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Performance of Fentanyl Immunoassays in an ED Patient Population.
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Guest: Dr. Nikolina Babic from the department of Pathology and Laboratory Medicine at the Medical University of South Carolina.

Randye Kaye: Hello and welcome to this edition of *JALM* Talk from *The Journal of Applied Laboratory Medicine*, a publication of the Association for Diagnostics & Laboratory Medicine. I'm your host, Randye Kaye. Fentanyl is a synthetic opioid that is currently at the center of the opioid crisis in the United States. Drug overdose deaths involving fentanyl more than tripled from 2016 to 2021. However, patients may be tested for fentanyl in as little as 5% of overdose emergency room visits.

Synthetic opioids such as fentanyl are not detected in routine urine opioid screens and rather require a dedicated separate test to be added. For these reasons, several states including California, Maryland, and Pennsylvania have adopted legislation requiring fentanyl to be included in standard urine drug screens.

The September 2024 issue of *JALM* features several articles focused on fentanyl testing, including one study that evaluated the performance of two FDA-cleared fentanyl immunoassays in patients at the Medical University of South Carolina's Emergency Department. The study also assessed the positivity rate for fentanyl testing and compared it to that of other substances included in the urine drug screen.

With this study, the authors aim to enhance the understanding of fentanyl's impact, guide future laboratory practices, and improve patient care through more effective detection and treatment of substance use disorders. Today we are joined by the article's senior author, Dr. Nikolina Babic. Dr. Babic is an Associate Professor in the Department of Pathology and Laboratory Medicine at the Medical University of South Carolina, where she also serves as a Medical Director of the Clinical Chemistry laboratories and the Point of Care testing. Welcome Dr. Babic.

Nikolina Babic: Thank you, I am very happy to be here.

Randye Kaye: First, why is it necessary for clinical laboratories to incorporate fentanyl into their routine urine drug screens?

Nikolina Babic: It has been widely reported and as we all know, there is an ongoing opioid crisis. It is mostly fueled by elicit fentanyl and

is arguably the most dangerous and deadly drug crisis yet. To compile the problem further, there are many fake pills in the drug market nowadays that are made to look like prescription opioids or stimulants but are laced with fentanyl. DEA has recently launched a campaign under the slogan "One Pill Could Kill."

This is in response to the agency finding lethal doses of fentanyl in 7 out of 10 fake pills seized in 2023. Let us put this into perspective. That means that 7 out of 10 individuals, say high school or college kids, would take a single fake Xanax or Adderall pill at a party may end up overdosing on fentanyl. It is beyond frightening. The clinicians cannot provide appropriate care if they have to speculate what the person was exposed to.

For this reason alone, I think at the very minimum, the laboratory servicing emergency department or EDs are obligated to provide fentanyl testing within routine drug screening.

Randye Kaye: All right, thank you. Those are really startling statistics. So what factors are important for labs to consider when they are selecting and evaluating fentanyl immunoassays?

Nikolina Babic: The most important question to ask is who is the patient or service that will be using this test. Is the test going to be used mainly to make immediate treatment decisions such as in the ED? Or is it going to be used for compliance monitoring such as in pain management patients? Obviously in the case of ED patients, test sensitivity is a very important aspect. We do not want to miss anyone with the cute fentanyl ingestion. On the other hand, if the assay is intended to monitor compliance, specificity becomes much more significant consideration, and also access to confirmatory testing is essential.

This is because the false-positive result may have much more significant repercussions for the patient. Another consideration that we typically take for granted is reagent stability. We know that manufacturers do robust stability studies and we rely heavily on their stability and handling claim. However, if the reagent is kept open on board the analyzer for long periods of time, it may start to deteriorate more rapidly as it approaches its stability limit.

Thus, a lab with low testing volume may need to replace the reagents more frequently to avoid missing low positive specimens. This issue of *JALM* actually provide several great resources for the labs considering implementing fentanyl testing. I would really encourage everyone to read the editorial done by Dr. Broussard on how to choose a fentanyl

immunoassay. It provides an excellent overview and a high-level summary of relevant publications.

Randye Kaye: All right, thank you. Those are important, considerations and let's talk about some of those resources and comparisons. The study compared the ARK Fentanyl II assay to the Immunalysis SEFRIA fentanyl immunoassay and you also evaluated specimens with discrepant results by liquid chromatography-tandem mass spectrometry as the confirmatory test. Can you summarize your findings?

Nikolina Babic: Absolutely, we evaluated these two immunoassays on Abbott analyzers. The population we selected were patients presenting to the ED since they account for approximately 70% of our testing volume and best represent our routine workflow and drug use prevalence in our service area.

We found that both assays had equivalent sensitivity at 95% with each missing only one of 20 total positive specimens. Specificity was also excellent. ARK II slightly outperforms that SEFRIA with respect to specificity as 99% and 97%. The false positive rates were also very low. As you mentioned, we use mass spectrometry-based method to confirm the presumptive immunoassay positive result and to resolve any discrepancy we observed. Aside from confirming the true positives and identifying false positive, to our surprise, we identified two false negatives, one by each immunoassay. The false negative result on ARK II can be explained by decreased cross reactivity of this assay toward the fentanyl metabolites, norfentanyl.

