Development and Implementation of One-Step, Broad-Spectrum, High-Sensitivity Drug Screening by Tandem Mass Spectrometry in a Pediatric Population

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Merih T Tesfazghi, Rick Bardelmeier, Al N Saunders, Sarah M Riley, Stephen M Roper, Dennis J Dietzen.
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Guest: Dr. Dietzen is a professor of pathology and immunology and pediatrics at Washington University in St. Louis and he's the medical director of laboratory services at St. Louis Children's Hospital.

Randye Kaye: Hello, and welcome to this edition of JALM Talk. This comes from the *Journal of Applied Laboratory Medicine*; a publication of the American Association for Clinical Chemistry and I'm your host, Randye Kaye.

Modern toxicology testing in the clinical laboratory is typically conducted with immunoassay-based screening tests. Drug immunoassays are readily available on automated analyzers and they yield rapid results, but they suffer from poor sensitivity and specificity. Immunoassay results are considered presumptive until confirmed by a targeted mass spectrometry-based method, and this confirmation can take hours to days. While false positive toxicology screens can be problematic in any clinical setting, these results pose particular challenges in pediatric settings, where positive results can have severe medical and legal consequences. An original article in the March 2022 issue of JALM describes the development and clinical impact of a novel liquid chromatography tandem mass spectrometry LC-MS/MS drug screening assay at a pediatric hospital.

The author has developed a one-step LC-MS/MS test that simultaneously measures 38 drugs and metabolites in order to avoid the reliance of immunoassay drug screening. On today's podcast, we are joined by the senior author of this article, Dr. Dennis Dietzen. Dr. Dietzen is a professor of pathology and immunology and pediatrics at Washington University in St. Louis and he's the medical director of laboratory services at St. Louis Children's Hospital. Welcome, Dr. Dietzen.

Dennis Dietzen: Thank you for having me.

Randye Kaye: You're welcome. First, what would you say are the unique challenges associated with toxicology testing in neonatal and pediatric settings?

Dennis Dietzen: So, toxicology testing in kids has a lot of very specific requirements and it's a very difficult thing to do. I mean, kids

cover a broad age range. There's a distinction between neonates and toddlers and teenagers and all of them come with very important challenges. Neonates tend to excrete very dilute urine. So, we're looking for things in a very dilute samples so we have to have very low limits of detection to find these things.

Typically, the things we're looking for there are things that they were exposed to in utero and using urine poses a challenge. Toddlers on the other hand start to explore their environment, pick things up, put them in their mouths. Those could be toxic substances for which they present to the ED. And teenagers have a whole other realm of experimentation that we have to be able to detect and they begin to use substances that adults use in terms of illicit substances. So, as the child tends to mature, so do the requirements for drug screening and the screen that we've developed for use in our hospital attempts to cover all of those developmental issues in children.

These also have -- when you're dealing with minors as well, these issues have legal implications. If you detect something that suggests the child is in an unsafe environment, that child may be separated from parental care for some period of time while an investigation goes on. All of this is done under the guides of keeping children safe and making sure we know why they are presenting to a clinical environment and because of those medical-legal implications, we can't be wrong. So, there's a lot of pressure. I lose a lot of sleep at night doing drug testing in children.

Randye Kaye: Yeah. I would imagine so. It's extremely important. So, let's talk about the common drug screening tools that are currently used for neonatal and pediatric patients. So, what are the limitations there?

Dennis Dietzen: So, the tools that most hospitals have at their disposal are immunoassays. Immunoassays are convenient. They're relatively cheap to run. They're fast. But using an antibody which has a very large binding pocket to identify very small substance, like a molecule of morphine for example, that's not a match made in heaven. The immunoassays that we typically use in the clinical environment were one upon a time developed for workplace drug testing. And those workplace drug tests were designed to interrogate the urine specimens from airline pilots, truck drivers, trained engineers and they were designed to detect quantities of illicit substances that impaired those individuals. Those are not appropriate in a lot of adult settings and they're certainly not appropriate in pediatric settings.

Furthermore, the immunoassays are far from perfect. They tend to identify substances that aren't there so you can get

false positives. Methamphetamine assays are the worst at these. False positive rates of 50% in some cases happen. But they're also -- because they're dialed in to try to detect higher quantities of things, there's also a false negative problem in children in particular because of the high cutoff concentrations and I already mentioned the dilute urines that children have. So, we're already fighting a battle that we're bound to lose under those circumstances.

