In toxicology, puzzling out the unexpected negative

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November 2022—In cases of unexpected negative results in toxicology testing, avoid overinterpretation, know your assays and providers, and don't put off definitive testing when it's needed, though it's not a panacea.

That's some of the advice Nicholas Heger, PhD, NRCC, medical director of clinical operations and lab support and co-director of clinical chemistry at Tufts Medical Center, shared in an AACC session in July on toxicology investigations, focused on urine drug screening for compliance and pain management and using his lab's patient cases.

"Hold yourself back from trying to suggest the patient may have diverted, sold the drug, done *x, y,* or *z*. It's tempting sometimes to come up with scenarios to potentially explain a negative or positive result," he said, but "that's not what our job is. Our job is to interpret the data we have in front of us, report it objectively, and move on from there."

For patients in medication-assisted treatment programs, if a result doesn't match what's expected, "we wouldn't want that patient to be discharged from that program," or an infant to be taken from its mother, "because the lab didn't do what it's supposed to do to confirm a presumptive positive result."

Ask yourself at the start whether the assay the laboratory is using "can do what it is I want it to do," he said.

Dr. Heger

For example, several structurally related compounds, such as codeine (at 500 µg/mL) or heroin (at 300 µg/mL), would test negative at the 100 ng/mL cutoff of a particular oxycodone urine immunoassay. "There are several other naturally occurring opiates that are not picked up well by the oxycodone assay, and that's perfectly expected," said Dr. Heger, who is also assistant professor of anatomic and clinical pathology at Tufts University School of Medicine. The concentrations listed for this assay's package insert are micrograms per mL, not nanograms per mL, so the oxycodone immunoassay wouldn't be expected to pick up compounds like morphine, codeine, heroin, hydromorphone, and others.

For Dr. Heger, "package inserts are gold" and the first place he goes when working up a case. "And even after having looked up the same package inserts year after year, I find myself gravitating back to them to answer a lot of those questions," he said.

If the laboratory is performing confirmatory testing in-house—generally with gas chromatography mass spectrometry or tandem mass spectrometry—"look at your in-house standard operating procedures, perhaps some of your validation data where you worked up cross-reactivities with other structurally related drugs," he said, noting, "That can be helpful as well."

As can personal experience. "As you start to review more urine drug screens, you will start to come across some interesting things at your own facility that you may not have realized aren't picked up by your assays," he said.

Review drug metabolism pathways, he advises. Residents and physicians may be unaware of drug interrelatedness, especially with opiates and benzodiazepines, for which there are many common pathways.

“There are lots of common drugs and common metabolites, and reviewing this can be important.” Because many screening assays are class assays—benzodiazepines, barbiturates, opiates, amphetamines—“it’s important to review the drug metabolism pathways to determine whether the assay you’re running will or will not pick up what you want.”

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Unexpected positive results

Detection varies by method and manufacturer. For example, one manufacturer of a commercially available immunoassay for fentanyl designed its assay to pick up fentanyl at 2 ng/mL and claims it doesn’t pick up norfentanyl at all; the other manufacturer’s assay detects fentanyl at 2 ng/mL and norfentanyl at 5 ng/mL. If the laboratory were using the first manufacturer’s assay and had a patient who was on a waning dose of fentanyl, the assay “might not pick them up as having used fentanyl recently because it wouldn’t pick up norfentanyl.”

Stay updated on assay versions, he said. “It has happened more than once where the manufacturer has released a new version of the assay parameters, and it was not updated on the instrument itself. Same thing with formulation.” Some third-party assays have “fairly elaborate” preparation steps—bringing the assay to room temperature or mixing part A with part B and letting it sit in the refrigerator for 24 hours, for example. “Follow all of that carefully and make sure your assay’s performing the way you expect.”

Lot-to-lot variability is not as common of an issue, but does happen occasionally. “There may be different lots of antibodies, and that could potentially explain an unexpected negative.”

