product	Drug detected is a process impurity of the expected drug Drug has longer liberation and elimination kinetics than expected Incorrect prescription/formulation was filled	Drug delivery was poor or variable Drug has shorter liberation and elimination kinetics than expected Incorrect prescription/formulation was filled
Immunoassay	Cross-reacting non-drug substance(s) Cross-reacting drug or drug metabolites Cutoff concentration lower than expected Test not performing appropriately	Poor cross-reactivity for drug analytes of interest Cutoff concentration higher than expected Signal suppression (e.g. adulteration) Test not performing appropriately
Targeted assay	Analytical error (e.g. carryover, noise) Unrecognized isobaric interference Unrecognized stereoisomer Reporting limit lower than expected Test not performing appropriately	Test not designed to detect analyte(s) of interest Sample not tested Inadequate sensitivity to drug metabolites Poor recovery from sample preparation Signal suppression (e.g. ion suppression) Reporting limit higher than expected Unresolved interference Test not performing appropriately

TABLE 2. Analytes and Cutoff Concentrations for Traditional SAMHSA Urine Drug Testing (1)

Initial test		Confirmation test	
Drug class	Cutoff (ng/mL)	Specific analyte	Cutoff (ng/mL)
Opiates	2000	Codeine	2000
		Morphine	2000
6-Acetylmorphine	10	6-Acetylmorphine	10
Amphetamines	500	Amphetamine	250
		Methamphetamine	250
MDMA	500	MDMA	250
		MDA	250
		MDEA	250
Cocaine metabolites	150	Benzoylcegonine	100
Marijuana metabolites	50	THCA	15
Phencyclidine	25	Phencyclidine	25

MDMA Methylenedioxymethamphetamine, ecstasy

MDA Methylenedioxymamphetamine

MDEA Methylenedioxyethylamphetamine

THCA Delta-9-tetrahydrocannabinol-9-carboxylic acid.

TABLE 3. Proposed Cutoff Concentrations for Selected Drugs of Interest to Pain Management Estimated to Identify Prescription Drugs in 97.5% of Pain Patients (10)

Drug class	Analyte	Cutoff (ng/mL)	Cutoff (μ g/g creatinine)
Opioids	Codeine	29	15
	Morphine	59	52
	Oxycodone	45	46
	Oxymorphone	44	38
	Hydrocodone	41	31
	Hydromorphone	34	26
	Fentanyl	2	2
	Buprenorphine	7	5
	Methadone	89	74
	Tramadol	147	70
	Tapentadol	42	58
	Meperidine	88	28
	Propoxyphene	60	42
Anxiolytics	Clonazepam metabolite	19	15
	Alprazolam metabolite	15	11
	Lorazepam	30	25
	Carisoprodol	56	35
	Meprobamate	92	113
Stimulants	Amphetamine	76	59

concentrations are standardized in the federal drug testing program (see Table 2), the ideal cutoff concentrations applicable to pain management patients are not currently defined. Examples of proposed cutoffs for selected prescription drugs of interest to pain management are shown in Table 3. For the drugs that overlap with SAMHSA, the proposed cutoffs for medication adherence monitoring are notably lower than those utilized by SAMHSA.⁹ While this proposed list is useful, it is important to recognize that the cutoffs cannot be universally compared, without also comparing the sample preparation and detection methods. For example, clean-up of a sample before analysis to minimize matrix effects, or a high resolution instrument may be required to achieve low cutoffs. This is one reason for the substantial differences in cutoffs that exists among clinical laboratories. An-

other reason for differences in cutoffs between laboratories is whether or not the laboratory incorporates hydrolysis in the sample preparation methods. Liberating glucuronide conjugates by preanalytical hydrolysis will produce higher concentrations of many drug analytes than would be observed with a method that does not include hydrolysis. The latter would be referred to by the laboratory as detecting "free" versus "total" drug.

Most drugs are extensively metabolized, and the detection of drug metabolites demonstrates that the drug has been taken/administered, and processed by the body. Some urine drug-testing methods now detect glucuronide metabolites specifically, an approach that has been shown to increase detection limits for some drugs.¹⁰ Incorporating several drug metabolites may also improve the likelihood of drug detection, increase confidence in results, and may guide interpretation of results. Normetabolites, for example, are common to the opioids and may be the only analyte detected for a drug. Table 4 summarizes data from two studies in which the patterns of opioid analytes were evaluated for thousands of chronic pain patients. The percent of urine samples for which only the parent drug was detected versus the percent of urine samples for which only the normetabolite of the parent drug was detected are shown. Thus, the positivity rates for drugs such as hydrocodone, oxycodone, meperidine, propoxyphene, and fentanyl are significantly improved when the normetabolite is detected.^{11,12} Based on the known enzymatic reactions responsible for generating the normetabolites, the ratio of parent and normetabolite may provide information akin to a metabolic phenotype for a patient, and once established, may help identify drug-drug interactions, and/or approximate time of last dose. Measuring drug metabolite patterns as well as parent drug can also identify urine samples to which drug have been added after voiding to mimic adherence.¹³

Urine drug testing for pain management need not be defined by a two-step process. Some immunoassays perform very well, and as such, results that are

TABLE 4. Detection Patterns for Selected Opioids Based on Parent Drug and/or Normetabolite (12,13)

Drug	Percent identified by normetabolite alone	Percent identified by parent alone	Percent identified with both parent and normetabolite
Oxycodone	71	17	12
Propoxyphene	53	4	43
Meperidine	50	5	46
Fentanyl	30	39	31
Hydrocodone	14	18	68
Buprenorphine	9	4	87
Methadone	8	19	73
Codeine	3	82	15

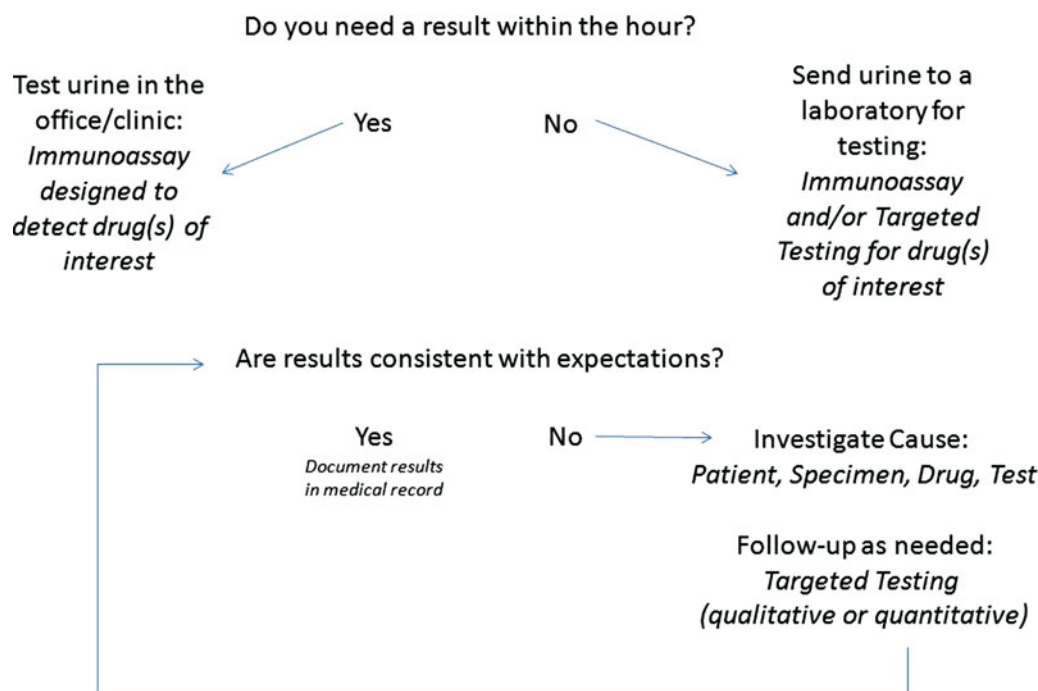


FIGURE 2. Algorithm for selection of urine drug testing for medication adherence monitoring.

consistent with clinical expectations need not be confirmed or quantitated. Often the choice to perform an immunoassay depends on the timeliness for which a result is needed. For immunoassays that perform well, a useful qualitative result can be obtained within an hour of urine collection. For drugs that are not well served by immunoassays, targeted analytical methods traditionally applied to confirmation testing may be used independent of any screening test to specifically detect the drugs and drug metabolites of interest. Quantitative tests are available for those scenarios wherein the concentration of the various drug analytes detected guides interpretation. However, determining the concentrations of the drugs and drug metabolites is not usually necessary if results are consistent with clinical expectations. Eliminating multi-step processes (e.g. screen and confirm) and minimizing the need for quantitative analysis will reduce the expenses associated with testing, and simplify the medical record. This may include some immunoassays, and some targeted testing, but not in a "reflexive" configuration.⁸ An algorithm for medication adherence monitoring is summarized in Figure 2.

