Retrospective Study

Lower Cutoffs for LC-MS/MS Urine Drug Testing Indicates Better Patient Compliance

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Background: Urine drug testing is used by health care providers to determine a patient's compliance to their prescribed regimen and to detect non-prescribed medications and illicit drugs. However, the cutoff levels used by clinical labs are often arbitrarily set and may not reflect the urine drug concentrations of compliant patients.

Objectives: Our aim was to test the hypothesis that commonly used cutoffs for many prescribed and illicit drugs were set too high, and methods using these cutoffs may yield a considerable number of false-negative results. The goals of this study were to outline the way to analyze patient results and estimate a more appropriate cutoff, develop and validate a high sensitivity analytical method capable of quantitating drugs and metabolites at lower than the commonly used cutoffs, and determine the number of true positive results that would have been missed when using the common cutoffs.

Study Design: This was a retrospective study of urine specimens submitted for urine drug testing as part of the monitoring of prescription drug compliance described in chronic opioid therapy treatment guidelines.

Setting: The study was set in a clinical toxicology laboratory, using specimens submitted for routine analysis by health care providers in the normal course of business.

Methods: Lognormal distributions of test results were generated and fitted with a trendline to estimate the required cutoff level necessary to capture the normal distributions of each drug for the patient population study. A validated laboratory derived liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis capable of achieving the required cutoff levels was developed for each drug and/or metabolite.

Results: The study shows that a lognormal distribution of patient urine test results fitted with a trendline is appropriate for estimating the required cutoff levels needed to assess medication adherence. The study showed a wide variation in the false-negative rate, ranging from 1.5% to 94.3% across a range of prescribed and illicit drugs.

Limitations: The patient specimens were largely sourced from patients in either a long-term pain management program or in treatment for substance use disorder in the US. These specimens may not be representative of patients in other types of treatment or in countries with different approaches to these issues.

Conclusions: The high-sensitivity method reduces false-negative results which could negatively impact patient care. Clinicians using less sensitive methods for detecting and quantifying drugs and metabolites in urine should exercise caution in assessing patient adherence using and changing the treatment plan based on those results.

Key words: Urine drug testing, patient adherence, clinical toxicology, immunoassay, LC-MS, definitive drug testing, REMS, negative test results, false negative

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It has been well-established that urine drug testing is an important component of treatment plans for patients on chronic opioid therapy (1-6). Much has been written about false-positive results (7,8), however in the clinical setting, false-negative results are just as important (8). Physicians monitoring patients on chronic opioid therapy presume that the cutoffs provided by their laboratory are like those reference intervals for specific analytes to establish possible health and disease states of a patient (9-11). Nevertheless, there are no such standards to test for compliance in patients being monitored for illicit drugs, pain, anxiety, or other scheduled medications. Most laboratories use cutoffs to determine patient compliance that have been established by the manufacturers of immunoassay reagents or the sensitivity of liquid chromatography tandem mass spectrometry (LC-MS/MS) instrumentation (12-15). In part, these cutoffs reflect those established for workplace testing or availability from immunoassay manufacturers. Complicating a simple interpretation of the drug test is the estimation that half of all medications are not taken as prescribed (16), and patients on chronic opioid therapy often take these medications depending on how they feel (17). This work was done to test whether clinically validated lower cutoffs would better describe patient compliance and detect illicit drug use.

We have described an algorithm using the frequency distribution of urine drug concentrations and suggest that this may be used to estimate appropriate cutoffs (18-20). By analogy to the usual clinical chemistry analytes, we chose to estimate if this mathematical model could estimate the lower cutoff. The problem in establishing such cutoffs is that pain patients take varying amounts of medication depending on how they feel or are prescribed. Because of this wide range of concentration values, transformation of the data to convert it to an approximation of a Gaussian curve is used.

It has been posited that 5 requirements must be met to identify appropriate cutoffs for pain management drugs and their metabolites. First, an analytical procedure that covers the wide dynamic range of urine excretion of these drugs and their metabolites must be developed and validated (12,13,21). Second, a large database of test results on this patient population must be obtained (22). Third, the urine samples must be clinically validated using creatinine and other validation tests (23). Fourth, mathematical models must be used to calculate the cutoffs (18-20). Finally, the results must be consolidated to provide the best estimates (18-20).

