Expanding the Utility of High-Sensitivity Dried Blood Spot Immunoassay Testing with Single Molecule Counting

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Guest: Dr. Jeff Bishop is Senior Vice President of Diagnostics Operation at Singulex in Alameda, CA.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

Clinical testing using whole blood dried on filter paper has been performed since the 1960s. The use of dried blood spots reduces the cost of sample acquisition and offers numerous benefits including simplified storage, transport, and reduction in the risk of infection for laboratory personnel.

Despite these clear advantages, there are significant pre-analytical challenges related to errors from improper collection: sample-to-sample variations in hematocrit, interference from blood cells, and the need for efficient analyte extraction. These challenges coupled with a small sample volume available for analysis have restricted the adoption of dried blood spots for analytes.

Overcoming the issues requires advancements in both dried blood spot quality control and analysis. Diagnostic applications of single molecule counting (SMC) technology make detection of low abundance biomarkers possible that were previously undetectable using dried blood spots.

Recently, a method was described that combines the sensitivity of single-molecule counting with the convenience of dried spot collection. The study evaluates three conventional protein analytes routinely measured in the clinical lab: Troponin I, Prostate Specific Antigen (PSA), and C-reaction protein. The authors detailed the assay method that incorporates spot-scanning to account for dried blood spot variability followed by analysis with single-molecule counting. "Expanding the Utility of High-Sensitivity Dried Blood Spot Immunoassay Testing with Single Molecule Counting" was published in the March 2018 issue of *The Journal of Applied Laboratory Medicine*.

The corresponding author is Jeff Bishop. Dr. Bishop is Senior Vice President of Diagnostics Operation at Singulex

and has worked in the diagnostics industry for more than 18 years. Dr. Bishop has led the development of many drug and biomarker assays on numerous platforms across a wide range of disease states. He is our guest for today's podcast. Welcome, Dr. Bishop.

So, what is new or special about the assay method described in this paper?

Dr. Jeffrey Bishop: The assay method is really, I think, a win-win situation. We took two things which are dried blood spots sample collection and then single-molecule counting technology and we combine them in a way that makes both of them better. So dried blood spot sample collection is easy and inexpensive but it has limited utility, because many of the assays require either more or better controlled samples in order to provide meaningful results. So single-molecule counting technology is a high-sensitivity immunoassay technology developed by my company, Singulex, that overcomes the limitations of dried blood spot sample and provide the clinically meaningful results for many protein assays that couldn't otherwise be measured with a small sample.

Randye Kaye: So it's a case of the whole is greater than the sum of its parts. Can you explain in case somebody doesn't know, what exactly is single-molecule counting technology and how does it work compared to other immunoassay methods?

Dr. Jeffrey Bishop: Sure, I'd be happy to. So single-molecule counting technology has about a thousand times more sensitivity than existing technologies, and it's great because it reveals the presence or absence of disease more clearly and definitively than was possible before it came along. To explain how it works, the technology uses a combination of a brightly fluorescent dye and a confocal microscope along with extremely sensitive single-photon avalanche photo diode detector to gather signals from individual molecules.

The design of the optical system combined with our proprietary analysis methods provides a much greater signal compared to the background noise than other traditional immunoassay methods. All that sounds complicated, so one of the examples I use to try and illustrate this is, imagine that you're in a concert hall with a thousand people and they all begin applauding at the end of a performance. If I ask you to listen to the sounds created by one individual in that group, you couldn't do it because there's too much background noise created by the other 999 people.

Now, imagine you're in that same concert hall and it's empty except for one person. Now if that person claps you

can hear them even though the noise that they're making is the same as it was before. So if I bring that back to single-molecule counting technology, what our optical system, what our instrument does, is it allows as to focus both the light source and the detector on a very small area so that we can count individual molecules and because of that we can see more of them than anybody else.

Randye Kaye: That's a great analogy, thank you. That really helped me to understand it. So, why would you combine this single-molecule counting technology with dried blood spot sampling? What's the advantage of that?

Dr. Jeffrey Bishop: Yeah. So as I said before, dried blood spot sampling has some limitations, so I think the biggest of that is the overall sample volume, so I think, probably, the most common reason people might have been exposed or have seen dried blood spot sampling before is in newborn infant screening. If you have a child, they grab the child, turn him upside down and prick their heel and get as much blood out of the screaming baby as they can, and you don't get a lot of blood. And also, it's not really easy to measure that amount, it's just there's a lot of limitations associated with that sample. And because single-molecule counting technology provides ultra sensitivity, one of the applications of that sensitivity is that a lot of people don't think about is you can then dilute a sample down if you need to have more volume there and then still we have enough sensitivity to measure analytes in that sample even if you didn't have much sample to begin with or you had to dilute it down.