The discussion below describes in more detail how immunoassays and targeted analytical methods can be applied to the pain management setting to achieve the goals of testing, while minimizing apparent false negative and positive results. Figure 3 provides a summary of these two analytical approaches to test-

ing, and some of the characteristics that make each unique.

Immunoassays

Immunoassay-based urine drug tests can be qualitative or quantitative, and are designed to be performed at the point of collection (POC) or using laboratory reagents and equipment.¹⁴⁻¹⁷ All immunoassays incorporate either monoclonal or polyclonal antibodies that "capture" drugs and drug metabolites via a unique binding affinity for an epitope. The capture antibody thereby defines the specificity for a test. A monoclonal antibody is designed to optimize specificity, by recognizing a defined epitope, whereas polyclonal antibodies will bind to many epitopes. In either case, a result produced using an immunoassay reflects the sum of the antibody binding that is detected, relative to all reactive substances present in the sample, including drugs, drug metabolites, endogenous matrix components, and other nondrug substances. Poor specificity may be a good characteristic when the goal of testing is to detect several drug analytes within a drug class, but each compound within a drug class will be detected at different concentrations, reflecting the unique affinity of the capture antibody for the individual compound. More often than not, poor specificity is reflected by the incidence of false-positive and false-negative results. The specificity of an individual

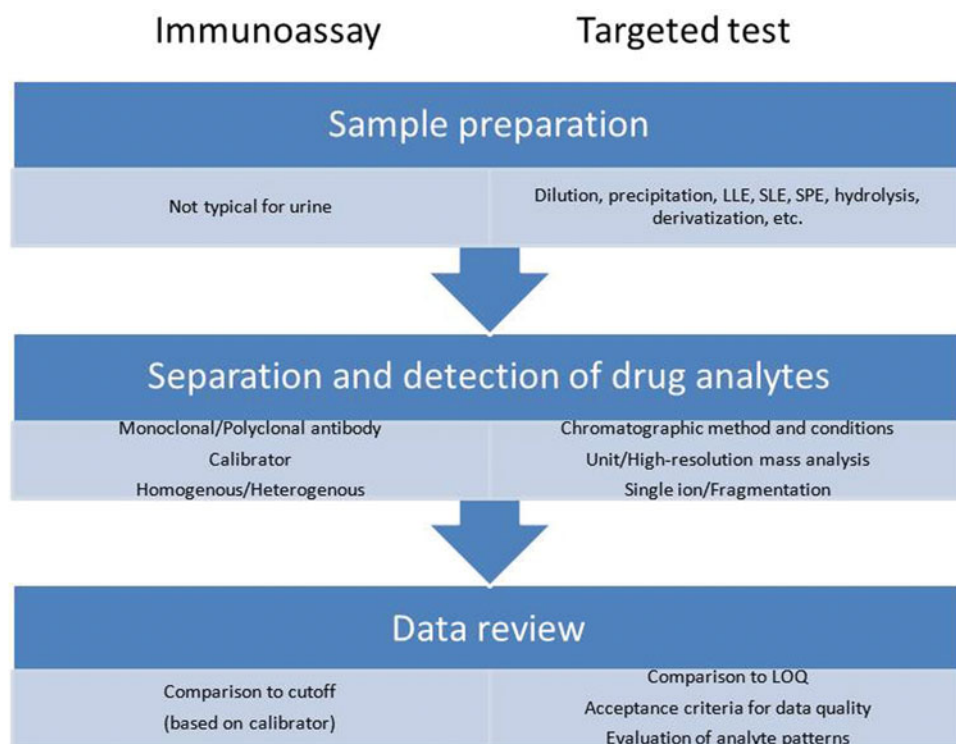


FIGURE 3. Comparison of immunoassay and targeted testing methods, and variables that could influence quality of urine drug testing results.

test is evaluated and published by the manufacturer of each immunoassay, and may be further characterized by independent investigators, as evidenced by post-market research and publications.

Every immunoassay has a calibrator. The calibrator is selected by the manufacturer and is usually representative of the drug class for which the test is designed. For example, morphine is a common calibrator for immunoassays designed to detect opiates. The actual affinity of the calibrator for the capture antibody is normalized to other drug analytes that exhibit affinity for the capture antibody, such that the calibrator will exhibit 100% cross-reactivity for the capture antibody, and will be detected reliably at the cutoff concentration. Drug analytes that exhibit greater than 100% cross-reactivity for the capture antibody produce a “positive” result at concentrations less than the cutoff for the test. Likewise, drug analytes that exhibit less than 100% cross-reactivity for the capture antibody produce a “negative” result at concentrations that may far exceed the cutoff concentration for the test. A cross-reactivity profile, reflecting the affinity of the capture antibody for various drug analytes within a drug class, or a table that provides the concentration of drug analytes required to trigger a positive result, is provided in the labeling (package insert) for any commercial immunoassay.

An example of this type of data is summarized in Table 5, illustrating that the cross-reactivity profiles vary tremendously among members of the benzodiazepine drug class, and among the commercial benzodiazepine assays shown, despite equivalent cutoff concentrations. Thus, the concentration of clonazepam required to trigger a positive benzodiazepine test at the 300 ng/mL cutoff is 500 ng/mL for the EMIT and Drug Check test, 650 ng/mL for the Triage test, and 5000 ng/mL for the NexScreen test. The common urinary metabolite of clonazepam, 7-amino-clonazepam, is only detected at extremely high concentrations that are not likely to be encountered clinically, or was not characterized by the manufacturer at all. For alprazolam, the laboratory test reagents are sensitive at concentrations much lower than the cutoff, whereas the POC cups require concentrations higher than the cutoff to trigger a positive result. As such, the same urine sample could test positive with one test and negative with another test, despite the same cutoff concentration. These differences in assay performance reflect the different capture antibodies and calibrators.

Another concern that should be considered, exemplified by the data in Table 5 for alprazolam, relates to potential alignment of these immunoassays with a “confirmation” test. If these immunoassays were

TABLE 5. Concentration (ng/mL) Required to Produce a Positive Result in Four Commercial Immunoassays with a Cutoff of 300 ng/mL*

Drug compound	Laboratory reagents		POC cups	
	EMIT	Triage	Nex Screen	Drug check
Calibrator	lormetazepam	oxazepam glucuronide	oxazepam	oxazepam
Alprazolam	79 ng/mL	100 ng/mL	400 ng/mL	400 ng/mL
Alpha-OH-alprazolam	150	100	N/A	N/A
Clonazepam	500	650	5,000	500
7-amino-clonazepam	11,000	N/A	N/A	N/A
Chlordiazepoxide	7,800	13,000	8,000	300
Nordiazepam	140	700	500	150
Diazepam	120	200	2,000	450
Oxazepam	350	3,500	300	300
Temazepam	210	200	200	200
Lorazepam	890	200	4,000	500

*Values obtained from respective kit package inserts, accessed September 15, 2013.