Our laboratory recently validated a more sensitive quantitative method for monitoring these drugs and metabolites. We compared the results of the high-sensitivity method to what would be reported using our previous cutoff levels, which in most cases reflected those commonly used by other laboratories to calculate a false-negative rate.

Methods

This is a retrospective study derived from specimens sent for urine drug testing from pain physician offices and rehabilitation centers. This study was approved by Aspire IRB (Santee, CA).

A high-sensitivity LC-MS/MS method was developed and validated, capable of quantitating low concentrations of 71 drugs and metabolites, which is important for monitoring medication adherence and substance use disorder.

A 20-XR series binary pump system (Shimadzu, Kyoto, Japan), well-plate autosampler, and temperature controlled column oven was paired with a 6500 triple quadrupole mass spectrometer (Sciex, Framingham, MA).

LC-MS grade water was obtained from an ultrapure water system (Sartorius, Bohemia, NY), LC-MS grade methanol was obtained from EMD Millipore (EMD Millipore, Billerica, MA), and LC-MS grade formic acid was obtained from Covachem (Covachem, Loves Park, IL). A Kinetex® phenyl-hexyl column (Phenomenex, Torrance, CA) with dimensions of 50 x 4.6 mm and 2.6 µm particle size was used for chromatographic separation. The binary pump system delivered a mixture of the mobile phases at the proportion and flow rate that is displayed in Table 1. Mobile phase A (MPA) was 0.1% formic acid in LC-MS grade water and mobile phase B (MPB) was LC-MS grade methanol containing 0.1% formic acid.

The mass spectrometers used the following settings common to all analytes: curtain gas of 35 L/min, collision gas of 10 L/min, positive mode IonSpray voltage of 2500 V, source temperature of 450°C, ion source gas 1 of 60 L/min, and ion source gas 2 of 50 L/min. Two transitions for each analyte were optimized for declustering potential collision cell energy and exit potential.

Analytes and internal standards were obtained from Cerilliant (Cerilliant, Round Rock, TX). Four-point calibration curves were prepared from the cutoff level to 30 times the cutoff level for each analyte. Two quality control samples were analyzed with each batch of specimens to ensure acceptability of results.

Table 1. *The false-negative rate of analytes across drug classes.*

Substance	Category	n	High-Sensitivity Cutoff (ng/mL)	Low-Sensitivity Cutoff (ng/mL)	False-Negative Rate
Amitriptyline	Antidepressants	431	10	100	14.6%
Nortriptyline	Antidepressants	456	10	100	23.9%
Venlafaxine	Antidepressants	397	2	50	9.8%
9-Hydroxyrisperidone	Antipsychotic	583	5	50	24.7%
Quetiapine	Antipsychotic	1155	5	50	9.7%
Norquetiapine	Antipsychotic	1142	25	50	2.5%
Clonazepam	Benzodiazapines	1343	5	50	94.3%
7-Aminoclonazepam	Benzodiazapines	2016	5	50	30.1%
Alprazolam	Benzodiazapines	3378	5	50	39.1%
Alpha-Hydroxyalprazolam	Benzodiazapines	3736	5	50	29.0%
Lorazepam	Benzodiazapines	1300	10	50	19.5%
Nordiazepam	Benzodiazapines	5473	5	50	14.6%
Oxazepam	Benzodiazapines	3315	10	50	22.4%
Temazepam	Benzodiazapines	2559	10	50	21.0%
Benzoylecgonine	Illicit Drugs	4252	5	50	44.9%
6-Monoacetylmorphine	Illicit Drugs	2183	5	25	19.3%
Carisoprodol	Muscle Relaxants	301	10	100	36.9%
Cyclobenzaprine	Muscle Relaxants	1193	5	100	44.8%
Hydrocodone	Natural Opiates	5667	5	50	32.9%
Norhydrocodone	Natural Opiates	5366	10	50	24.1%
Hydromorphone	Natural Opiates	9444	5	50	37.4%
6-Beta Naltrexol	Opioid Inverse Agonist	305	10	50	4.9%
Naloxone	Opioid Inverse Agonist	2472	10	100	22.0%
Zolpidem	Sedatives	464	1	50	81.0%
Buprenorphine	Semi-Synthetic Opiates	15142	5	10	3.3%
Norbuprenorphine	Semi-Synthetic Opiates	10194	5	10	1.5%
Oxycodone	Semi-Synthetic Opiates	1241	10	50	10.6%
Noroxycodone	Semi-Synthetic Opiates	4488	25	50	4.7%
Oxymorphone	Semi-Synthetic Opiates	2973	10	50	11.9%
Duloxetine	SNRI/SSRI	373	10	50	29.0%
Norfluoxetine	SNRI/SSRI	802	10	50	34.5%
Paroxetine	SNRI/SSRI	124	5	50	29.8%
Sertraline	SNRI/SSRI	555	10	50	7.2%
Amphetamine	Stimulants	3166	25	100	15.0%
Fentanyl	Synthetic Opiates	1418	1	5	31.7%
Norfentanyl	Synthetic Opiates	2246	2	5	10.2%
Tapentadol	Synthetic Opiates	119	2	50	18.5%
Tramadol	Synthetic Opiates	1548	25	50	6.1%

