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Robert D Maynard.
Personalized Reproductive Hormone Monitoring in Sweat
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Guest: Dr. Robert Maynard from Albert B. Chandler Hospital at the University of Kentucky in Lexington, Kentucky.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Estradiol, the primary female sex hormone, is routinely measured by automated immunoassay or liquid chromatography mass spectrometry for the evaluation of ovarian function and overall reproductive health. As estradiol concentrations fluctuate throughout the typical menstrual cycle, the timing of specimen collection and result interpretation can sometimes be challenging, particularly in patients with menstrual irregularities. Furthermore, traditional laboratory testing requires travel to a phlebotomy site, disrupting the patient's daily routine, and does not provide results in real time, which is suboptimal for patients undergoing time sensitive treatments.

Recently, researchers have evaluated the feasibility of measuring estradiol in sweat using wearable biosensors. If successful, this approach might address limitations of serum based measurement by providing continuous assessment of estradiol concentrations anywhere, anytime. A News & Views article appearing in the October 2024 issue of *Clinical Chemistry* highlights this new approach to estradiol measurement, compares its performance to that of traditional laboratory methods, and describes considerations for eventual implementation. In this podcast, we're excited to welcome the article's author.

Dr. Robert Maynard is an Assistant Professor of Clinical Pathology and the Director of Point of Care Testing, Special Chemistry, and Blood Gases at Albert B. Chandler Hospital at the University of Kentucky Medical Center in Lexington. So, Doctor, let's start here. Could you explain why estradiol can be challenging to measure using traditional laboratory methods?

Robert Maynard: Yeah, sure. First, I just want to thank you for having me on the podcast. You know, it's an absolute pleasure to be here. And so, to answer your first question, yes. So, there are challenges and limitations to measuring estradiol, and that really goes for both the most commonly used methods in the clinical laboratory. And when I say most common, you know, I'm referring to immunoassays and mass spectrometry assays. And so, immunoassays are the most commonly used

to measure estradiol in the blood. You know, they're FDA approved methods. They don't require complex sample preparation, you know, which makes them relatively straightforward to implement.

They can be incorporated into an automated chemistry platform, which helps with throughput. And, overall, they're reasonably well suited for most clinical applications. That includes, you know, routine measurements and, for example, fertility assessment in premenopausal females. However, you know, challenges arise when attempting to measure estradiol at the very low end of the concentration. Estradiol concentrations are much lower in children, for example, and in cisgender males. And so, the low end sensitivity of these assays really starts to become a limitation of these assays, typically in, you know, these scenarios. And so, a different method may be required in order to obtain the information necessary to make certain clinical decisions.

Liquid chromatography tandem mass spectrometry assays, you know, they overcome the sensitivity issue. They have a much better low end sensitivity, and so, they can be used to measure estradiol in cisgender males and pediatric patients. These assays are also very specific. However, there are different challenges and limitations here versus immunoassays. The instrumentation and startup costs are quite expensive. Sample preparation is more challenging, it's time consuming. There's usually additional extraction steps, possibly even the need for derivatization depending on the method that's used. So, the technical expertise required to perform these assays is often significantly higher. And then, on top of all that, these are laboratory developed tests, or LDTs. And so, they have far more regulatory requirements, and there's definitely some uncertainty there given the new FDA final rule regarding the oversight of LDTs.

And so, regarding these challenges, the Endocrine Society position statement on the measurement of estradiol, it highlights many of these technical challenges and limitations for both of these methodologies. You know, they rightly point out that repeatability or precision of the measurements, accuracy, specificity, you know, these are all crucial for the reproducible measurement of estradiol. I highlight the critical need to establish reference intervals for estradiol, and that goes for all ages and sexes, while considering that many pathological and non-pathological conditions can impact estradiol concentrations, which, of course, further complicates our ability to differentiate normal from abnormal. And so, they've recommended standardization of estradiol measurement much in the way that it was done for testosterone. You know, that includes the creation of the reference material, and that will, of course, help with the overall, quality of testing.

Bob Barrett: Well, just to follow up on that, how might a wearable nanobiosensor address some of these challenges?