N/A indicates that the concentration was not available.

used initially to detect positive urine samples, and then the positive results were confirmed by another test, the required cutoff concentrations for the various benzodiazepines would need to align with the cross-reactivity profile for the specific immunoassay rather than with the cutoff concentrations. As such, a confirmation method for an EMIT test would need to be sensitive to concentrations lower than 79 ng/mL to confirm a low positive result, but could be sensitive at 400 ng/mL for the POC cups shown here.

Sensitivity of an immunoassay is based on the cutoff concentration for the calibrator, and whether the assay is of homogeneous or heterogeneous design. Heterogeneous assays incorporate several wash steps to minimize nonspecific binding and maximize sensitivity, and may include competitive or noncompetitive reactions with the antibodies employed. This design format may require sample preparation steps, is laborious, time-consuming, and relatively expensive. For drug testing, heterogeneous assays are used most often for specimen types that require greater sensitivity than urine, such as blood, hair, and oral fluid. Homogeneous assays do not incorporate wash steps, and usually do not require sample preparation. This design format is relatively simple, fast, and less expensive than a heterogeneous design format. Because there are not wash steps, a homogeneous assay requires that a unique signal be generated when an analyte is bound to the capture antibody, versus not bound. Most homogenous urine drug tests are based on a competitive reaction between a known amount of labeled drug analyte that is included in the test, and any drug analyte that may be present in a urine sample. The cutoff concentration reflects the amount of labeled drug, and some immunoassay manufacturers offer more than one cutoff concentration for a test.

Opiate test reagents, for example, may be available with SAMHSA cutoff concentrations, and with lower cutoff concentrations, targeted for clinical use. In any case, when a drug analyte concentration produces signal that falls below the signal required to exceed the cutoff, the test result would be considered negative, even though the drug is present.¹⁸

Point of collection (POC) formats for urine drug testing include independent test strips (dip sticks) or test strips incorporated into a cartridge, card, or urine collection cup and may not require skilled personnel to perform (e.g. CLIA-waived, FDA-cleared test devices). All POC tests and most laboratory urine drug tests are of homogeneous formats. For a typical POC test, the urine comes into contact with an absorbent material on the test strip and is pulled across the strip. The test strip contains immobilized antibodies that are specific for a drug class, parent drug, or metabolite. If the urine sample does not contain the drug or metabolite that is tested by the test strip, the test strip may display a colored line for each drug that is not detected. If the urine sample contains (a) drug(s), the test strip for the specific drug does not display a color and the test result is a presumptive positive. POC tests should contain a "control" to prove that the device is working correctly. If the control line does not appear on the respective test strip, the test is invalid. The advantages of using POC tests is convenience, ease of use, low per-test costs, and rapid time to result. Results are usually available within 5 to 10 minutes.

A limitation of POC tests is that results must be interpreted and recorded manually at a defined period after the test is initiated. Results may be invalid as little as 10 minutes after adding urine to the device, making it a challenge to archive results and impossible to identify transcription errors.^{19,20} Performance

of POC tests has been shown to exhibit wide variation between manufacturers and between manufactured lots/batches. Training to handle, utilize, and interpret results generated by these devices should be conducted, and proficiency of skills verified annually, even in a non-CLIA (Clinical Laboratory Improvement Amendments) environment. The labeling for any CLIA-waived device should also be consulted to assure compliance with the CLIA-waived status. For example, a device label may indicate that any positive result can be confirmed per the federal drug testing model. Violation of the manufacturer's instructions may invalidate the CLIA-waived status of the device. Some performance characteristics may be sample specific, and may not be recognized without additional testing. For example, urine samples can be adulterated with chemicals to degrade the drug in urine or to reduce antibody binding in immunoassays, leading to false-negative results.²¹ Some devices evaluate urine quality by including test strips for temperature and common adulterants. A discussion of adulterants is beyond the scope of this review.

Immunoassays performed in a clinical laboratory setting are typically based on liquid reagents that are handled using common automated chemistry analyzers, and are performed/supervised by skilled, licensed laboratory personnel. As with POC tests, each testing product/system will vary in cross-reactivity profiles for drugs of interest. Detection chemistries may include colorimetry, fluorescence, fluorescence polarization, turbidimetric, chemiluminescence, nephelometric, agglutination, or enzyme activity. Automated immunoassay testing is more expensive than POC tests, and is classified as "nonwaived" by CLIA, due to its moderate or high complexity regulatory status, but is reimbursed at higher rates than POC testing.²² Laboratories routinely verify performance of testing and personnel with previously characterized quality control materials, and peer-reviewed proficiency testing. In addition, the automation component of testing is typically computerized and associated software can facilitate analysis, archiving of results, and may interface results directly into the medical record.

Separation of Urine Components for Targeted Methods

Urine as a sample

Human urine is composed largely of water (~95%) with the remainder including a variety of organic molecules such as urea, uric acid, creatinine, carbohydrates, and trace amounts of protein. Inorganic ions such as chloride, sodium, potassium, and magnesium are also common components of urine, as are drugs and drug metabolites. Individual health status and/or

disease state, as well as hydration of the urine donor at the time of specimen collection can affect the actual composition and concentration of urine between individuals and even within a given individual throughout the day. The urine pH can vary considerably depending upon diet and disease state; urine pH can affect extent and efficiency of drug elimination. Therefore, urine is a highly variable and somewhat unpredictable specimen for drug detection.

Strategies have been employed to "normalize" urine, to account for the known variation in hydration status, using creatinine or specific gravity. Creatinine excretion in healthy individuals is considered constant regardless of other kidney processes and hydration state.²³ Creatinine concentration is used by SAMHSA to evaluate whether or not a donor has attempted to subvert the test by excessively diluting the specimen, such as by deliberate overhydration, or by physically manipulating the donated specimen. Specific gravity is also recognized by SAMHSA to determine if a urine specimen is excessively dilute, and to qualify an individual specimen as valid.²¹ These considerations are also important when qualifying specimens and interpreting results of drug testing for pain management patients because the composition of the urine can alter the concentration of a drug present for testing.

Normalization of urine drug concentrations to creatinine or specific gravity has been applied to adherence testing in the chronic pain management population. When comparing results to a cutoff concentration, the result may change from negative to positive or from positive to negative, as a consequence of normalization. For example, the percent of samples positive for the common alprazolam metabolite increased (27%) when results were normalized to specific gravity, and increased (15%) when results were normalized to creatinine; the detection of codeine was increased (6.7%) when normalized to specific gravity, but was decreased (−5.2%) when normalized to creatinine.²⁴ Proposed cutoff concentrations adjusted for creatinine are included in Table 3.⁹ Creatinine and specific gravity normalization are particularly useful to help interpret serial results for drugs with a long half-life, such as marijuana.^{25–27}

Preparation of Urine for Targeted Analysis

All targeted analytical methods for urine drug testing incorporate preanalytical sample preparation steps and/or reactions.²⁸ The goal of sample preparation is to minimize potential interferences in the sample matrix that will hinder detection of drug analytes, or potentially damage the analytical equipment. Sample preparation is sometimes used to improve detection of drug analytes, such as through derivatization or

hydrolysis reactions. A sample may also be concentrated to promote detection of trace amounts of drug analytes. For quantitative analysis, the sample preparation process includes addition of internal standards that facilitate calculation of the unknown drug concentration(s), and may reflect quality of the data. Some of the common sample preparation techniques are described below.

Dilution

Sometimes drugs are excreted into urine in sufficient amounts that dilution is a useful approach, so as not to saturate the chromatographic column, detector or other components of the analytical instrument. The other outcome is that potential interferences (natural urine components) are diluted to the point of not interfering with the analysis. This is a fast and simple process whereby a fixed volume of urine is combined with a fixed volume of diluent (e.g. water, dilute buffer, or other compatible solvent). Often, the urine is centrifuged prior to dilution, to separate any particulates or precipitate suspended in the collected specimen. In that case, the clarified urine is collected for dilution and the sediment is avoided or discarded. This approach is often called “dilute and shoot” in laboratory jargon.

