What’s going on? Interpreting urine toxicology cases

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March 2024—For urine toxicology screening, immunoassays are automated and rapid but have variable sensitivity and specificity and results are considered presumptive. Mass spectrometry, used for confirmation, has superior sensitivity and specificity but is labor-intensive and slow and requires significant expertise.

“In my lab, it takes at least a year for a technologist to be comfortable troubleshooting and working a mass spec,” said Joe El-Khoury, PhD, D(ABCC), director of the clinical chemistry laboratory, Yale New Haven Health, and associate professor of laboratory medicine, Yale University School of Medicine.

He was speaking in a session at last year’s Association for Diagnostics and Laboratory Medicine meeting where he shared complex urine toxicology cases that made use of both assay types and bear lessons worth noting. One of those lessons: “Don’t assume because it’s mass spec that the result is the absolute truth.” Three of his cases follow.

The first is that of a 59-year-old male with a history of chronic low back pain, opioid use disorder, and type 2 diabetes mellitus who presented to an outpatient clinic for pain-contract-related urine drug screen testing. At that time the patient was taking metformin, oxycodone, and Suboxone (buprenorphine-naloxone combination). Five days before the visit, he stopped taking Suboxone due to nausea and vomiting (Choucair I, et al. *Clin Chem*. 2022;68[10]:1344–1345).

The immunoassay was positive for oxycodone, which was expected, and positive for opiates, which was not expected, Dr. El-Khoury said. “Buprenorphine is not one that would react with an opiates assay.” That the test was positive for amphetamine was concerning, he said.

By mass spectrometry, the laboratory saw buprenorphine and norbuprenorphine as expected. Naloxone at 206 ng/mL (positive cutoff, 50 ng/mL) “was right around where it should be for somebody taking it,” Dr. El-Khoury said. Morphine was negative at 37 ng/mL (positive cutoff, 50 ng/mL). Oxycodone at >2000 ng/mL (positive cutoff, 50 ng/mL) “was through the roof.” And “oxymorphone was reasonable,” at 80 ng/mL (positive cutoff, 50 ng/mL). “But then we had 6-monoacetylmorphine [6-MAM], which is heroin’s metabolite, at 269 ng/mL” (positive cutoff, 10 ng/mL). Amphetamine, too, was “through the roof,” at 5400 ng/mL (positive cutoff, 500 ng/mL).

“There is clearly something going on here,” Dr. El-Khoury said, and it was the unusual pattern that was concerning. “When you’re taking heroin you should have a very high morphine concentration compared to 6-monoacetylmorphine, so this is very unusual. You start thinking about other types of inhibitors, things that could be going on.” Several instances in which patients on heroin had low morphine concentrations have been reported in the literature, he said, mostly attributed to inhibitors of carboxylesterases from the heroin synthetic process. “So not everybody on heroin will show up as positive for morphine because certain enzymes can be present in the preparation process that inhibits the normal metabolic pathway.” Typically, the 6-MAM-to-morphine ratio is less than 0.26; in this sample it was 7.27.

A reference laboratory confirmed the Yale laboratory’s results. In their published case report, Dr. El-Khoury and coauthors wrote, “The fact that 2 laboratories using different mass spectrometry-based methods showed detectable 6-MAM supported heroin use, instead of a false positive due to an interference.”

Follow-up testing ordered by the physician five days after the initial urine sample collection found the sample was positive for fentanyl and norfentanyl. At the time, fentanyl wasn’t part of the laboratory’s general urine drug screen. As of August 2023 it is, “so things like this wouldn’t get missed,” Dr. El-Khoury said in a recent interview.

Before the laboratory added the fentanyl immunoassay to its urine drug screen, it switched from one immunoassay to another with a lower false-positive rate, based on a University of California, San Diego, study published last year

(Menlyadiev M, et al. *J Mass Spectrom Adv Clin Lab*. 2023;28:105–113).

The UCSD authors conducted a method comparison between the Thermo Fisher DRI assay and the Roche FEN2 assay, cleared by the Food and Drug Administration in 2022. They used a liquid chromatography tandem mass spectrometry laboratory-developed test as a reference method, which demonstrated that the FEN2 assay has greater clinical sensitivity and is less prone to false-positives than the DRI assay.

If the Yale New Haven laboratory had added the fentanyl immunoassay to the general urine drug screen before switching to the Roche FEN2 assay, Dr. El-Khoury says, "we would be flagging false-positives left and right." Topiramate is among the drugs that can cause interference, he noted. "And if you're testing generally on patients without consideration, you're going to end up with a false result."

Like Dr. El-Khoury, who stresses the importance of laboratory-developed tests in clinical toxicology ("The race to keep pace with drug use changes"), the UCSD authors do the same. They wrote, "Understanding the performance characteristics of the fentanyl immunoassays in this work would not have been possible without the use of LDT-based mass spectrometry techniques, demonstrating their key role in laboratory medicine."

