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Robert Nerenz et al.
Analytical and Clinical Evaluation of the NOWDiagnostics ADEXUSDx Human Chorionic Gonadotropin Test Using Whole Blood.
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Guest:

Dr. Nerenz is an assistant professor in the Department of Pathology and Laboratory Medicine at Dartmouth Hitchcock Medical Center.

Randye Kaye: Hello! Welcome to the first edition of "JALM Talk." My name is Randye Kaye and I'll be your host. In most healthcare settings, pregnancy testing is performed using urine, but urine testing has been shown to give false negative results in certain patients. The July 2016 issue of *The Journal of Applied Laboratory Medicine* published a paper from a group led by Dr. Rob Nerenz at the University of Kentucky Medical Center, that evaluated a new pregnancy test approved for use with finger stick whole blood, a sample type that presents many advantages over urine-based testing.

Our guest for today's podcast is Dr. Nerenz, the lead author of this research study who is now an assistant professor in the Department of Pathology and Laboratory Medicine at Dartmouth Hitchcock Medical Center. Welcome Dr. Nerenz!

Dr. Rob Nerenz: Thank you!

Randye Kaye: Can you start by giving us a quick overview of how pregnancy testing is performed in most healthcare settings?

Dr. Rob Nerenz: Absolutely! Pregnancy testing is simply performed in many different locations within hospitals and outpatient clinics. In a hospital setting, most testing is carried out in the emergency department to rule out pregnancy before performing either imaging studies or initiating other types of treatment that may harm a fetus. In the clinic setting, testing is often done to confirm a positive home pregnancy test result or to rule out pregnancy prior to prescribing a medication that may cause birth defects.

The key point is that in both of these settings, doctors normally want the pregnancy test results within minutes so that they can either make rapid treatment decisions based on the patient's pregnancy status, or discuss the result with the patient during the same clinic visit. And in order to do this, pregnancy testing is most frequently performed by testing urine on point-of-care devices, because urine can be easily collected for most patients and test results are

available usually less than five minutes after the initiation of the test.

Randye Kaye: Well, there's a lot to that. But you've spoken in the past about false-negative results in urine-based pregnancy testing. So, can you tell me why these false-negatives occur?

Dr. Rob Nerenz: Sure. Pregnancy tests use two different antibodies to recognize the pregnancy hormone hCG. And when the hCG is present in the sample, the antibodies are brought together and you get a colored line. When hCG is absent, the antibodies remain separate and no colored line develops. Now, this sounds like a pretty simple and straightforward approach, but testing urine actually presents a bit of a challenge because there are actually multiple different forms of hCG present in urine. And in fact, beginning in week six or seven of pregnancy, one of these forms beta core fragment is actually present at tenfold higher concentration than the intact hCG hormone.

Now, in certain women, for reasons that we don't fully understand yet, the hCG beta core fragment concentration can be very high, and this can interfere with antibody recognition of the intact hCG hormone. This results in a negative test result even though the woman is pregnant and intact hCG is definitely present in her urine sample.

Randye Kaye: So that could definitely present a problem. So, are there any alternatives in urine-based testing?

Dr. Rob Nerenz: Well, there's certainly are. Hospital labs can measure hCG in plasma or serum. Laboratorians actually prefer this approach because the quantitative measurement is more sensitive than point-of-care urine testing, meaning, pregnancy can be detected earlier which hCG concentrations are low. And as the name implies, it provides a quantitative result which allows doctors to detect abnormal pregnancy by following the change in hCG concentration over time. Now, another big advantage of this approach is that hCG beta core fragment is not present in plasma, it's only found in urine, and this eliminates the concern for false-negative results caused by high concentrations of hCG beta core fragment.

The downside is that plasma or serum hCG testing must be performed in a central hospital laboratory rather than at the point of care, this means that the blood sample must be sent to the lab, processed, and tested, all of which that delay the availability of the test result. So even though we know there are limitations associated with urine-based testing and there are alternatives available, urine-based

testing is performed most frequently because it's faster and considered by most to more convenient.

Randye Kaye: So, in this study, you evaluated a new point-of-care pregnancy test which is designed for use with whole blood. So can you just tell us a little bit more about how this test is different from other test methods that are currently on the market?