The advantage of sample dilution is that it is fast, simple, and subsequently is the least costly sample preparation method with respect to both labor and supplies. The potential disadvantage is that dilution does not improve or otherwise clean up the sample, and the optimum dilution for any individual sample is not known. A highly dilute (over-hydrated) urine specimen further diluted by the laboratory can result in a false-negative test because the additional dilution has rendered the measured drug concentration undetectable. Conversely, a urine sample with large relative concentrations of physiological wastes or disease biomarkers (e.g. urea, uric acid, protein) can contribute to analytical interferences that may not be sufficiently reduced by dilution alone.

Precipitation

Proteins are “sticky” molecules that can clog or damage delicate instrumentation. Proteins can effectively bind and thereby “trap” some drug analytes, preventing or reducing detection by an analytical method. While routinely applied to samples containing a lot of protein (e.g. serum and/or plasma), precipitation may be applied to a urine specimen as a precaution, to remove protein that may be present. The procedure may be considered an extension of dilution, because the solution(s) used for precipitation will, by default, dilute the sample. Typically precipitation is accomplished by combining a fixed volume of organic

solvent or acid with a fixed volume of urine; insoluble components such as proteins precipitate forcefully out of solution. The sample is then clarified by centrifugation and the supernatant is collected and used for analysis or additional preanalytical sample preparation.

Liquid-Liquid and Supported Liquid Extraction

Another common sample preparation technique is liquid-liquid extraction (LLE), a modification of which is supported liquid extraction (SLE). The fundamental principle of this technique is solvent partitioning of urine components. An aqueous (and therefore polar) urine specimen is mixed with a nonpolar organic solvent (e.g. hexane). Polar drugs and metabolites in the urine are chemically manipulated to make them more nonpolar by altering the pH of the urine specimen. Through the process of mixing, nonpolar urine components (drugs/metabolites of interest and other urine components) are transferred into the organic phase. The phases are physically separated by centrifugation; the organic phase is collected and evaporated. One concern with evaporation, which often involves heat, is that some analytes may degrade and volatile analytes may be lost. A laboratory needs to carefully validate the recovery efficiency of the extraction method. After evaporation, the residue is reconstituted in an appropriate solvent for instrument analysis. Depending on the reconstitution volume, the analytes may become more concentrated than in the original urine sample, which may facilitate detection. For SLE, a high purity, inert diatomaceous earth sorbent helps to facilitate the extraction process, and improves the potential for automating the extraction. Automation of LLE methods has historically been difficult, but the introduction of SLE has greatly reduced that challenge. Depending on the scenario, SLE may provide cleaner extracts than traditional LLE as well, and is therefore growing in popularity.

Advantages of liquid extraction include relatively simple sample preparation using easily obtained laboratory solvents. Liquid extraction methods can be relatively nonselective which can be an advantage in that it is useful when a large number of analytes need to be detected. This can also be a disadvantage in that undesirable urine components can also be extracted adding potential interferences to the analysis. Drugs and metabolites typically need to possess an ionizable functional group (e.g. amino or carboxylate group) to successfully obtain a clean extract. Liquid extractions can be time consuming and costly due to the many steps required to isolate the sample extract, and opportunities for error when performed manually. Organic solvents also pose hazards to laboratory

personnel and the environment, and must be handled with engineering controls for safe storage and handling, in addition to appropriate waste disposal protocols.

Solid Phase Extraction

Solid phase extraction (SPE) is another commonly used sample preparation technique. SPE shares the basic principles of liquid chromatography (see below) wherein the desired drug analytes are held or “retained” with a solid support material (silica- or polymer-based particles, packed in a column) via ionic and/or hydrophobic chemical interactions while the undesirable matrix interferences are washed through the support material with various solutions, to a waste receptacle. The support material is typically washed with an appropriate buffer to remove any incidentally retained matrix interferences. Retained analyte molecules are removed from the support material by washing through a nonpolar solvent at an appropriate pH to disrupt the hydrophobic and/or ionic interactions that was originally established to retain the analytes. This collected eluent is then evaporated (concentrated) and the residue is reconstituted for analysis as with LLE above.

Advantages of SPE are that it can be highly selective for the chemical properties of the drug analyte of interest and can therefore produce clean extracts, virtually free of matrix interferences. From that perspective, SPE is the preferred sample preparation for most traditional confirmation assays. However, some drug analytes are more efficiently extracted by LLE.²⁹ SPE is amenable to automation and can therefore be accomplished reasonably quickly and consistently by the laboratory. Disadvantages, as compared to the other sample preparation methods discussed thus far include the increased costs of the SPE materials (i.e. columns), particularly when special chemistries are required to retain and/or elute specific drugs and metabolites.

Hydrolysis

As mentioned previously, hydrolysis is a common preanalytical reaction that is performed to liberate glucuronide conjugates for drugs that are eliminated in a glucuronidated form, including many opioids and benzodiazepines. For example, the morphine-3-glucuronide conjugate is converted to morphine by hydrolysis, to increase the amount of morphine in the urine, and thereby increase the likelihood of detection in a targeted assay that detects morphine, but not the glucuronide. Hydrolysis will not benefit detection of drugs such as hydrocodone, because it is not eliminated as a glucuronide conjugate.

Hydrolysis can be performed enzymatically or chemically. Variables in the efficiency of hydrolysis reactions include temperature, time, and concentrations of both the glucuronides and the enzyme and/or chemical reagents added to perform the hydrolysis. The major disadvantages of hydrolysis include variable efficiency of the reaction and the possibility of destroying the analyte of interest. Thus, the percent recovery of morphine-3 and morphine-6-glucuronides as well as hydromorphone glucuronide, was 95% to 100% when hydrolyzed with hydrochloric acid, but zero to 50% when hydrolyzed with *H. pomatia* or *E. coli* β -glucuronidase.³⁰ However, enzymatic hydrolysis is preferred for opioid assays if one hopes to detect 6-acetylmorphine, because that heroin metabolite is destroyed by chemical hydrolysis reactions³¹ preventing detection of heroin use. Enzymatic hydrolysis is also preferred for benzodiazepines because chemical hydrolysis has proven too harsh for this class of drugs, leading to degradation of the drug analytes. Of interest, enzymatic hydrolysis has also been shown to transform oxazepam to nordiazepam, thus preventing accurate identification of parent drug.³²

Laboratories that utilize hydrolysis reactions as a component of routine sample preparation should include glucuronide conjugate material as a quality control sample for every analytical batch, to assure that the reaction occurs according to assay specifications. Alternatively, measurement of the “free” drug analyte, in conjunction with direct analysis of glucuronide conjugates eliminates the need for hydrolysis reactions, can improve positivity rates of some drugs, and can offer information about a patient’s metabolic phenotype that may not be realized otherwise.^{10,13}

Chromatography

In spite of a laboratory’s best effort to create a sample free from potential analytical interferences using the preparation methods described above, urine still presents as a complex mixture of components that can potentially confound the ability to reliably measure drugs and metabolites. In addition, specific drug analytes typically need be separated from one another to achieve the specificity expected of targeted assays. Chromatography is a separation method that incorporates a solid phase, and a mobile phase.^{28,33} The solid phase is contained within the analytical column, and the mobile phase is most commonly gas (GC) or liquid (LC). Drug analytes interact with the solid and mobile phases in a characteristic way that is measured relative to the time required to elute the analytes from the solid phase and be detected (e.g. retention time).