Robert Maynard: Well, it has several advantages. So first, there's no sample preparation required. The biosensor is simply strapped around the patient's fingertip, and it performs automated induction of sweating. Now, it has its own power supply, it's reagentless, and so, once sweating is induced by iontophoresis, the sweat fills up inside the chamber, the sensor then detects and quantitates the estradiol. And so, if you factor in all the things that contribute to turnaround time, including your collection time, transport to the lab, the testing time, and so on, this device is relatively quick compared to immunoassay and mass spec assays.

It actually only takes about 10 minutes to perform the measurement in sweat. And so, you can get your result pretty quickly. From the preliminary studies, it appears to be quite specific to estradiol as well. It doesn't cross-react with other steroid hormones, even at supraphysiologic conditions or concentrations, excuse me. For sensitivity, the authors of the study demonstrated that these nanobiosensors, they really have excellent sub-picomolar sensitivity. Another benefit is that testing is noninvasive, so it obviously doesn't require a blood draw. And so, I think that opens up the door to the possibility to perform, you know, more rigorous reference interval verification. So, obviously, you know, that still requires recruitment of study participants, but I think it may be easier to identify willing participants, especially healthy children, since there is no venipuncture involved.

And so, from a practical standpoint, that might mean that this device can, you know, open the door to measure estradiol in patient populations that really have been challenging to acquire in the past. I think that could really help us to overcome some of the roadblocks there. Now, of course, there are, you know, there's a lot of unknowns at this point. It remains to be seen how repeatable this nanobiosensor is beyond the initial characterization studies that were performed by the authors.

And also, you know, how truly accurate this device is. And so far, the initial studies, they correlated sweat estradiol to serum estradiol, you know, which is an entirely different matrix, it's a different specimen type. And so, the agreement in these studies was quite poor, for the mere fact that estradiol is present in much higher concentrations in the blood relative to the sweat, you know, orders of magnitude higher.

Bob Barrett: Doctor, speaking as a laboratory medicine professional, what are some additional studies you'd recommend to obtain FDA clearance?

Robert Maynard: Well, I think really in line with the Endocrine Society's recommendations, what we as clinical laboratory professionals typically need to demonstrate when validating a new method or a new device, you know, that includes an assessment of precision. You know, is this nanobiosensor capable of reproducibly measuring estradiol, you know, across a wide range of concentrations, you know, is it repeatable? Accuracy, is the sweat estradiol measurement comparable to blood? Are they interchangeable despite the large magnitude of difference between the two? You know, in other words, do they provide similar clinical information? I mentioned that the initial studies, they show that this device is sensitive, but I think additional studies would be needed to determine the limit of blank, limit of detection, and limit of quantitation also, you know, before putting this into clinical use.

Specificity assessment, you know, so the authors performed some studies already demonstrating that this synthetic aptamer is specific for the secondary structure of estradiol. It did not seem to have interference from, you know, similar compounds and other hormones, but further evaluation would definitely be beneficial to show that there is no cross reactivity with other estrogens, estrone and estriol, for example. For the reportable range, they have to demonstrate the limits in which this quantitative result is valid. Establishment of reference intervals, I think that's a big one, right? I think this is one of the most intriguing for this device. Since it's non-invasive, I think with proper planning, it should be feasible to measure sweat estradiol in all ages and sexes, you know, in presumably healthy individuals, I think that's huge.

Assessment of potential interferences would be important. You know, testing is performed in sweat, not blood. And so, what interferences might be there that we aren't even aware yet? And there are differences in sweat composition between individuals? You know, is this going to present a challenge? And does the location that the device has even placed on the patient matter? Now the authors already performed some preliminary studies to show that the aptamer is specific for estradiol. It doesn't cross-react with other steroid hormones. They've also created the device to account for differences in pH for body temperature among other things, and they also included real-time calibration. So, I think more on-body trials would definitely be necessary to validate this device. So, the initial studies, they only -- you know, use quite a small handful of patients in the study, I think there were two on-

body trials in patients, so they're going to need a lot more than that.

I think, importantly, what would be the intended use of this device? This is going to be critical in determining its clinical utility. Now could this be approved as wave testing? I think I would envision that for a device like this, it's potential use would be at the point of care, more specifically at-home testing of reproductive hormones. And as the authors point out, it certainly has a potential application for fertility monitoring, you know, really where repeated monitoring is acquired in order to detect the rise and fall of estradiol. I think that's going to provide a lot of -- or we're going to need to provide a lot of clinical evidence of that prior to its use in the clinical setting.