As part of this, we also introduced all of the known and documented quality control steps that others have used before us in order to ensure the quality as much as possible of these samples. So this is things such as punching out a precise area of the paper, of the dried blood spot paper using efficient extraction buffers and then visually reading the optical density of the blood spot before we begin in order to know how much blood we actually started with. So we did all of the things that other people have done but then by combining that with the sensitive assay technology of single molecule counting, we just get better and more meaningful results than a lot of people have gotten in the past.

Randye Kaye: So you chose to validate this method with specific assays that you chose. Why did you choose to do that?

Dr. Jeffrey Bishop: Yeah. So, the three analytes, the three biomarkers that we measured in this specific paper were cardiac troponin, cTnI, Prostate Specific Antigen, or PSA, and then C-reactive protein, or CRP. So we had several reasons for choosing

each one of these and maybe I'll just explain each one really quickly.

First, these three biomarkers are all very well known in the clinical chemistry field. I think anyone reading the journal or listening to this podcast has probably heard of each of these three markers, so that was first. Specifically cardiac troponin I, it's really the gold standard biomarker for detecting cardiac injury in something like a heart attack or also known as a myocardial infarction.

So this test is been an important one and if you look at any issue of *Clinical Chemistry* or *Journal of Applied Laboratory Medicine* in the last five years, there's almost undoubtedly an article about cardiac troponin in there. It's a very popular and very good marker. We have a cardiac troponin assay in our laboratory here at Singulex so we know a lot about it and it made it easier for us to gauge the performance of our dried spot assay because we had something already in hand to compare it to, so that's troponin.

PSA, Prostate Specific Antigen, it's an important marker in the detection of prostate cancer and it's not a perfect marker in terms of specificity, meaning just because you have an elevated PSA doesn't mean you have prostate cancer, but it's still an important marker and its widely used. But in prostate cancer patients that have had their prostate removed either through surgery or to radiation treatments, it has been shown that very small amounts of PSA remaining after that procedure are predictive of cancer recurrence. Therefore, it would be beneficial to periodically monitor these patients, and the dried blood spot collection method is a simple inexpensive way that could make this periodic monitoring much easier.

And then, C-reactive protein, CRP, it's a very widely used marker of inflammation across a wide variety of disease states and it's present at relatively high concentrations compared to troponin and PSA, so it seems like just a good addition to the paper to show that we can measure analytes at low concentration and high concentrations and that the method is not limited to any one marker or one range.

Randye Kaye: Wow. So you've actually already started to answer my next question I think, which is about some of the unique applications for this assay technology. Are there any more you'd like to add to what you've all ready told me?

Dr. Jeffrey Bishop: Sure. This application of the technology is ideal in situations where blood is either difficult to obtain in large quantities or when it's going to be needed frequently like in the case of monitoring that I just mentioned or when the biomarker to

be measured is present in very low concentrations, for example, cardiac troponin.

One example is chronic disease management. If I wanted to test your blood for a specific biomarker, let's say, cardiac troponin, and I needed to do so on, let's say, a weekly or a monthly basis, you'd quickly get tired of having someone sticking a needle in your arm every time you needed blood to do that. Also, depending upon the volume that was needed and the frequency that you are going to do that, it might actually pose a health risk to you in terms of anemia or your general wellbeing.

Another example might be for use in resource-limited setting such as under-resourced countries of any other location where the patient and the laboratory are not in the same place. The dried blood spot samples are stable at room temperature. They can even be mailed through regular postal couriers for the cost of a stamp, so it makes transporting blood much easier.

One of my personal hobbies is running marathons, and so, one of my favorite examples of an application is a paper that we're hoping to publish later on, but we did a study where we actually went to a marathon and we collected samples from runners both before and after they ran the race. Many of these runners wouldn't wanted to have had a traditional blood draw prior to running the race but they were happy to have a small finger stick sample taken. We were then able to measure their cardiac troponin levels before and after the race and compared those results to some of the training and health history questions that we asked them. So studies like that show the promise of this technology and help us see things that would have been difficult or impossible to do in the past.

To summarize, the new method makes sample collection cheaper, easier and more accessible and then the assay technology makes it possible to measure biomarkers that were not previously measurable and this study validates the method and then we also suggest the number of these possibilities in the paper for how it might be used in the future.

Randye Kaye: Wow, that is so interesting and so much less invasive to the person being tested, especially before and after a marathon. I can't even imagine that. Thank you so much for this very interesting interview and have a great day.

Dr. Jeffrey Bishop: Thanks, you too.

Randye Kaye: That was Dr. Jeff Bishop from Singulex talking about the JALM publication, "Expanding the Utility of High-Sensitivity

Dried Blood Spot Immunoassay Testing with Single Molecule Counting" 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.