TABLE 6. Examples of Molecular Formulas and *m/z* for Selected Opioids

Opioid	Molecular formula	Nominal <i>m/z</i>	Accurate <i>m/z</i>
Morphine	C ₁₇ H ₁₉ NO ₃		
Hydromorphone			
Norcodeine		285.1	285.13649
Norhydrocodone			
Noroxymorphone	C ₁₆ H ₁₇ NO ₄	288.3	288.31842
Nornaloxone			
Codeine	C ₁₈ H ₂₁ NO ₃	299.1	299.15214
Hydrocodone			
Oxymorphone	C ₁₇ H ₁₉ NO ₄		301.13141
Noroxycodone	C ₁₈ H ₂₃ NO ₃		301.16779
Morphine <i>n</i> -oxide		301.1	
Dihydrocodeine			
6-monoacetylmorphine	C ₁₉ H ₂₁ NO ₄	327.1	327.14706
Naloxone			

As such, a chromatogram that displays signal intensity over time is generated, and components appear as discrete peaks that when examined closely, resemble a Gaussian-shaped bell curve.

Chromatographic separation is particularly important when the urine sample contains multiple drugs and metabolites of analytical interest, and is critical when drug analytes are of the same or similar masses. Table 6 lists examples of opioids that share the same or similar molecular formulas and masses, leading to isobaric interferences. When compounds share the same mass, the analytical instrument cannot distinguish them and may not be able to correctly identify a drug or drug metabolite. Some isobaric compounds can be separated chromatographically, such as morphine and hydromorphone, but others, such as noroxymorphone and nornaloxone, which are chemically identical, cannot.³⁴ After separation of the compounds by chromatography, compounds are identified with a variety of detectors (e.g. UV-VIS, FID, ECD, fluorometric, NPD). For the purposes of this discussion, only mass spectrometry, the predominant high sensitivity & specificity detector, coupled to chromatographic separation, will be reviewed.

Gas Chromatography (GC)

Separation of analytes using gas chromatography (GC) has been applied to drug testing for decades, and was once the only chromatography system suitable for detection by a mass spectrometer. The principle of separation is selective interaction of gas phase drug molecules traveling through a narrow-bore column with the film coating on the inside of the column. The mobile phase is an inert gas, such as helium, and heat is often used to modify the rates of travel through the column. Analytes with little affinity for the film

elute quickly from the column, analytes with a high affinity for the column film elute later.

While somewhat supplanted by the widespread adoption of LC in recent years, GC still offers some important advantages as a chromatographic technique. For example, an extensive database of mass spectral data used in the identification of unknown compounds has been compiled for GC-MS, based on historical data from many sources. Laboratories utilizing GC-MS can apply “library matching” software to identify unknown compounds in a urine specimen. The experiments used to generate these libraries are very reproducible allowing inter-laboratory comparison of collected data. Also, there still exist several classes of hydrophobic drug molecules of clinical interest (e.g. cannabinoids, barbiturates, alcohol, etc.) for which GC-MS remains the preferred chromatographic method.

A primary disadvantage of GC is the need for complex sample preparation. Samples must be volatile, or otherwise able to exist in the gas phase, under conditions of heat. Many drugs degrade with heat, and have polar, nonvolatile metabolites. As such, urine is typically extracted and reconstituted with a volatile organic solvent. Chemical derivatization is routinely used for drug analysis to chemically alter compounds of interest to make it stable enough and volatile enough to be introduced into the GC-MS system. These typically manual, time-consuming steps add to the analytical time and cost.

Liquid Chromatography

Liquid chromatography (LC) provides chromatographic separation of matrix components using a liquid mobile phase and this is the preferred separation technology for most drug assays performed today. The reason for this is simple solution chemistry.

If a sample analyte can be dissolved into an aqueous liquid (which the overwhelming majority can), it can be analyzed using LC. By using multiple liquids, and the many options for analytical columns, LC can effectively separate mixtures of high molecular weight components like proteins, peptides, and nucleotides. Specialized chromatographic conditions allow for the separation of stereoisomers as well, such as D- and L-methamphetamine, or levorphanol and dextrorphan.

Separation principles for LC are similar to SPE sample preparation, because LC involves hydrophobic and ionic interactions between the solid support material inside the LC column with the drugs dissolved in the liquid that is being pumped through the column. The mobile phase is typically a mixture of aqueous and organic solvents that may change during analysis, to maximize resolution of closely related compounds. The complex mixture of a urine sample extract is separated on the basis of relative affinity of a component for the LC column material, and the mobile phase pumping through. In spite of the advantages of LC in urine drug testing, there are some disadvantages to consider. LC may generally be described as more complex than GC in that there are more variables to be considered and optimized. With that complexity, LC systems tend to be more expensive than GC systems. In addition, liquid handling systems (pumps, injectors, etc.) physical dimensions and chemistry of the LC column, the choice of mobile phase and the optimal mobile phase mixtures must all be optimized for a particular analysis.

A major concern with LC-MS systems is a phenomenon called ion suppression, in which components in the sample, or the mobile phases, can cause a suppression of the ionization (see below) and subsequent detection of the analytes of interest. This can be a particular problem with urine specimens due to the highly variable nature of urine from one specimen to the next and must be thoroughly evaluated by the laboratory to prevent the misinterpretation/misreporting of a urine drug test.

Mass Spectrometry

Ionization

A fundamental prerequisite for detection of drug molecules by mass spectrometry^{28,33}, regardless of the chromatography system, is that the molecule must present to the instrument as a gas phase ion. The most common ionization techniques used in clinical urine drug testing are electron ionization for GC-MS, and atmospheric pressure ionization for LC-MS. Although chemical and other ionization techniques are sometimes used for drug detection, such alternate techniques are not discussed here.

Electron ionization is most commonly associated with GC-MS. Sample molecules are vaporized at the point of injection into the GC-MS system and are therefore separated chromatographically while in the gas phase. Once the separated molecules reach the end of the chromatographic column, they exit into a high-vacuum region of the GC-MS called the ion source. Here the molecules are bombarded with high energy electrons. This bombardment tends to fragment the molecules into ionized pieces that can then be detected by the GC-MS. The pattern and complement of these pieces create a mass spectrum which relates signal intensity over a mass range. The mass spectrum is very characteristic of a particular molecule and constitutes the data that makes up the mass spectral libraries used to identify unknown compounds in a particular sample (see above). The mass spectrum is often referred to as the “molecular fingerprint” of a drug analyte.

Atmospheric Pressure Ionization (API) is associated with LC-MS. Liquid phase molecules in the prepared urine specimen are injected into the LC-MS system and separated (in the liquid phase) as they travel through the LC column. The pH of the mobile phase may be controlled to promote ionization of the molecules of interest. Once the ions exit the LC column, they are introduced into the ion source of the LC-MS, this time at atmospheric pressure. The most common ionization technique at atmospheric pressure for LC-MS applications is electrospray. Here the LC flow is sprayed from the end of a thin metal capillary with a voltage applied. The tiny droplets formed by this electrospray process are rapidly evaporated with a combination of heat and drying gas to the point that ionized sample molecules are ejected from the droplet into the gas phase. These gas phase ions are then directed deeper into the LC-MS for detection.

A significant disadvantage compared to electron ionization is the phenomenon known as ion suppression.^{35–37} The electrospray process can be thought of as competitive. A very high concentration of an interference in an electrospray droplet will out-compete the relatively low concentration of the drug molecule of interest in the same droplet and therefore not be ionized and subsequently, not detected. This phenomenon must be carefully and thoroughly evaluated and subsequently controlled in order for any electrospray method to produce reliable and quality data, but could be a reasonable analytical explanation for an unexpected negative result, if the assay performed is vulnerable to relevant ion suppression.

Nominal Mass Analysis

Once the sample molecules are ionized they travel further into the MS. Here the ions are sorted and filtered

on the basis of their mass to charge (m/z) ratio. Ions of interest are selected and subsequently detected. Any other ions that represent contaminants in the sample are filtered out of the instrument because they do not correspond to a selected m/z . Several configurations of mass analyzers exist and each provides unique data that are better suited for a particular result depending upon the particular goals of the analysis. For the purposes of this discussion, we can group them into two basic categories: unit resolution analyzers and high-resolution (also known as accurate mass) analyzers. See the discussion on high-resolution analyzers below.