The final problem with the immunoassay paradigm is the menu of immunoassays has remained static for the last 30 years or so, and it develops and it evolves very, very slowly. So, when new compounds come onto the market, these immunoassays aren't going to see those. So, there has developed this immunoassay screen-then-confirm paradigm that many hospital laboratories employ. The problem with that is that the time between immunoassay screen and confirm can be lengthy. If you don't have facilities at your laboratory to do this, it can be hours to days to as much as a week.

The issue in the pediatric emergency room is you've got a physician who's got to make decisions pretty quickly about the disposition of a child. And you're sending them a result with an immunoassay that says, "I think that this substance might be present in that urine, but I'm going to make you wait for three days so I can figure it out and make sure that I'm telling you the right thing." So, that doesn't help anybody. So, that's the current situation into which we did our work.

Randye Kaye: So, let's talk about that work. You put a solution in place at St. Louis Children's Hospital and you described it in the JALM article. So, can you summarize that for us?

Dennis Dietzen: I can very briefly. Essentially what we've done is we've cut the immunoassay screen part out of the whole operation. It's no longer a screen and confirm approach. We go directly to a mass spectrometry technique, and we'll talk in a little bit -- there are many, many hurdles to implementing mass spectrometry as a primary tool here. Mass spectrometers are typically located in the back dark corners of laboratories where only a few people put their hands on them and a few very highly specialized operators run them.

To do this, we had to put in a 24/7, 365 process in order to do drug screening around the clock every day, all day, which presents a number of hurdles. But in this technique, what we do is we do very simple sample preparation. We dilute urine with a series of internal standards as a control and we -- in a 15-minute LC run -- we get a panel of about 40 different compounds that we can look at. Those compounds are identified at the bench by laboratory technologists.

There are at least four criteria that they evaluate the presence of each drug for. It has to have the right LC retention time and it has to have the right precursor mass and for most of the drugs that we identify, it has to have two product masses that match and those product ions have to be present in the right ratio in order for us to identify a compound. So, there are four distinct hurdles that the technologist must walk through before they identify a substance in that urine.

The final sort of adjudication of that is a medical director review. I have a partner here and we review all of those drug screens, not in real time, usually once a day, to make sure that we did not call something incorrectly which we almost never do. What we usually do is err on the side of not identifying something until we can do some further investigation and then call the physician later and say, "Look, we found another substance in this urine." That usually takes place within a few hours to a day or so afterwards. But what it is then, if we give them an answer that meets these criteria, it is a definitive answer for them. It's not we think this is there. We are very, very sure about the presence of those compounds that we identify in urine specimens and physicians can therefore make decisions comfortably knowing that we've gone to that rigorous sort of analysis to identify those compounds in urine.

Randye Kaye: Wow. So, along those lines in the article, you compared the findings from reviewing the data one year prior to and one year after you implemented the LC-MS/MS screening assay. So, can you share some more of the major differences you saw after the implementation?

Dennis Dietzen: Yeah, absolutely. So, we saw a higher positivity rate. We had a couple of hypotheses going in that we would identify more compounds and that was an easy hypothesis because we are screening for many more things.

The mass spectrometer allows us to identify substances that the immunoassays are not targeted for. So, that was an easy hypothesis. We also hypothesized that we would find higher positivity rates in more compounds, and we did exactly that. We found some other things that we didn't expect to find like immunoassay detection of cannabinoids in neonatal urine for example. Prior to implementation of mass spectrometry, when we did confirmatory analyses on those, we found that almost all of those, 90% of those were falsely positive, and you can get the reaction that a parent might undergo being told that their child has a cannabinoid in their urine when it's really not there.

The other thing that we identified a lot more of, because of our lower cutoffs, because of our lower limits of detection, we

identified a lot more cocaine metabolite in neonates than we did previous to the implementation of this technique. So, we set out to have a broader menu and lower detection limits, and though that was in a nutshell, that was sort of the impact that we found. We found much bigger variety of substances. Some of them where there for therapeutic reasons. Some of them were not there for therapeutic reasons. So, we did achieve what we set out to do.