This specimen had no detectable fentanyl but only very low amounts of norfentanyl were detected by confirmatory assay and so it was missed by our immunoassay. The false negative on SEFRIA is a bit more of a conundrum. As the level of fentanyl present were well above the detection cut off. Since the specimen was analyzed more than 20 days after the reagent was placed on board, we can now only speculate that the reagent degradation is a contributing factor. In conclusion, we found that both assays performed great, with excellent concordance.

Randye Kaye: All right, thank you. Let me just follow up with this. How did the results compare to other fentanyl assay evaluations that have been published?

Nikolina Babic: So in our hands, SEFRIA performed much better than what was previously reported. While the test sensitivity is comparable, specificity, not so much. Our false positivity rate of only 3% are in stark contrast with previously reported rates that were as high as 45%.

Our finding on ARK II performance are however much more in line with other. There are two additional publication in current *JALM* issue that describe performance of ARK II fentanyl assays. Both use Roche, so that offers evaluation on an alternate platform to add that was used in our lab but note one of the two papers, authored by Lam and colleagues, reported somewhat lower sensitivity than the rest of us. This group found negative rates to be roughly two times higher compared to us.

This is most likely reflection of the differences in population testing. Majority of the mixed patients in that study had no detectable fentanyl, very low norfentanyl. So this suggests most likely remote fentanyl exposure in these patients. In contrast, the ED patients most likely will present with acute fentanyl exposure. There's yet another excellent contribution to this issue addressing just that. The paper specifically looks at the performance of immunoassays in specimens with low fentanyl and low norfentanyl level. I found the paper very informative and highly recommend it.

Randye Kaye: Thank you. Can you comment now on the importance of the detection of fentanyl analogs?

Nikolina Babic: Having access to fentanyl analog testing is very important. It could help inform the providers about the local drug supply and affect how unexpected fentanyl positive immunoassay results are addressed.

We know that current confirmatory mass spec assays typically only test for fentanyl and norfentanyl but historically different fentanyl analogs have been detected in drug supply in different regions of the country. We also know that different immunoassays have different cross reactivities with various fentanyl analogs. All this is significant due to the fact that in the recent years, we are seeing resurgence of a fentanyl analog called para-fluorofentanyl, or pFF, as an adulterant in the illicit drug supply.

Although this analog is less potent than fentanyl, it could actually lead to overdose and death in concentrations similar to those that we have seen involving fentanyl. There are a few studies ARK II fentanyl assay exhibits almost 70% cross reactivity with pFF. So you can imagine a scenario where we could have a presumptive positive immunoassay result from a patient who inadvertently ingested stimulant drug for example, that was adulterated with pFF.

This result would likely be unconfirmed by the fentanyl mass spec assay and thus, would be considered a false positive by us. More importantly however, we potentially inadvertent opioid co-ingestion would be missed.

Randye Kaye: All right, thank you. So obviously, very important and in fact several states have now mandated fentanyl testing to be included in urine drug screens but fentanyl testing in emergency rooms across the U.S. still seems to remain low. Why do you think that is?

Nikolina Babic: This is most likely due to a significant lag in availability of quality immunoassays. We know that mass spectrometry is a gold standard and it has been around for a while. The problem is that this technology is still very much inaccessible to majority of community, or regional, or even though rural hospital laboratories.

Also, turnaround times associated with mass spec testing are not necessarily appropriate for acute patient management. To support urgent clinical testing, rapid straightforward immunoassay has typically been the best option. The first FDA-cleared immunoassay for fentanyl was SEFRIA, which I believe it became available in -- some time in 2017.

And as we already discussed, the early evaluation studies showed that this assay had less than adequate specificity. Fortunately, the new generation of immunoassay is much improved and we currently now have four different immunoassays to choose from. They cover most major clinical analyzers in addition point-of-care testing has also become available lately.

So the issue is by the time we were able to start offering the test, physicians got used to managing the patients without it. So they are now pressuring the labs to bring out the testing and in turn, the labs are slow to respond.

Randye Kaye: I see. Okay, so finally, can you share with us your experiences since you implemented the routine fentanyl testing in your hospital?

Nikolina Babic: Overall, I am very happy to state that this has been a very positive experience for us. Both from the standpoint of past adoption and position satisfaction. We do continue to monitor our positivity rate, which are staying consistent somewhere right around 10% which I suppose is a good thing, at least they are not going up. Also, we continue to monitor the polysubstance detection rate involving fentanyl. Cocaine or amphetamines are drugs most frequently detected along fentanyl in our patients.

It is very important to monitor these trends, especially given the recent rise in stimulant-related overdoses. Personally, though for me, the most rewarding are those cases where we know we really made a difference. For example, helping inform care for vulnerable populations, such as the case of detection of fentanyl exposure in a two-year-old child.

Because fentanyl is integrated in our routine urine drug screen, we were able to perform the right test and the patient received the appropriate therapy.

Randye Kaye: And those are the days that make it all worthwhile. Thank you so much for joining us today doctor.

Nikolina Babic: Thank you so much for having me, it was a pleasure.

Randye Kaye: That was Dr. Nikolina Babic from the Medical University of South Carolina describing the *JALM* article, "Performance of Fentanyl Immunoassays in an ED Patient 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.