Unit resolution analyzers are also known as single-stage quadrupoles or triple quadrupoles (see below). These names are derived from the physical configuration and specific architecture of the MS. These instruments can reliably distinguish between single, whole mass units. In other words they can distinguish between m/z of 350 and 351 reliably and reproducibly when used correctly. Historically, GC-MS instruments have been unit resolution single-stage quadrupole instruments. These are very useful in identifying compounds, and determining concentrations in urine specimens. Some limitations have emerged when using single quadrupole instruments in LC-MS applications. Due to the nature of atmospheric pressure ionization, fragmentation is limited, as compared to electron ionization used in GC-MS. Because of this, selectivity of the “molecular fingerprint” can be severely compromised in LC-MS. Also, because LC-MS applications can be prone to ionization suppression from co-eluting interferences (see above), the available signal for detecting the drug molecule may be diminished sufficiently to prevent detection. Single quadrupole LC-MS instruments may not have the sensitivity to overcome this suppression.

An evolution of the single quadrupole mass analyzer is the triple quadrupole mass analyzer, also known as the tandem MS or MS/MS. This mass analyzer uses three quadrupole-based assemblies arranged in series and is well suited to overcome the limitation of single-stage quadrupole mass analysis seen with LC-MS. Briefly, the first quadrupole mass analyzer (Q1) is used to select intact ionized molecules of interest. Other ions are filtered to waste. The selected ions are sent into the second quadrupole-based section (Q2), called the collision cell. Here, the selected ions are subjected to high voltage and a collision gas to create an analogous situation seen in electron ionization. In the collision cell, selected ions are fragmented and these ionized fragments are sent into the third quadrupole mass analyzer (Q3). Laboratories monitor the abundances of

paired “transitions” from precursor mass, detected in Q1, to the product ion masses that are detected in Q3. It is preferred that laboratories monitor two transitions per compound to assure specificity of results. This creates a useful molecular fingerprint for the identification of drug molecules, somewhat analogous to the molecular fingerprint generated with GC-MS. However, the mass spectra (molecular fingerprint) generated by MS/MS cannot be compared with the mass spectra generated by GC-MS and thus, libraries used for identifying unknowns are currently being generated within specific laboratories, and are not necessarily useful or appropriate for inter-laboratory comparisons.

High Resolution Mass Analysis

High-resolution mass spectrometry measures the m/z of an ion with more accuracy than traditional unit resolution instruments, typically in the milliDalton (1 mDa = .001 Da) range. This increased mass accuracy is achieved by using time-of-flight, also known as “TOF”^{38,39}, or orbitrap^{40–43} technology. The more accurate mass resulting from a high-resolution instrument provides better specificity than unit resolution data generated by LC-MS or GC-MS instruments. High-resolution instruments have the ability to distinguish compounds of the same unit mass based on their fractional mass. For example oxymorphone and dihydrocodeine both have a molecular mass of 301 amu. The exact mass of oxymorphone is 301.13141 and the exact mass of dihydrocodeine is 301.16779. While this fractional difference could not be readily distinguished with a single-stage quadrupole MS, a TOF or orbitrap can easily make the distinction.

High-resolution instruments can also distinguish between different isotopes of specific atoms that make up a compound, for example being able to distinguish Carbon-12 from Carbon-13. Since the natural abundances of atomic isotopes are known, the expected abundance and the mass of compounds containing isotopes can be calculated from the chemical formula. The exact mass of a drug including the mass and the abundance of its isotopes can be used as additional criteria to validate the identity of a compound (in addition to retention time, etc). TOF and orbitrap instrumentation also continually collect full-spectrum data, as opposed to methods wherein a specific m/z is selected for detection and other ions are eliminated. One of the advantages of full spectrum data collection is the ability to retroactively search for compounds that may not have been of interest when the data was initially collected. This is not possible with traditional GC-MS or LC-MS/MS.

It is still possible to collect fragmentation data (molecular fingerprints, see above) using TOF or

orbitrap technology. Quadrupole- time-of-flight mass spectrometers (QTOF) and quadrupole-orbitrap hybrids add a collision cell similar to LC-MS/MS and collects high-resolution accurate mass data for an ionized molecule in addition to fragment data. High-resolution mass spectrometry has been used to detect drugs of interest in pain management in serum/plasma⁴⁴ and urine⁴⁵. High resolution techniques can be used for qualitative detection of drugs and metabolites, or compounds can be quantitated if required, depending on the application and clinical need. There is currently research underway to utilize high-resolution mass spectrometers to accurately identify drug analytes without complicated chromatographic separation (e.g. direct injection), which could reduce complexity of testing and costs significantly.⁴⁶

Quantitative versus Qualitative Testing

Depending upon the nature of the analytical test design, results can be qualitative or quantitative. Qualitative results report the presence or absence of a drug above a method-specific threshold (defined by a commercial manufacturer or the laboratory). The type of instrument used for the analysis can be optimized to provide qualitative versus quantitative results. Immunoassays, as discussed above, are largely qualitative, but could be designed to provide quantitative results by adding calibrators and evaluating the change in signal with concentration of drug analyte. TOF and other high-resolution instruments can also be used to replace a traditional screen, and detect a large menu of drugs and drug metabolites. Such targeted drug detection panels are particularly useful when immunoassays do not perform well, such as for opioids and benzodiazepines.

The primary difference between targeted qualitative and quantitative analysis is the inclusion of several calibrators and internal standards, which are designed to generate a concentration versus response curve. In a qualitative test, the assay will be optimized to perform best at the cutoff. For a quantitative test, the assay is optimized to perform within laboratory-established precision and accuracy criteria over a range of concentrations defined as the analytical measurement range (AMR). The lower limit of the AMR is the limit of quantitation (LOQ), and the concentrations across the range are mathematically calculated, usually based on the analyte signal divided by the signal for an internal standard. Concentrations below the LOQ may be determined, at least to the limit of detection (LOD) for the assay system, but concentrations that fall between the LOD and the LOQ may not meet quality standards established by the laboratory for acceptance and reporting

of the data. Examples of acceptance criteria include accuracy and precision expectations, a manual review of the chromatography and retention times, evaluation of ion response ratios, instrument performance, review for evidence of analytical carryover, quality control performance, etc. In order for the calculations to be accurate, the instrument must have a linear response across a broad range of relevant concentrations. Single quadrupoles and triple quadrupoles are particularly well suited for quantitative analysis.

Analytical Explanations for Unexpected Results

As discussed above, a fully compliant patient with a well-preserved and appropriately collected specimen could be faced with an unexpected drug testing result. An unexpected result should stimulate an investigation of potential explanations such as those listed in Table 1. When we consider analytical causes of unexpected results, it is first important to know what test was performed and what the test was designed to detect, at what concentrations. When analytical processes are suspected as a cause of the unexpected result, the laboratory should be notified so that they can participate in the investigation. Some examples of unexpected results that the laboratory has helped resolved, or could help investigate are described below.

A finding that may have been viewed as an interference initially is the observation that patients who are prescribed Suboxone may test positive for noroxymorphone. Because Suboxone is a co-formulation of buprenorphine and naloxone, it is possible for patient urine to contain both parent drugs, and associated metabolites. A common naloxone metabolite, nornaloxone, is chemically identical to noroxymorphone.³⁴ As such, laboratories cannot distinguish whether the metabolite came from naloxone, oxymorphone, or oxycodone. This example illustrates how measuring patterns of analytes can prevent misinterpretation of an unexpected drug test results.