The samples were prepared by a "dilute and shoot" method described briefly here. A Freedom EVO® (Tecan, Maennedorf, Switzerland) was used to add 120 µL of urine specimen to a 96-well plate. Then, 30 µL of β-glucuronidase solution (IMCS, Columbia, SC) and 30 µL of 2 mM ammonium acetate buffer (Fisher Scientific, Waltham, MA) in water were added. The plates were incubated at 60°C for 30 minutes. Finally, 300

μL of methanolic internal standard solution and 500 μL of water were added to each specimen. Five μL of prepared specimen was injected onto the analytical column for LC-MS/MS analysis.

The results were analyzed using ASCENT software (Indigo BioAutomation, Inc., Indianapolis, IN). A 4-point calibration curve was used with a linear fit and one/x weighting. Calibrator acceptability was within ± 20% of the expected concentration with an R^2 value of greater than 0.98. The area ratio of the analyte to a deuterated internal standard was used to account for ion suppression. All analytes had a signal-to-noise calculation of greater than 10 at the lower limit of quantitation.

The precision and accuracy of the assay was evaluated over 5 days for both intraday and interday variability, and all analytes were within 20% CV. Recovery was determined to be within ± 20% for all analytes.

An estimate of the high-sensitivity cutoff levels required were determined by plotting previous patient data on a semi-logarithmic plot and using the intersection of an applied trend line and x-axis for each drug or metabolite in Microsoft Excel (Microsoft Corp., Redmond, WA). The lower limit of quantitation (LOQ) was set at the higher level of the trendline intersection and where $S/N \geq 10$.

Results

The Sciex 6500 LC-MS/MS is one of the most sensitive instruments available to the clinical laboratory. Its dynamic range is about 10^5. Compared to other instruments by the same manufacturer, it has a signal-to-noise and LOQ improvement of up to 5-fold over the widest range of compounds and about a 20-fold increase in detector dynamic range. The result is that the limit of quantitation can be set to be considerably lower than many other laboratories performing the same drug test. These lower limits of quantitation, often termed cutoffs, were used in this study (please see Table 1).

The lognormal distribution of hydromorphone (Fig. 1) shows the typical truncated bell curve found for most drugs analyzed with greater than 100 positive patient results during the study period. The trendline indicated a more appropriate cutoff level of 10 ng/mL. The analytical system was validated to 5 ng/mL for hydromorphone, so this lower cutoff value was used instead.

A retrospective analysis of 83,205 anonymized patient results, of which 46,717 had at least one positive analyte, was performed by plotting the concentrations on a lognormal distribution graph for compounds with greater than 100 positive results. The number of positive results with concentrations below the typical industry cutoff level (4,5,14), but above the high-sensitivity cutoff level, were determined. The ratio of these results to the number of low-sensitivity positives were used to calculate the false-negative rate.