The second case is that of a 62-year-old female with a history of stress and insomnia who had been prescribed a nightly dose of 6.25 mg zolpidem (Ambien) and presented to an outpatient clinic for a routine follow-up and prescription refill (Shang E, et al. *Clin Chem*. 2023;69[12]:1435–1436).

A urine drug test was ordered to confirm adherence to the prescribed drug. "Unexpectedly, the test came back negative for zolpidem," Dr. El-Khoury says of the mass spectrometry test result from a reference laboratory. The reference laboratory had a positive cutoff of 20 ng/mL and measured only the parent drug, not the metabolites.

Dr. El-Khoury's laboratory sent the sample to a second reference laboratory, which had a positive cutoff of 4 ng/mL and reported the sample positive, at 4.7 ng/mL. It also reported very high levels of the metabolites phenyl-4-carboxylic acid (>800 ng/mL) and 6-carboxylic acid (22 ng/mL).

Zolpidem (Ambien) is not excreted in urine predominantly as zolpidem, he says. "It's excreted as the metabolite. And many laboratories today, reference laboratories, toxicology laboratories, are measuring the parent drug zolpidem."

"Basically, you have labs doing the wrong thing," Dr. El-Khoury says. He advises checking with a reference laboratory that reports a negative result for zolpidem so as not to miss patients, "especially on low-dose Ambien."

In their case report, Dr. El-Khoury and coauthors write, "Zolpidem phenyl-4-carboxylic acid, the major metabolite of zolpidem, can be detected at high levels in urine even 72 hours after ingestion of low doses of zolpidem (5–10 mg). Zolpidem 6-carboxylic acid can also be detected in the patient's urine at 72 hours but at lower concentrations."

Case No. 3 is that of a 28-year-old male with a history of opioid abuse who was prescribed Suboxone to manage the disorder and submitted a urine specimen for a drug screen to verify compliance with the prescribed drug and abstinence from illicit compounds (Gall B, et al. *Clin Chem*. 2019;65[10]:1332–1333).

With Suboxone, buprenorphine is expected in the immunoassay result, and in this case the sample tested positive for buprenorphine by cloned enzyme donor immunoassay. Surprisingly, Dr. El-Khoury said, the screen was positive for oxycodone by homogeneous enzyme immunoassay, which is not expected. "Oxycodone is not in Suboxone."

The physician then ordered confirmation testing by mass spectrometry and requested help in interpreting the

results.

The mass spectrometry results were positive for buprenorphine (>1000 ng/mL; cutoff, 2 ng/mL), norbuprenorphine (15 ng/mL; cutoff, 2 ng/mL), and naloxone (>1000 ng/mL; cutoff, 100 ng/mL).

“You have over 1,000 [ng/mL] buprenorphine, you have very little norbuprenorphine, which is a major metabolite of buprenorphine, and there’s a lot of naloxone,” Dr. El-Khoury said.

“Norbuprenorphine is almost always more than twofold buprenorphine. The fact that in this case we had way higher buprenorphine and barely any norbuprenorphine is indicative of the patient spiking the urine with the pill of Suboxone.” If they spike it, he said, “you get a very high buprenorphine and almost no norbuprenorphine because they didn’t metabolize it—it didn’t go through their system.”

Naloxone in this case is also indicative of adulteration because it is not supposed to be seen in urine at such high concentrations. In patients taking Suboxone orally, usually it’s less than 300 ng/mL, Dr. El-Khoury said, noting there are different mechanisms for Suboxone use. “In the common form, you don’t see it that high.” These results show “the sample had been adulterated.” And in this case, he said, this would have been missed if not for the cross-reactivity of the immunoassay with naloxone and the oxycodone result.

Dr. El-Khoury cites a study published late last year of simulated adherence in 3,950 long-term buprenorphine-naloxone treatment patients, in which the authors report “simulated adherence is a recurring phenomenon” even among long-term treatment seekers (Rahman N, et al. *Subst Use Misus*e. Published online Nov. 10, 2023. doi:10.1080/10826084.2023.2275559). This is the first longitudinal study to analyze patients’ simulated adherence practices over a period of 18 months, the authors write. They found half of the patients positive for simulated adherence had multiple occurrences.

They write: “Out of 3950 patients, 411 (10.4%) had a history of one or more simulated adherence. On average, patients with multiple simulated adherences had 48.1% of their tests simulated, while on the contrary, patients with a single occurrence of simulated adherence had 17.6% of their tests simulated.” A quantitative urine drug toxicology profile in frequent visits will help address this issue, they conclude.

Similarly, Dr. El-Khoury and his coauthor write in their patient’s case of adulterated urine: “[Q]uantitative definitive testing by mass spectrometry is essential in cases like this because the ratios of parents to metabolites can help distinguish compliance from adulteration.”

“Metabolite/parent ratios matter,” Dr. El-Khoury said. “It’s not just about seeing certain things or the compound. The concentrations matter, and you need to investigate those.”

For high-risk patients with a history of opioid use disorder and who have been prescribed buprenorphine or methadone, Dr. El-Khoury’s recommendation for those who can do it in-house: “Mass spec is preferred because you can miss sample spiking with immunoassays.”

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