Run the right assay for the right drug. A person can hear or transcribe the wrong test, write the wrong test on the requisition, or choose the wrong test from the medical record.

Dr. Heger presented the case of a 30-year-old female who was seen for chronic pain post-cesarean section. The patient was prescribed 2 mg of Dilaudid (hydromorphone, a semi-synthetic opiate) every six hours PRN. The urine drug screen was negative for opiates, and the provider didn’t understand why.

A pathology resident who looked into the case found no problems with calibration or quality control, no recent lot changes of the assay, and no labeling problem. The specimen had been automatically aliquoted by the track system.

The laboratory sent the specimen out for confirmatory testing by liquid chromatography–tandem mass spectrometry. The result: 900 ng/mL of hydromorphone. “So clearly there’s hydromorphone in the sample, which we would expect,” Dr. Heger said.

The resident then pulled the package insert: “This particular assay for opiates does not strongly cross-react with hydromorphone,” Dr. Heger said. “We need concentrations of 1,400 ng/mL at least or higher to report a positive result for the opiates assay.”

The laboratory explained to the provider that the assay was best designed to pick up naturally occurring opiates, such as morphine and codeine, and less so for hydrocodone and hydromorphone. The laboratory decided to improve its communication with and education of providers by using links on its intranet site about common cross-reactants and at what concentrations for some assays.

In another case, a 57-year-old female with a history of multiple sclerosis, chronic pain, depression, and ADHD was prescribed 10 mg of Ritalin (methylphenidate) three times a day. During an appointment, the patient’s physician assistant ordered a urine drug screen, and the patient tested negative for amphetamines. The PA asked the laboratory why the urine drug screen was negative for amphetamines in a patient prescribed a stimulant.

The laboratory first confirmed with the provider that the patient was taking methylphenidate. They next reviewed the package insert of the amphetamines assay. This assay, according to the insert, will pick up amphetamine and

methamphetamine as well as MDMA (3,4-methylenedioxy-methamphetamine) or ecstasy, and MDA (methylenedioxyamphetamine), the metabolite. Structurally, methylphenidate and *d*-amphetamine are different, Dr. Heger said. The package insert also says the assay does not cross-react with Ritalin, "so we would not expect to detect it."

The laboratory sent the specimen out for definitive testing by LC-MS/MS, and the result was methylphenidate (3,920 ng/mL) and the metabolite ritalinic acid (>2,000 ng/mL), "which is the expected finding in a patient taking Ritalin. The negative amphetamines result was explainable—the amphetamines assay does not pick up methylphenidate." The laboratory talked with the PA to explain the assay performed as designed and that future testing for Ritalin compliance should be performed with the definitive test specifically for Ritalin. So it was a case of the wrong assay.

Other likely culprits of unexpected negative results: dose and frequency. "We see this one a lot," Dr. Heger said, noting a 2012 study that found approximately 50 percent of all drugs are not taken according to how they are prescribed. Formulation matters too—immediate, sustained, or extended release—as does route of administration.

In a third case, a 60-year-old male with a history of chronic pain after a motor vehicle accident was taking one to two tablets of Percocet (5 mg of oxycodone, 25 mg of acetaminophen) every four to six hours. The patient had expressed that he'd been experiencing loss of work, financial trouble, and stress at home and had just recovered from norovirus. When the patient's urine drug screen ordered by his primary care provider in a routine clinic visit came back negative for oxycodone, the provider questioned the laboratory and its assay because the patient had long been compliant with his medication. The laboratory decided to send the specimen out for confirmatory testing, Dr. Heger said, "but before that we reviewed with the provider the things he or she should normally do as part of the workup." No. 1 is a pill count, which in this case looked okay. And the patient had consistently picked up his prescription at the same pharmacy and paid through insurance with a copay, according to the state's prescription drug monitoring program database. "So nothing looked strange at all."