With increasingly more sensitive instrumentation, laboratories have been detecting minor metabolites and pharmaceutical impurities. For example, small amounts of codeine are observed with large amounts of morphine.⁴⁷ This pattern of results is consistent with pharmaceutical impurity. Quantitative analysis, along with evaluation of the patterns of results, can resolve this unexpected result. Another example of an unexpected result that was best resolved with input regarding the clinical scenario is the observation that hydromorphone occurs in small quantities, with high concentrations of morphine. Hydromorphone was subsequently characterized as a minor metabolite

of morphine.⁴⁸ Alternatively, examples such as these may be evidence that cutoff concentrations are being pushed too low, and should be established to minimize potentially confusing results such as these.

Analytical interferences or poor assay performance can also contribute to unexpected results. For example, spectrophotometric detection methods common to immunoassays and some chromatographic detectors will absorb light at the same wavelength as the drug/compound of interest or interfere with the reaction. Cross-reactivity of an immunoassay can occur with unexpected drugs or substances such as herbal products, dietary supplements, or over-the-counter medications. Analytical interferences, leading to apparent positive results, are well described for antihistamines, antidepressants, antipsychotics, antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), and select drug screen assays, particularly with immunoassays designed to detect amphetamine-like drugs.^{49,50} NSAIDs have also been reported to cause false-positive urine drug screen results for cannabinoid, barbiturate, benzodiazepine, and PCP immunoassays.⁵¹

With both targeted testing and immunoassays, analytical carryover caused by analyzing a very concentrated sample prior to a negative sample, has been reported to produce an apparent positive result in the negative sample. Carryover can occur during sample preparation, liquid handling, chromatography, or as a result of instrument contamination.^{52,53} Another instrument-based source of inaccurate results is the unfortunate production of 6-acetylmorphine through heat or chemical reaction, when testing highly concentrated morphine samples.^{21,54} Laboratories need to assure that their analytical methods do not create drugs, the interpretation of which would negatively impact the patient. In some cases, 6-acetylmorphine has been detected in the absence of morphine.⁵⁵ When this occurs and is not expected, it should be questioned and investigated.

Unexpected results such as those described above are best defined through communication between the laboratory and the provider. The laboratory should offer repeat testing, or perform secondary testing using a different, perhaps more specific methodology. These data, along with the clinical and pharmacy history, as well as input from the provider, can help characterize and prevent the contribution of analytical methods to unexpected results, and improve interpretation for all. In conclusion, the analytical choices to support urine drug testing for the purpose of adherence monitoring are diverse, and not well standardized. The best utilization of immunoassays and targeted testing will align clinical needs, actual test capabilities, and costs of testing, and may thereby represent

a combination of tests that each perform as needed.

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REFERENCES

- [1] Benzon HT, Kendall MC, Katz JA, Benzon HA, Malik K, Cox P, et al. Prescription patterns of pain medicine physicians. *Pain pract: the official journal of World Institute of Pain*. 2013;13(6):440–450. PubMed PMID: 23228095.
- [2] Manchikanti L, Abdi S, Atluri S, Balog CC, Benyamin RM, Boswell MV, et al. American Society of Interventional Pain Physicians (ASIPP) guidelines for responsible opioid prescribing in chronic non-cancer pain: Part 2—guidance. *Pain Physician*. 2012;15(3):S67–S116. PubMed PMID: 22786449.
- [3] Manchikanti L, Abdi S, Atluri S, Balog CC, Benyamin RM, Boswell MV, et al. American Society of Interventional Pain Physicians (ASIPP) guidelines for responsible opioid prescribing in chronic non-cancer pain: Part I—evidence assessment. *Pain Physician*. 2012;15(3):S1–S65. PubMed PMID: 22786448.
- [4] Cone EJ, Caplan YH, Black DL, Robert T, Moser F. Urine drug testing of chronic pain patients: licit and illicit drug patterns. *J Anal Toxicol*. 2008;32(8):530–543. PubMed PMID: 19007501.
- [5] Couto JE, Romney MC, Leider HL, Sharma S, Goldfarb NI. High rates of inappropriate drug use in the chronic pain population. *Popul Health Manag*. 2009;12(4):185–190. PubMed PMID: 19663620.
- [6] Owen GT, Burton AW, Schade CM, Passik S. Urine drug testing: current recommendations and best practices. *Pain Physician*. 2012;15(3):ES119–ES133. PubMed PMID: 22786451.
- [7] Mikel C, Almazan P, West R, Crews B, Latyshev S, Pesce A, et al. LC-MS/MS extends the range of drug analysis in pain patients. *Ther Drug Monit*. 2009;31(6):746–748. PubMed PMID: 19935363.
- [8] Melanson SE, Ptolemy AS, Wasan AD. Optimizing urine drug testing for monitoring medication compliance in pain management. *Pain Med*. 2013. PubMed PMID: 23899241.
- [9] Pesce A, West C, Egan City K, Strickland J. Interpretation of urine drug testing in pain patients. *Pain Med*. 2012;13(7):868–885. PubMed PMID: 22494459.
- [10] Dickerson JA, Laha TJ, Pagano MB, O'Donnell BR, Hoofnagle AN. Improved detection of opioid use in chronic pain patients through monitoring of opioid glucuronides in urine. *J Anal Toxicol*. 2012;36(8):541–547. PubMed PMID: 22833646.
- [11] Depriest A, Heltsley R, Black DL, Cawthon B, Robert T, Moser F, et al. Urine drug testing of chronic pain patients. III. Normetabolites as biomarkers of synthetic opioid use. *J Anal Toxicol*. 2010;34(8):444–449. PubMed PMID: 21819788.
- [12] Heltsley R, Zichterman A, Black DL, Cawthon B, Robert T, Moser F, et al. Urine drug testing of chronic pain patients. II. Prevalence patterns of prescription opiates and metabolites. *J Anal Toxicol*. 2010;34(1):32–38. PubMed PMID: 20109300.
- [13] McMillin GA, Davis R, Carlisle H, Clark C, Marin SJ, Moody DE. Patterns of free (unconjugated) buprenorphine, norbuprenorphine, and their glucuronides in urine using liquid chromatography-tandem mass spectrometry. *J Anal Toxicol*. 2012;36(2):81–87. PubMed PMID: 22337776.
- [14] Kricka LJ, Park JY. Principles of immunochemical techniques. In: Burtis CA, Ashwood ER, Bruns DE, editors. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 5th edition. St. Louis, MO: Elsevier Saunders; 2012. p. 379–99.