The distribution of hydromorphone (Fig. 2) using the high-sensitivity method shows a bell curve that extends further down the normal distribution of results. This demonstrates that a greater proportion of the positive population is being captured by the high-sensitivity LC-MS/MS method. The low-sensitivity method with a cutoff level of 50 ng/mL has a 37.4% false-negative rate. The rates were calculated for all analytes with sufficient positive results and are displayed in Table 1, along with the cutoff levels used for the calculation.

Discussion

Our observations are not surprising. Previous urine drug testing work has shown that using lower cutoffs increases the number of positive results (24-26). The central problem is figuring out the most objective method of determining the optimal cutoff. We suggest using frequency distributions after log transformation offers a solution. This work shows that the lower cutoffs enabled by a more sensitive LC-MS/MS analysis allow good estimates of medication cutoffs from their observed distributions. We show that these cutoffs can readily be derived from patients' data. Visual inspection of the frequency distribution can be used to establish whether the cutoff is appropriate. Laboratory-derived cutoffs offer better clinical insights than arbitrary administrative cutoffs currently accepted by health care providers.

These data show the common cutoff levels are insufficient to adequately detect and quantitate most common prescription medications and illicit substances. This can result in false-negative reports being sent to the treating health care provider. Therefore, false-negative results can lead the health care provider to doubt a patient's adherence to the prescribed regimen and reduce or not prescribe needed pain medications. Also, drug diversion may be incorrectly suspected when cutoff levels are inappropriately set.

Our analysis of the distributions of the high-sensitivity urine drug concentrations show that a method with even greater sensitivity should be investigated for fentanyl, norfentanyl, cyclobenzaprine, oxazepam, temazepam, and zolpidem. Our data set was insufficiently large to determine cutoff levels for less com-

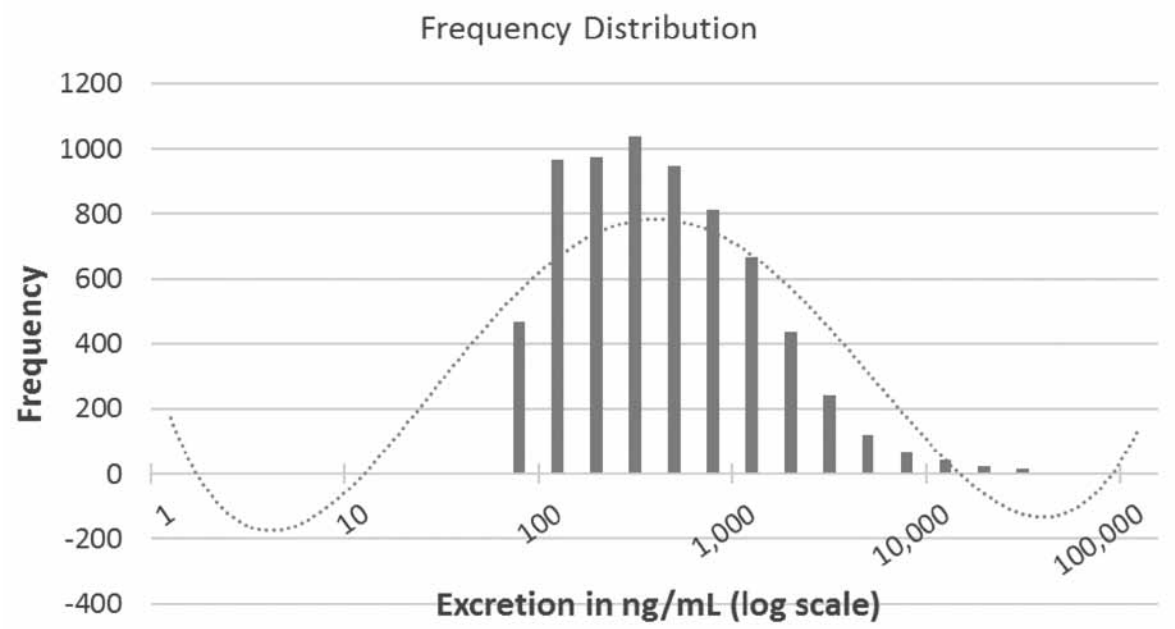

Fig. 1. *A plot of hydromorphone concentrations in urine. The lognormal distribution of positive patient samples shows a truncated bell curve at low concentrations when using the typical LC-MS/MS cutoff for hydromorphone. It shows that false-negative results are being reported due to incorrectly set cutoff levels.*

