In toxicology, unraveling the unexpected positives

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December 2022—In toxicology testing, cross-reactive compounds, incomplete medical records, immunoassay performance, calibrator drift, and human error all play into unexpected positives. “Always be critical of your testing. Be hard on it,” Danyel H. Tacker, PhD, D(ABCC), said in an AACC session in July.

Patient interference should be on the radar, but drug and supplement cross-reactivity is the most likely culprit in urine drug screens, said Dr. Tacker, medical director of clinical chemistry and mass spectrometry laboratories at West Virginia University and J.W. Ruby Memorial Hospital. In a session on toxicology investigation, she tackled the unexpected positives and Nicholas Heger, PhD, NRCC, co-speaker, the unexpected negatives (see CAP TODAY, https://bit.ly/3AFIKrZ). Toxicology testing is a “continuous quality control process,” Dr. Tacker noted, one from which the laboratory “can’t look away.”

But it’s sometimes something else. Patients transferred to WVU Medicine from one of many independent clinics that don’t use Epic or document medications the same way may assume their data transferred too, but it hasn’t. “Then we get this unexpected positive and you end up doing all this work and you find out, yes, it was supposed to be there. These unexpected results can be that wolf in sheep’s clothing,” she said.

In the first case she described from her clinical experience, the laboratory began to use a new fentanyl screening test that quickly resulted in a cluster of false-positives in behavioral medicine substance use disorder clinic patients who had used fentanyl before or could have had a contamination. Patients were adamant about compliance with the program’s rules for nonuse, and confirmatory compliance checks were consistently negative.

Dr. Tacker

“I always say, ‘Do you have an example?’ Because generalities don’t work,” Dr. Tacker said. The clinic’s first example was a patient whose listed medications were acetaminophen, buprenorphine, naloxone, and risperidone, and it was the risperidone that was causing interference. A look online at an image of the structure of risperidone and a comparison with fentanyl’s structure revealed “just enough likeness in the center of the nucleus.” But then other examples came and among them were obstetric patients on beta-blockers, also with false-positive fentanyl screens. The laboratory found cross-reactive associations with diphenhydramine and other antihistamines too. “Fentanyl has a very low cutoff, at 2 ng/mL. It’s the lowest cutoff we have in our entire urine drug screening panel—two orders of magnitude lower than the usual opiates screen and 50 times lower than the oxycodone cutoff,” Dr. Tacker said. Combine a very low threshold with cross-reacting compounds, and “it’s super easy to trigger a fentanyl, at least in my lab.”

The laboratory set out to educate by updating result comments to inform physicians that beta-blockers and antihistamines are compounds that can potentially provide “crazy results.” They encouraged questions and research requests. The laboratory also educated its staff.

Assay performance is another likely cause of unexpected positives. “Consider when the assay was made. Consider what’s come behind it,” Dr. Tacker said. Many drugs have come out in the past 30 to 40 years, but the classical drug tests, especially those with high cutoffs (for opiates and possibly amphetamines, barbiturates, and benzodiazepines), were likely FDA cleared decades ago, she said, and then subsequent devices followed with the

same configurations by comparison to their predicates.

“The internal studies don’t show any variation, but you put them in clinical use and you really start testing it.” Patients are taking new compounds that haven’t been tested in the routine way, she said, but changing the package insert to provide updates on new drugs requires a new FDA filing. “It’s a little bigger and takes more review, so a lot of places just say, ‘Let’s go with the predicate.’”

Specificity and cross-reactivity for class drugs often depend on the calibrator. “Do you know which compound is used for the calibrator in your opiates assay? Is it morphine? It should be,” she said. But there are different groupings of benzodiazepines, and depending on which benzodiazepine is in the calibrator, other class drugs in legitimate benzodiazepines are not going to cross-react as well, she said. “So you’ve got to go back to the standard operating procedures and instructions for use” when working up unexpected positives.

Very high concentration drugs may cross-react unexpectedly, Dr. Tacker said. “I have five opioids in my urine drug screen panel, and if I have one particular drug that’s present in very high concentrations, it’s going to trigger at least two positive results, not just one.”

The second case she cited is that of an HIV-positive patient who came to the HIV clinic for routine care and had a Δ9-THC-COOH-positive result on a urine drug screen that was negative by definitive testing. The patient denied using marijuana. The physician questioned the laboratory’s test and provided the requested patient medication list: amlodipine, efavirenz, emtricitabine, and tenofovir. “Efavirenz is our little culprit,” Dr. Tacker said, because of its similar structure to tetrahydrocannabinol (Saitman A, et al. *J Anal Toxicol.* 2014;38[7]:387–396). THC is prone to cross-reactions with some prescription drugs, “so it’s one where you’re probably going to do some THC checks if you keep it on your drug-screening panel,” she said. Whether THC checks are still clinically important is a question some are debating, but “we still do.”

The laboratory updated its local job aids and catalog and reached out to HIV clinic physicians. “Consider this a possibility, check your meds, talk to your patient. There’s probably an explanation and this could be it,” the laboratory told them. Since efavirenz is one of the cocktail compounds for high-intensity antiretroviral therapy, this situation could be common in HIV patients.