- [15] CLSI. Toxicology and drug testing in the clinical laboratory. *Approved Guide*. 2007;27(15).
- [16] CLSI. Immunoassay interference by endogenous antibodies. *Approved Guide*. 2008.
- [17] CLSI. Immunoassay systems: radioimmunoassays and enzyme, fluorescence, and luminescence immunoassays. *Approved Guide*. 2004;24(16).
- [18] Magnani B, Kwong T. Urine drug testing for pain management. *Clin Lab Med*. 2012;32(3):379–390. PubMed PMID: 22939297.
- [19] Greene DN, Lehman CM, McMillin GA. Evaluation of the integrated E–Z split key((R)) cup II for rapid detection of twelve drug classes in urine. *J Anal Toxicol*. 2011;35(1):46–53. PubMed PMID: 21219703.
- [20] Lin CN, Nelson GJ, McMillin GA. Evaluation of the NexScreen and DrugCheck Waive RT urine drug detection cups. *J Anal Toxicol*. 2013;37(1):30–36. PubMed PMID: 23144203.
- [21] SAMHSA. Washington DC: Substance Abuse and Mental Health Services Administration; [cited 2013 September 13]. Division of Workplace Programs Information on Drug Testing]. Available at: <http://workplace.samhsa.gov/DTesting.html>.
- [22] CMS. Centers for Medicare & Medicaid services; [cited 2013 September 15]. Clinical Laboratory Fee Schedule]. Available at: <http://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/ClinicalLabFeeSched/clinlab.html>.
- [23] Narayanan S, Appleton HD. Creatinine: a review. *Clin Chem*. 1980;26(8):1119–1126. PubMed PMID: 6156031.
- [24] Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL. Normalization of urinary drug concentrations with specific gravity and creatinine. *J Anal Toxicol*. 2009;33(1):1–7. PubMed PMID: 19161663.
- [25] Huestis MA, Cone EJ. Differentiating new marijuana use from residual drug excretion in occasional marijuana users. *J Anal Toxicol*. 1998;22(6):445–454. PubMed PMID: 9788519.
- [26] Schwilke EW, Gullberg RG, Darwin WD, Chiang CN, Cadet JL, Gorelick DA, et al. Differentiating new cannabis use from residual urinary cannabinoid excretion in chronic, daily cannabis users. *Addiction*. 2011;106(3):499–506. PubMed PMID: 21134021. Pubmed Central PMCID: 3461262.
- [27] Smith ML, Barnes AJ, Huestis MA. Identifying new cannabis use with urine creatinine-normalized THCCOOH concentrations and time intervals between specimen collections. *J Anal Toxicol*. 2009;33(4):185–189. PubMed PMID: 19470219. Pubmed Central PMCID: 3159564.
- [28] Flanagan RJ. Fundamentals of Analytical Toxicology. Chichester, England: John Wiley & Sons; 2007.
- [29] Juhascik MP, Jenkins AJ. Comparison of liquid/liquid and solid-phase extraction for alkaline drugs. *J Chromatogr Sci*. 2009;47(7):553–557. PubMed PMID: 19772726.
- [30] Wang P, Stone JA, Chen KH, Gross SF, Haller CA, Wu AH. Incomplete recovery of prescription opioids in urine using enzymatic hydrolysis of glucuronide metabolites. *J Anal Toxicol*. 2006;30(8):570–575. PubMed PMID: 17132254.
- [31] Coles R, Kushnir MM, Nelson GJ, McMillin GA, Urry FM. Simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, oxycodone, and 6-acetylmorphine in urine, serum, plasma, whole blood, and meconium by LC-MS-MS. *J Anal Toxicol*. 2007;31(1):1–14. PubMed PMID: 17389078.
- [32] Fu S, Lewis J, Wang H, Keegan J, Dawson M. A novel reductive transformation of oxazepam to nordiazepam observed during enzymatic hydrolysis. *J Anal Toxicol*. 2010;34(5):243–251. PubMed PMID: 20529458.
- [33] CLSI. Mass spectrometry in the clinical laboratory: general principles and guidance. *Approved Guide*. 2007;27.
- [34] Fang WB, Chang Y, McCance-Katz EF, Moody DE. Determination of naloxone and nornaloxone (noroxymorphone) by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *J Anal Toxicol*. 2009;33(8):409–417. PubMed PMID: 19874646.
- [35] Annesley TM. Ion suppression in mass spectrometry. *Clin Chem*. 2003;49(7):1041–1044. PubMed PMID: 12816898.
- [36] Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem*. 2003;75(13):3019–3030. PubMed PMID: 12964746.
- [37] Remane D, Wissenbach DK, Meyer MR, Maurer HH. Systematic investigation of ion suppression and enhancement effects of fourteen stable-isotope-labeled internal standards by their native analogues using atmospheric-pressure chemical ionization and electrospray ionization and the relevance for multi-analyte liquid chromatographic/mass spectrometric procedures. *Rapid Commun Mass Spectrom: RCM*. 2010;24(7):859–867. PubMed PMID: 20196193.
- [38] Ferrer I, Thurman EM. Liquid Chromatography-Time of Flight Mass Spectrometry: Principles, Tools and Applications for Accurate Mass Analysis. New York, NY: Wiley; 2009.
- [39] Mirsaleh-Kohan N, Robertson WD, Compton RN. Electron ionization time-of-flight mass spectrometry: historical review and current applications. *Mass Spectrom Rev*. 2008;27(3):237–285. PubMed PMID: 18320595.
- [40] Hu Q, Noll RJ, Li H, Makarov A, Hardman M, Graham Cooks R. The orbitrap: a new mass spectrometer. *J Mass Spectrom: JMS*. 2005;40(4):430–443. PubMed PMID: 15838939.
- [41] Makarov A. Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis. *Anal Chem*. 2000;72(6):1156–1162. PubMed PMID: 10740853.
- [42] Makarov A, Denisov E, Kholomeev A, Balschun W, Lange O, Strupat K, et al. Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer. *Anal Chem*. 2006;78(7):2113–2120. PubMed PMID: 16579588.
- [43] Perry RH, Cooks RG, Noll RJ. Orbitrap mass spectrometry: instrumentation, ion motion and applications. *Mass Spectrom Rev*. 2008;27(6):661–699. PubMed PMID: 18683895.
- [44] Marin SJ, Hughes JM, Lawlor BG, Clark CJ, McMillin GA. Rapid screening for 67 drugs and metabolites in serum or plasma by accurate-mass LC-TOF-MS. *J Anal Toxicol*. 2012;36(7):477–486. PubMed PMID: 22802572.
- [45] Crews BO, Pesce AJ, West R, Nguyen H, Fitzgerald RL. Evaluation of high-resolution mass spectrometry for urine toxicology screening in a pain management setting. *J Anal Toxicol*. 2012;36(9):601–607. PubMed PMID: 22995480.
- [46] Leveridge M, Buxton R, Argyrou A, Francis P, Leavens B, West A, et al. Demonstrating enhanced throughput of RapidFire mass spectrometry through multiplexing using the JmjD2d demethylase as a model system. *J Biomol Screen*. 2013. PubMed PMID: 23896685.
- [47] West R, Crews B, Mikel C, Almazan P, Latyshev S, Pesce A, et al. Anomalous observations of codeine in patients on morphine. *Ther Drug Monit*. 2009;31(6):776–778. PubMed PMID: 19935365.
- [48] Cone EJ, Heit HA, Caplan YH, Gourlay D. Evidence of morphine metabolism to hydromorphone in pain patients chronically treated with morphine. *J Anal Toxicol*. 2006;30(1):1–5. PubMed PMID: 16620524.
- [49] Brahm NC, Yeager LL, Fox MD, Farmer KC, Palmer TA. Commonly prescribed medications and potential false-positive urine drug screens. *Am J Health Syst Pharm: AJHP: official journal of the American Society of Health-System Pharmacists*. 2010;67(16):1344–1350. PubMed PMID: 20689123.

- [50] Reisfield GM, Goldberger BA, Bertholf RL. 'False-positive' and 'false-negative' test results in clinical urine drug testing. *Bioanalysis*. 2009;1(5):937–952. PubMed PMID: 21083064.
- [51] Herring C, Muzyk AJ, Johnston C. Interferences with urine drug screens. *J Pharm Pract*. 2011;24(1):102–108. PubMed PMID: 21507878.
- [52] Lippi G, Romero A, Cervellin G, Mercadanti M. Unusual false-positive case of urinary screening for buprenorphine. *J Clin Lab Anal*. 2011;25(4):244–245. PubMed PMID: 21786326.
- [53] Hughes NC, Wong EY, Fan J, Bajaj N. Determination of carryover and contamination for mass spectrometry-based chromatographic assays. *The AAPS J*. 2007;9(3):E353–E360. PubMed PMID: 18170982. Pubmed Central PMCID: 2751487.
- [54] Paul BD, Shimomura ET, Smith ML. A practical approach to determine cutoff concentrations for opiate testing with simultaneous detection of codeine, morphine, and 6-acetylmorphine in urine. *Clin Chem*. 1999;45(4):510–519. PubMed PMID: 10102911.
- [55] Crews B, Mikel C, Latyshev S, West R, West C, Pesce A, et al. 6-Acetylmorphine detected in the absence of morphine in pain management patients. *Ther Drug Monit*. 2009;31(6):749–752. PubMed PMID: 19745789.

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