Bob Barrett: Okay. Let's expand on that a bit. What are other possible applications of aptamer based nanobiosensors?

Robert Maynard: Yeah, sure. So, while this study focused on estradiol, there are a wide range of other analytes that could potentially be targeted using synthetic aptamers. So, other studies and literature have demonstrated the ability of, you know, nanobiosensors to measure other hormones, metabolites like glucose, lactate, and creatinine, electrolytes. I've even seen nutrients like certain amino acids, other non-hormone proteins such as cytokines or c-reactive protein, and even other certain drugs.

And so, there are a lot of possibilities with this technology. You know, this device -- this specific device, it incorporates iontophoresis, which is already in use for sweat chloride testing for cystic fibrosis, so the technology is, you know, somewhat familiar, and from that perspective, technically speaking, you know, an aptamer could theoretically be synthesized, you know, that is specific for the secondary structure of a wide range of analytes. The challenge here is, you know, what analytes are present and measurable in sweat. So, while you can very likely synthesize an aptamer for most analytes, there may be some of those analytes that are simply below the limit of detection even though the sensitivity of nanobiosensors is really quite impressive.

Bob Barrett: Speaking practically, do you foresee challenges in the validation or implementation of this device for routine clinical use?

Robert Maynard: The short answer is, yes. I think a lot of that will depend heavily on the test complexity status of this nanobiosensor. You know, should it be considered for FDA approval or clearance? So, for example, if this were approved as non-wave testing, how would you assess linearity as laboratory director? Remember, this is a quantitative assay, and so we

would need to demonstrate linear results across the analytical measurement range. You know, this device, it's a closed system, so, you know, can you apply an exogenous solution to the device to verify that position? You know, if so, how variable would that validation testing be? Would there be a need or is it even possible to have external quality control? You know, I think internal quality controls or calibrators would have to be the way forward here, and this device actually incorporates the use of internal calibrators.

It will likely require, I think it's gonna require some outside the box thinking really to envision how this would look, you know, using a 3D printed wearable nanobiosensor, how that would be compared to, you know, our traditional blood testing. Importantly, what did you compare this device to? Currently, estradiol is measured in the blood. This device is using sweat. And so, is it enough to demonstrate comparability to a different specimen type? You know, perhaps not. Also, is there agreement between the two specimen types? I think the preliminary studies from the authors really suggest that there's not. However, there is agreement in the pattern of the rise and fall of estradiol.

And so, I think this device may end up -- kinda forming its own niche. And it's gonna require a lot of thought as to how it can be validated prior to clinical use. And so, there may be some difficulty in, you know, clearing certain regulatory hurdles in order to be able to implement this testing really as anything other than wave testing.

Bob Barrett: But finally, Dr. Maynard, with such granular monitoring of an individual's reproductive hormones, are there any social concerns or implications that we should be mindful of?

Robert Maynard: There are, I think many of these, you know, they're not necessarily unique to estradiol, but they're important to discuss. So let's say, anytime you're measuring reproductive hormones such as estradiol, you know, extra care needs to be taken really not to reveal sensitive information about a person's hormonal health, you know, their reproductive status, or, you know, any other potential health conditions in the patient. Privacy concerns, you know, those immediately come to mind. There you've got to worry about third party access. That could be a concern given that this device can wirely transmit data to an app that's on your smartphone. You know, presumably, this data would also be transmitted to an electronic medical record.

So, I think data security is going to be really important and, you know, that goes for both the patient's confidentiality, you know, as well as for health care systems, for cybersecurity purposes. So, I think a cybersecurity assessment is going to be a very important piece of this and to determine if clinical

laboratories and health care systems are even comfortable allowing whether it's one way or two-way communication to their laboratory information system. So, really, what type of security features available for this device, you know, and what other protections are going to be put into place to protect this data?

Bob Barrett: That was Dr. Robert Maynard from the University of Kentucky in Lexington, Kentucky. He wrote a News & Views article describing reproductive hormone monitoring in sweat in the October 2024 issue of *Clinical Chemistry*, and he's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.