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Angela M. Jimenez Valencia, Lindsay Kryszak, Joshua Goheen, Willem Van Roy, Gabrielle Woronoff, Evelien Mathieu, Hildur Gudjonsdottir, Tim Stakenborg, and William Clarke. *Rapid Quantification of Plasma Creatinine Using a Novel Kinetic Enzymatic Assay.* J Appl Lab Med 2021;6:3 688-701. https://doi.org/10.1093/jalm/jfaa157

Guest: Dr. William A Clarke is a Professor in the Department of Pathology at the Johns Hopkins University School of Medicine and is the director of point of care testing and clinical toxicology for the Johns Hopkins Hospital.

Randye Kaye: Hello and welcome to this edition of JALM Talk. It's from the *Journal of Applied Laboratory Medicine*, a publication of the American Association for clinical chemistry and I'm your host, Randye Kaye. Enzymatic reactions are among the most common used in the clinical chemistry laboratory. Enzymatic method are commonly employed to measure creatinine, a biomarker of kidney function. Most clinical laboratories use end point measurements for enzymatic methods like creatinine, which means that the measurement of product formulation occurs at the completion of the reaction. However, kinetic assays which assess the rate of product formulation between time points may allow for significantly faster measurements. An article in the May 2021 issue of JALM describes the development and validation of a novel and rapid kinetic assay for the detection of creatinine. The senior author of the article and our guest for this podcast is Dr. William Clarke. Dr. Clarke is a Professor in the Department of Pathology at the Johns Hopkins University School of Medicine and is the director of point of care testing and clinical toxicology for the Johns Hopkins Hospital. Welcome Dr. Clarke. Can you start by describing the motivation for this project? Why is a new method for measuring creatinine needed?

William Clarke: It's kind of interesting. Obviously, creatinine has been around for a long time and I certainly never envisioned myself having to work on an assay for that but this was part of a bigger project. In the paper, we mentioned that we were – this may be compatible with silicon photonic methods for measuring analytes and that's actually the project we were working on. And so, we wanted to develop and enzymatic assay and creatinine we thought would be an ideal target for this.

Randye Kaye: Alright, thank you. So, what is different about the creatinine assay you described in the article relative to other assays commonly used by clinical laboratories or point-of-care devices?

William Clarke: Well, we wanted to try to stay as closely as we could to some of the existing approaches that were enzymatic in nature and

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so we mimic several of the enzymatic cascades that are out there for the methods. But I think there are couple of key differences. The first one is that from our understanding, this was the first one that used sarcosine oxidase, as the limiting enzyme and we wanted to do that so that we could get rid of some of the creatine background in the sample the way that we had set up the assay. Then the other thing that is different for us is that we used a different report of molecule called MCDP and so the reason we selected this molecule rather than just monitoring say a standard enzyme cofactor is we were really thinking about the end product if we ever were to get there, so we wanted to be able to use a higher wave length so that the light source or the LED source would be less expensive and so we were targeting in the 650 to 680 nanometer range. And so we were able to find this particular molecule that generated methylene blue when it interacts with horseradish peroxidase and that allowed us to monitor the absorbance at 660 nanometers which was ideal for our application.

Randye Kaye: Alright, thank you. Now, I think you've already answered this question but I want to give you an opportunity to say more about it if you want. The article does the describe MCDP used as the chromogen or the reporter molecule for the reaction. Anything else to say about why it was chosen?

William Clarke: I guess I did cover that a little bit in my previous answer. And so, the big driver was the target of this. The other one is that we found some literature where we had some planned experiments that never really came together but we wanted to, eventually, start looking at some derivatives of MCDP so that we could minimize some of the background or modify the reaction rate to try to perhaps speed up the reaction or add in some selectivity. Unfortunately, those experiments never took shape. We didn't get to take this project as far as we wanted so you know where the project ended is what's published in the paper which is with the MCDP molecule.

Randye Kaye: Alright, thank you. So, why did you focus on a rate-based or kinetic assay, rather than an end point assay? We know kinetic assays can be faster, can you expand on that or are there any other benefits of the kinetic assay?

William Clarke: Well, I think, you know, speed as you mentioned is one of the things that we were going for because our ultimate goal was to develop a disposable point-of-care platform. And so when we think about this point of care testing, we wanted the reaction to go fast. We didn't want to wait 10 or 15 minutes for a result. We were hoping to keep the reaction under 5 minutes in terms of doing this.

So, by using a rate-based reaction, we knew we wouldn't have to wait for the reaction to go to completion and so that

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really was a driving factor. But the other piece was to think how we might be able to compensate for any background absorbance or any interferences that may come from inter-patient variability. And we assumed that if we went on a rate-based reaction, we could compensate for any baseline absorbance that might be present.

Randye Kaye: Alright, thank you. Finally, can you leave us with some key takeaways from the project and any future plans to develop the work further?

William Clarke: Well, I think one of the takeaways even some of the most basic assays can be harder than you think. I really actually came away from this project with a newfound respect for my industry colleagues that work on this type of assays because this was way harder than I thought it was going to be. So as we try to optimize the different enzyme ratios and to tune the assay, this was really a much longer project than we anticipated and really sort of cemented in my mind the good work that our industry colleagues do as they develop diagnostics. So, it's certainly not for the faint of heart and so it was something that we really enjoyed. I think for what's next, I would've hoped to be able to give you the story, this was the beginning of the many assays that we were going to develop. This project actually came out of a collaboration with some colleagues in EMAC in Europe. And while it was a fruitful collaboration, there is a company that came out of this collaboration. That company is really more focused right now on cellular imaging and nucleic acid testing which is outside of what I really do, so for now we are watching closely to see how that company does with their technology focus. But unfortunately for us this is the end of the road for now.

Randye Kaye: Alright, thank you. Well, thank you for your time. Thanks for joining us today.

William Clarke: Alright, thank you.

Randye Kaye: That was Dr. William Clarke from Johns Hopkins University describing the JALM article "Rapid Quantification of Plasma Creatinine Using a Novel Kinetic Enzymatic Assay." Thank you for tuning into this episode of JALM Talk. See you next time and don't forget to submit something for us to talk about.

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