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Development and Clinical Validation of a Sensitive Lateral Flow Assay for Rapid Urine Fentanyl Screening in the Emergency Department

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Guest: Dr. Ping Wang is currently Chief of Clinical Chemistry and Director of the Core Laboratory at the Hospital of the University of Pennsylvania and Associate Professor of Pathology and Laboratory Medicine at the University of Pennsylvania.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Fentanyl is a highly potent opioid originally synthesized for analgesia but has become a major contributing factor to the current opioid epidemic in the United States. The National Forensic Laboratory Information System reported dramatic increases in fentanyl-related encounters since 2014, especially in the Northeast and Midwest regions of the United States.

As an example, fentanyl recently surpassed heroin as the leading drug involved in overdose deaths accounting for 84% of opioid-related deaths in Philadelphia, resulting in the Department of Public Health notification to recommend implementing routine fentanyl testing in emergency departments.

Clinical chemists in Philadelphia responded by developing and validating a sensitive lateral flow assay for the rapid screening of urine fentanyl suitable for use in emergency departments. Their work appears in the February 2020 issue of *Clinical Chemistry* and we are pleased to have the senior author of that paper as our guest in this podcast.

Dr. Ping Wang is currently Chief of Clinical Chemistry and Director of the Core Laboratory at the Hospital of the University of Pennsylvania and Associate Professor of Pathology and Laboratory Medicine at the University of Pennsylvania.

So, Dr. Wang, we've read about the increase in fentanyl-related deaths in Philadelphia. Is that what stimulated development for your assay and what other factors were involved?

Dr. Ping Wang: Well, the outbreak of fentanyl overdoses and the increase in fentanyl-related deaths locally is, for sure, one factor for our work. But our motivation also goes beyond local trends. So, as the audience may know, we are in an unprecedented national opioid epidemic. Fentanyl is a major culprit of this epidemic. What is especially dangerous about fentanyl is that it may be linked into other drugs of abuse and the user may ingest it without understanding or any knowledge of its presence.

Fentanyl is also a very potent opioid able to cause fatality at quite low doses.

So, combining all of these together, for people not experienced in quickly recognizing opioid syndromes and giving the antidote, naloxone, a rapid screening assay to detect fentanyl could be very useful to guide triage and rescue in an emergency, and this is a rationale and a motivation for us to develop the assay.

Bob Barrett: So, can you briefly tell us the principle of your assay and what you and your team did to validate it?

Dr. Ping Wang: We designed a competitive lateral flow assay. The operation of the assay is actually quite simple. So, the user mixes a small amount of patient samples with the pre-aliquoted fentanyl antibody gold nanoparticle conjugates. Invert the tubes for a few times to mix and then transfer the mixture to a lateral flow strip.

On the strip the sample flows through, fentanyl competes with BSA conjugated fentanyl for antibody binding. And the presence of fentanyl corresponds to red colors as gold nanoparticles in control line only and absence corresponds to red colors in both control and test lines. We optimized the assay so that it can detect fentanyl at or higher than 1 ng/mL and the norfentanyl at or higher than 10 ng/mL in the clinical relevant ranges in as short as five minutes.

The stability of the strip assay is excellent with the results valid for at least 24 hours after testing and the strips maintaining performance after at least 3 months in storage. After we test that the assay worked in spiked samples, we then perform validation study in consecutive emergency department patient samples we received during over a month period. We test the samples using both the lateral flow assay and the gold standard liquid chromatography mass spectrometry assay. We show that the lateral flow assay has high clinical sensitivity and specificity in the ED samples.

Bob Barrett: And why did you choose a lateral flow assay format?

Dr. Ping Wang: Well, we know that lateral flow assay is a classical format in point of care testing. It can give rapid and intuitive results in a very short amount of time in the hands of amateur users. And these are exactly the features we think fentanyl testing calls for and also that's the reason we choose it.

Bob Barrett: So, what about any other drugs or compounds that may cross-react and give false positive results?

Dr. Ping Wang: Well, we show that the assay has no cross-reactivity with common drugs of abuse such as amphetamine, cocaine, morphine, THC, methadone, buprenorphine, naloxone, and

acetaminophen as high as 1000 ng/mL, but does have some cross-reactivity with carfentanil, risperidone, and 9-hydroxyrisperidone.

Bob Barrett: What is the bottom line here? Is the assay useful in the emergency department outside of the walls of the laboratory and have you deployed it in routine practice?

Dr. Ping Wang: So, in the study, the prevalence of fentanyl in the emergency department at all institutions was about 5.5%. We show that in those populations, the assay has a positive predictive value of 92% and an active predictive value of 100%. So, in a population of relatively low prevalence, the assay can be used for ruling out fentanyl due to its high negative predictive value.

On the other hand, in a population of high prevalence, for example, with known drugs of abuse history, the assay can be used for ruling in fentanyl as the positive predictive value would be even higher than 92%. Besides the emergency department, I think other locations in which the assay could be very useful include first response vehicles, safe injection sites, or even patient homes. So, we definitely look forward to working with our ED to explore deploying the assay in routine practice.

There are some regulatory considerations we need to think about. We have recently implemented an FDA-approved defense analysis screening assay on our automated instruments in the core laboratory, but we also recognize that the turn around time and the logistics of the automated assay would not be sufficient to support on the spot antidote administration in emergency situations.

Bob Barrett: Do you believe that a rapid screening method for fentanyl has an effect on use of the drug or treatments?

Dr. Ping Wang: I do believe the rapid screening of method for fentanyl will impact the triage and rescue of overdose to patients especially by people who are less experienced in recognizing the clinical symptoms and presentations of fentanyl overdose, and this was the goal of our study. We did not actually focus on impacting the use of fentanyl drug itself. Commercially available strips are available and can detect fentanyl in much higher concentrations in drug products, not in clinically valid ranges, and that was not the focus of our study here.

Bob Barrett: Well, it sounds like this has all been quite successful. Let's take a look ahead. What are the future directions of you and your laboratory following this work?

Dr. Ping Wang: For this particular project, future directions may include further shortening the assay time and expanding the sample matrices. Currently, the minimum time from applying the sample mixture to assay readout is about five minutes.

With further development, we hope to decrease the assay time even further as we know every second counts in emergency overdose situations. And there was also potential to optimize and validate the assay for other sample matrices such as saliva. We are in the process of applying for funding from NIH and potentially other funding sources to continue this work. If the audience knows of a good funding source who could be interested, please also let us know.

But in general, my lab is working on several innovative diagnostic technologies in the areas of point of care testing as well as wearable technologies. The overarching questions we ask are, what are clinically unmet diagnostic needs, and what are the next generation diagnostic technologies like in the area of precision medicine and preventive medicine? So, our goal here is to hopefully eventually bring these technologies into clinical practice.

Bob Barrett: That was Dr. Ping Wang from the University of Pennsylvania in Philadelphia. She has been our guest in this podcast about development of a rapid screening method of urine fentanyl suitable for use in emergency situations. That article appears in the February 2020 issue of *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.