Dr. Rob Nerenz: Well, this new test is manufactured by NOWDiagnostics and it really has a pretty similar design to that of urine-based point-of-care pregnancy tests in that it relies on two different antibodies that bind to hCG. And when the hCG is present, you get the development of this line, very similar to urine devices and the new device provides the same kind of pregnant/not pregnant qualitative result. Where this device is different, is that it has a filter that separates blood cells from the surrounding plasma that contains hCG, which means that it can test whole blood without a separate processing step.

Now, because there is no specimen processing step and only a drop of blood is required, testing can still be performed close to the patient with the finger stick sample. And because it doesn't use urine, many of the concerns associated with false-negative test results caused by high concentrations of hCG variance can be eliminated.

Randye Kaye: Wow! That sounds like it will eliminate some potential problems. So in your study, what aspects of this new pregnancy test did you evaluate and how did you carry out that evaluation?

Dr. Rob Nerenz: Well, we wanted to see if the device was susceptible to the hook effect which is false-negative results caused by high concentrations of hCG. And on the other end of the concentration spectrum, we wanted to make sure that it was sensitive enough to generate a positive result early in pregnancy when hCG concentrations are low. We also tested the minimum blood volume required, as well as the maximum hematocrit. All of these benchtop studies were done using whole blood samples collected from non-pregnant volunteers, to which we added purified hCG at known concentrations.

Now, in addition to benchtop studies, we also wanted to see how this test performs in actual patients. So we recruited 40 pregnant and 40 non-pregnant volunteers, and we performed finger stick hCG testing using the new test, urine hCG testing using our hospital's point-of-care device. We compared both of these results to plasma quantitative hCG testing performed in the main hospital lab.

Randye Kaye: What did you find?

Dr. Rob Nerenz: In our hands, the new test performed quite well. It did show some susceptibility to the high-dose hook effect with decreasing signal at high hCG concentrations. But positive results were still seen at the highest concentration tested, which was about two million international units per liter. For point of reference, the highest hCG concentration that we ever saw at University of Kentucky was one million IUs per liter. On the low end, positive results using this device were observed at 18 IUs per liter which is less sensitive than the large hospital lab instruments, but about as sensitive as currently available urine point-of-care tests.

The minimum sample volume was 30 microliters and the maximum hematocrit was 46%. And then in the patient volunteers, the result of the new finger stick test agreed quite well with the urine point-of-care test and the main hospital lab, they agreed giving the same clinical answer for all 80 patients.

Randye Kaye: All 80 patients, wow! It sounds like those results are really pretty positive. So, how do you see this test being used in clinical practice?

Dr. Rob Nerenz: Well, in my view, I think it's really best suited as a replacement for urine-based point-of-care pregnancy test. As we talked about, it's not quite as sensitive as the test available on the large lab instruments and it doesn't give you a quantitative result that allows doctors to follow the change in hCG concentration over time. This means that quantitative serum and plasma testing is really still the gold standard and should be used anytime a point-of-care result doesn't fit with the clinical picture, or if there's concern for ectopic pregnancy or just or gestational trophoblastic disease.

That said, the new test does provide a rapid result. It can be used at the point of care without a specimen processing step and it's not susceptible to false-negative results associated with urine-based testing. It's particularly well-suited to the emergency department setting at where treatment decisions must be made quickly and where urine collection can sometimes be a problem, particularly in trauma patients, women who can't produce urine due renal failure or dehydration, or even those who simply choose not to provide a urine sample.

So I think the bottom line going forward is that it's still a new test. We'll definitely require more evaluation. But I think it really represents a big positive change in the field of point-of-care pregnancy testing.

Randye Kaye: That's great! Thank you so much for joining us today, Dr. Nerenz!

Dr. Rob Nerenz: Absolutely! Thank you for having me!

Randye Kaye: That was Dr. Rob Nerenz from the Dartmouth Hitchcock Medical Center talking about the JALM article, "Analytical and Clinical Evaluation of the NOWDiagnostics ADEXUSDx Human Chorionic Gonadotropin Test Using Whole Blood" for this podcast. Thanks for tuning in for "JALM Talk." See you next time and don't forget to submit something for us to talk about!