One case prompted questions of simulated compliance or surreptitious addition of oxycodone to the urine: A pain clinic patient who was prescribed oxycodone tested positive for opiates and oxycodone, and definitive testing confirmed a high positive level of oxycodone but a negative level of noroxycodone (the primary oxycodone metabolite). The WVU Medicine pain, substance use disorder, and obstetrical settings allow cup-based point-of-care screens to facilitate real-time consultations with the patients. All positives and unexpected negatives are confirmed, and physicians pay close attention to results and talk with their patients. “They don’t seek to punish,” Dr. Tacker said. “They only want to help, and we’ve got to do what we can when we can to capture them clinically to give them the help they need.”

The immunoassay package insert revealed that oxycodone cross-reacts with the opioid test in and around 10,500 ng/mL, and the high concentration triggered suspicion about the patient spiking his urine to trigger the positive result. “Our mass spectrometry screen is geared to calculate ratios between the oxycodone and noroxycodone present” and indicate a problem: insufficient metabolite. The patient admitted to diverting and selling his oxycodone pills, saving one pill to drop in his urine at the pain clinic to get a positive result and continue receiving his prescriptions. He was referred to the behavioral medicine substance use disorder clinic for an evaluation and to start a compliance regimen. “Instead of barring him from the pain clinic, we said, ‘We’re going to give you another avenue. Let’s have a talk about compliance and how to keep you on it,’” Dr. Tacker said.

In a toxicology investigation, it helps to go into a clinic and observe the specimen flow, Dr. Tacker said. “If you get an unexpected positive, was it really that patient’s urine? Potentially no.” Limited numbers of clinical assistants handle multiple urine cups. “They’re lining them up, trying to get them labeled, trying to read results. They can make mistakes. Things need to be asked at a fundamental level all the time.”

Ask too about the local laboratory's manual aliquoting steps. "Did someone in the lab aliquot this, send it down the track, or send it to confirmation? Or did they do it in the clinic setting and then send us a labeled tube for confirmatory testing and keep the urine cup?" Know how a specimen moves, where it goes, and who touches it, "so we're not pegging people with blame or discussions with the substance use disorder clinic because we didn't put the label on the right tube," Dr. Tacker said.

Assay-related issues are less likely causes of false-positive results, she said, though "things might switch on you overnight if a calibrator has drifted and it's detected later." Calibrator drift resulting in unexpected weak positives is more common for lower-cutoff screens, such as for fentanyl and buprenorphine, she said, and potentially if there's an interference on top of a low cutoff screen. "So look at your raw signals from your analyzer. It could give you good information" when investigating.

With a definitive-method false-positive, carryover is the most common issue. Also possible: the presence of an isobaric ion or ion ratio settings that aren't sufficiently stringent. "If something new comes on the scene and your ion ratios aren't tuned exactly right, you might get a false-positive." You find out you have an isobar, and now it needs to be differentiated from the target compound. "There's always a learning curve in definitive testing," Dr. Tacker said. "It's ever-evolving."

She cited dihydrocodeine and noroxycodone as an example. They share an ion at 302 m/z and have a close retention time on her laboratory's assay. "We have implemented two to three levels of ion ratio checking to differentiate them so that when technologists are looking at them, they can see it looks strange and that it failed the ion ratio check."

Dr. Tacker shared a case in which evening shift technical maintenance and quality control led to a buprenorphine urine screening failure. Her laboratory's buprenorphine urine screening test has a cutoff of 5 ng/mL. The technologist did a 24-hour look-back and found eight weak-positive raw results to review. All eight were negative on retests performed after the calibration was set up and rechecked and QC was in place. "It happens," Dr. Tacker said. "You get drift."

The laboratory corrected the results, made calls to explain, and offered definitive testing at no charge and credits for any unnecessary definitive reflexes. And, of course, it examined its QC and calibration intervals (for example, perhaps calibration is set at 30 days in the instructions for use, but a more stringent calibration interval of 21 to 28 days could be sufficient to prevent drift).

Validation is another but less likely explanation for a definitive false-positive. "You cannot cross-check every single drug in every single matrix at every concentration and see where all your cross-reactivities lie. It's something you acquire over time," she said. And while mass spectrometry is good, it's not perfect. "I love mass spec. I run that part of the laboratory. But we all realize what the limitations can be and try to be hyperaware of them and always course-correct as we move through our work." And sometimes unexpected positives stem from combinations of things—"a twisted mess," as Dr. Tacker puts it.

In another case from her laboratory, a patient with diabetes and a urinary tract infection tested ethyl glucuronide (EtG) positive and ethyl sulfate negative (alcohol metabolites). The patient denied a relapse. Her urine specimen, which had post-collection fermentation, had been stored at the clinic overnight before it reached the laboratory. There was no ethyl sulfate present ("no evidence that alcohol went through her liver," Dr. Tacker said) but the low EtG-positive signal was unusual in this scenario, so the laboratory confirmed the result: false-positive.

Another case involved a patient with urinary retention in a prolonged clearance of prescribed oxycodone by about two days. The window for an oxycodone positive result is usually up to three to five days, but there was trace positivity in the specimen one week later. The laboratory traced the decreasing oxycodone level, but the patient said they had urinary retention. "We thought, 'No way,'" Dr. Tacker said, "but it was actually enough to concentrate it and give us enough signal to give us that false-positive."

Keep lists and logs of investigations and confirmations, Dr. Tacker said in closing. Her own book of investigations is

always at arm’s reach, with notes in it about what test results were confirmed. “So when the phone rings, I open it up, ask for the medical number, and [say] let’s look at the meds. And I record those things because it helps me retrace my steps and find patterns.”

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