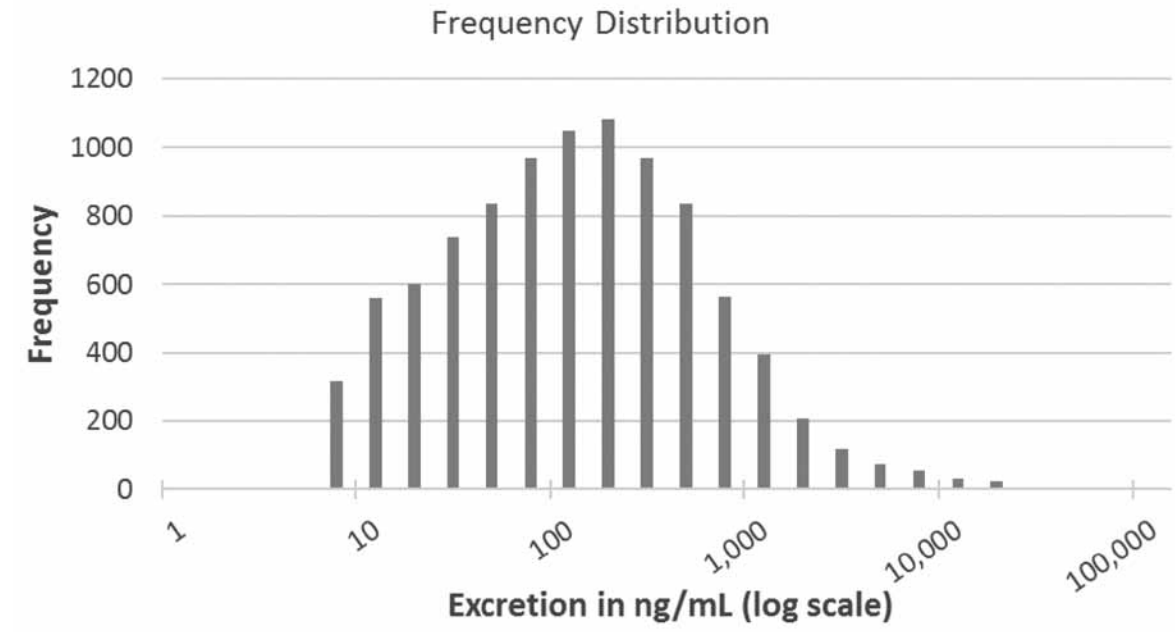

Fig. 2. *A plot of the same hydromorphone data using a lower cutoff. The lognormal distribution of positive patient samples show a more complete bell curve at low concentrations when using a high-sensitivity LC-MS/MS analysis, validated to 5 ng/mL.*

monly prescribed medications. Caution should be used when evaluating adherence based on urine drug test results for antidepressants and antipsychotics.

A high-sensitivity method was not investigated for the natural opiates morphine and codeine due to concerns about incidental ingestion from food sources. The cutoff levels for methadone and its metabolite EDDP were not lowered because a full distribution was observed at typical cutoff levels of 50 ng/mL.

It is important to consider that the distribution data comes from a patient population that is not representative of the world at large, but is indicative of patients who are frequently tested in the American health system. The population presented in this paper is likely over-representative of patients with chronic pain who are prescribed opioid analgesics and patients with substance use disorder.

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Dr. Kevin Krock, Dr. Agnes S. Cua, and Mr. Dennis Ritz designed the study protocol. Dr. Kevin Krock and Dr. Amadeo Pesce managed the literature searches and summaries of previous related work and wrote the first draft of the manuscript.

Dr. Kevin Krock provided revision for intellectual content and final approval of the manuscript.

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