The reference lab sent back a result of 120 ng/mL of noroxycodone, one of the metabolites of oxycodone. "Oxycodone is metabolized into oxymorphone, noroxymorphone, and predominantly noroxycodone, which is a major metabolite from oxycodone administration," Dr. Heger said. The small concentration of noroxycodone suggested the patient hadn't taken oxycodone recently, which may be understandable in light of the norovirus and its accompanying nausea and vomiting, "and perhaps not taking the drug as the patient typically would."

"And it turns out that our urine immunoassay for oxycodone does not pick up noroxycodone particularly well."

A less likely explanation for unexpected negatives is specimen adulteration, "and we have ways of working that up"—looking at urine creatinine and specific gravity, having thresholds that reveal whether a specimen is dilute or "potentially even physiologically impossible," Dr. Heger said. Like other labs, his lab has received specimens in which the specific gravity in the urine is 1.0 and there's no creatinine in the urine. Other signs of adulteration: oxidants, bleach, detergents, and additives, for which assays are available. "Substitution can also happen," he said. And even synthetic urine will work with some immunoassays, but "if you do a little digging, you'll find it doesn't work consistently."

Collection and engineering controls help, such as temperature monitoring strips on urine containers, blue dye added to toilets, or restroom faucets that have external shut-offs to restrict water flow during the patient collection. "A lot of things can be done," he noted.

An even less likely explanation for unexpected negatives: physiology. "Review your metabolic pathways," he advises, and understand some patients produce more or less of some metabolites because of their metabolizing status, and there are others who excrete less well. "We have patients who come in to do urine drug screens but they're anuric because they're in end-stage renal disease," he said. "So we have to find ways to get around those kinds of situations."

Requests for pharmacogenomic testing are made only on occasion, Dr. Heger said. Some patients are ultra-rapid or

very slow metabolizers or “may have supratherapeutic concentrations of a particular drug in their bloodstream because they don’t metabolize well. These patients may test unexpectedly negative if you’re screening only for the metabolite.”

Dr. Heger’s final case was that of a 40-year-old male with a history of insomnia, anxiety, and depression who was prescribed 2 mg of lorazepam in the morning and 2 mg in the evening as needed. The primary care provider reached out to the laboratory when the patient’s urine drug screen was negative for benzodiazepines. The laboratory sent the specimen to the reference laboratory for analysis by tandem mass spectrometry. The result: more than 3,000 ng/mL of lorazepam, “so plenty of lorazepam in this particular sample,” Dr. Heger said. The immunoassay package insert said the laboratory should detect lorazepam at concentrations above 650 ng/mL.

“We did a little digging and went into one of our toxicology books that reminded us that lorazepam is rapidly conjugated with glucuronic acid,” he said. “And about 75 percent of the dose is eliminated in the urine as lorazepam-glucuronide. So this is heavily glucuronidated for excretion.”

In talking with the reference laboratory about its assay, Dr. Heger and colleagues learned that the reference laboratory performs a hydrolysis step with glucuronidase to break glucuronide off the parent compound. So the reference laboratory’s result—3,181 ng/mL of lorazepam—is actually total lorazepam. “It’s both the free and the bound. It’s everything, and it’s all there,” he said.

Buried in a different section of the package insert was this statement: “Glucuronide metabolites of benzodiazepines may not react with the . . . Benzodiazepines assay at certain concentrations. Individuals known to be taking therapeutic doses of benzodiazepines may show negative urine assay results.”

“This is classic,” Dr. Heger said. “We see this time and time again with a particular assay that my institution runs for benzodiazepines for patients on therapeutic doses of lorazepam who will simply test negative.”

One of the managers searched and found other immunoassays for large analyzers that will incorporate a hydrolysis step with glucuronidase. “So it is incorporated into the reaction for spectrophotometric analyzers that can help break that glucuronide off of the parent compound and might improve detection,” he said.

There are many options to get to the bottom of such puzzles, he added. “So no need to fear.”

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