Randye Kaye: So, it sounds like it's working really well for your institution. Let's talk about implementation more widespread. What are the hurdles to implementing this to pediatric drug screening at other institutions?

Dennis Dietzen: There are an enormous number of hurdles to make this work, and this could not have been done without support from supervisory staff and bench technologist who all -- who I warned this would be a difficult thing to do, but they embraced the opportunity. In retrospect, they're happy that they did. Mass spectrometers on the whole are not as user-friendly and analytically mature as some of our other automated platforms. Our automated chemistry platforms run for hours and days without needing much user intervention.

Mass spectrometers are not that way. They're a little touchy. They get dirty. They must be maintained on a regular basis. The trouble-shooting, they can fail at a lot of different places, so this is a hard thing to do. So, we -- for six months prior to turning this on, we did little training sessions. We took urines that were submitted for drug screening and we ran dummy samples and we had groups gathered around the mass spectrometer to interpret those things. So, we trained people that way.

The presence of the secondary review also led to a higher level of comfort for the bench technologists. So, we were pretty well-prepared by the time we turned this thing on. The approach here, putting a mass spectrometer in a very time-sensitive environment also required a lot of help from the vendors. We have redundant platforms. We have two platforms on which to do this. The vendor that was identified to supply this equipment had to make me a promise that I would never be without both of those instruments. So, you need very, very timely and comprehensive support from your mass spectrometry vendor as well if you're going to make something like this happen, and we made sure that all of those ingredients were in place prior to implementing the assay.

Randye Kaye: Thank you. So, it sounds like those hurdles have been dealt with and -- or at least, in the process. So, let's talk about how this has been received by practitioners, say, in the

emergency department, the nursery, other clinical settings at your institution?

Dennis Dietzen: So, this project was stimulated in a number of different ways. It was stimulated by my longstanding dislike of immunoassays and their limitations, but it was also stimulated by frustration on the part of who we refer to as child protection physicians. It's their job to assess situations where there's a suspicion that a child that lives in a dangerous environment or for some reason, should not be discharged into that environment. They have a really hard job. If they think that a child is in a dangerous environment, then they might take temporary custody of that particular child, and if they're wrong, there are likely legal ramifications for that.

If they discharge a child into an unsafe environment, there's a downside there too. The child might get hurt. So, this process started more than a decade ago with a case of a child here who was administered clonidine by a parent. At the time, our drug screen did not identify clonidine. So, the drug screen came back clean, the child was discharged into an unsafe environment and there was harm at the end of the day.

The child protection physician said, "We need to do this better." I agreed, and this is better. So, we consult on a routine basis with our child protection physicians, our toxicology physicians, our neurologists and all of the people that take care of children who may have mental status changes indicative of an ingestion. We have a constant conversation with them about the menu of compounds that are present. We tap into our local DEA in toxicology, forensic toxicology laboratories to monitor what's present there. So, our menu is fluid. We can adjust it. We don't like to adjust it every day. We don't do it every day, not even every month, not even every year. But we do have the ability to adjust the menu if necessary, to expand the menu, to contract the menu if necessary.

It has required a fair amount of re-education of physicians, because physicians know immunoassays have limitations. And I still get phone calls. We've been doing this for a few years now and I still get phone calls with the question, "You identified methamphetamine in the urine of this child, what is it really?" The answer used to be, "I don't know what it is. We'll figure it out." But now, the answer is, "it's methamphetamine." So, it's helped a lot with handling children under these unfortunate circumstances. Even children that present with unknown symptoms, you know, we've prevented a lot of unnecessary laboratory work by identifying an ingestion that explains their symptoms at very early stages rather than having to go through a very long protracted workup for other causes of that clinical

presentation. So, in my mind, this has been an overwhelming success.

Randye Kaye: Wonderful. Well, clearly, accuracy is key and some people need to adjust to change, and I hope this article will help to make that happen. Thank you so much for joining us today, Dr. Dietzen.

Dennis Dietzen: You're quite welcome. Thank you.

Randye Kaye: That was Dr. Dennis Dietzen from Washington University in St. Louis discussing the JALM Original Article entitled: "Development and Implementation of One-Step Broad-Spectrum High-Sensitivity Drug Screening by Tandem Mass Spectrometry in a Pediatric Population." Thanks for tuning into this episode of JALM Talk. See you next time, and don't forget to submit something for us to talk about.