

Interview

## Saving Lives One Genotype at a Time

When lives are on the line, timing and accuracy are crucial. This message resonates strongly with Dr. Susan Crocker of the Children's Hospital of Eastern Ontario. As a postdoctoral fellow in the Ontario Newborn Screening Laboratory, Dr. Crocker develops molecular diagnostic assays for screening newborn blood for various diseases in an effort to prevent infant mortality and morbidity from difficult-to-diagnose diseases. Currently, she is developing multiplexed assays for clinically significant mutations using VeraCode™ technology from Illumina.

**i: Can you tell us a little bit about the Ontario Newborn Screening Laboratory and some of your reasons for screening?**

**SC:** The Ontario Newborn Screening Laboratory screens all the blood spots for babies born in Ontario, so we get about 135,000 samples per year and currently screen for twenty-four different disorders on each sample. We choose various diseases for screening based on the Wilson and Jungner criteria to prevent morbidity and mortality from rare diseases that are difficult to diagnose in newborns before the damage is done. With this information, we can intervene, provide results for future reproductive counseling, or quantify the incidence of the disease in the population. The classic example of early diagnosis success is phenylketonuria (PKU), where dietary intervention can prevent profound mental retardation.

**i: What are some of the biggest issues you confront for developing assays?**

**SC:** The biggest issues, in terms of which technique we are going to be using, are the false positive and false negative rates, and the timeliness factor. We have to get the results as quickly as possible so we can provide treatment before the onset of symptoms. We try to report within eight days of birth. There is a lot of variability

within the dates that we get the samples because they are mailed to us from whatever area the baby was born in. This means we usually have a 24 to 48 hour window to get our results out because that eight-day window is critical in cases of diseases like PKU. There's not time for lengthy retesting. Early intervention and effective treatment of PKU, MCAD, and congenital hyperplasia can make a big difference in these babies' lives.

**i: What are some of the factors you consider when looking at new technologies?**

**SC:** There are a number of considerations that we use for determining what tests have to be used for carrier reporting with current screening approval from the Ministry of Health. We also have quality control packages for each of the methods we currently use in the laboratory. For our assays, we have to ensure they are within range of our internal quality control requirements. If they aren't within those particular quality control ranges, we end up having to rerun those samples for that entire day, which in some cases can be up to 700 blood spots. Things such as the number of minimum steps and the ease of use of the different techniques are critical, as well as the reporting of the results being clear and concise.



Dr. Susan Crocker, postdoctoral fellow at the Newborn Screening Laboratory, Children's Hospital Eastern Ontario

"The assays I am developing using the VeraCode Universal Capture Beads worked the first time I tried them."

**i: What did your lab find attractive about VeraCode technology?**

**SC:** There are a few features about VeraCode technology that we found interesting. The assay flexibility allows us to determine which bead types we want to put together or not put together depending on what the Ministry of Health has determined we are allowed to screen for. The advantages of moving to VeraCode microbeads from fixed arrays are greater as far as time. For us time is extremely critical, so the fluid dynamics of the VeraCode assay are very appealing. The multiple replicates in the assay are extremely good for quality control.

I've found it easy to examine the spread of the data with BeadStudio software. I am anticipating that it will be easy to set up standards or rules for the performance of these assays using BeadStudio; for example, if all the GenCall scores are below a certain range. The VeraCode microbead holographic code makes the trackability much better if you are running multiple assays using different bead types on the same machine. Additionally, the BeadXpress™ Reader takes up to twelve measurements across each bead so you know that the fluorescent intensity readings you are getting are specifically related to that bead of interest.

There's a level of quality control that has greater potential with the Illumina system over the Luminex system. We can custom order beads to have additional information incorporated into the bead types such as a lot number. We can also be assured that any measurements that we obtain are related to that specific bead type. There isn't the same delay between reading the bead identifier and reporter associated with that bead. Also, there is the appeal of unlimited multiplexing. Theoretically, if we are going to move up and try to do any sort of large gene or multi-gene screening, not being limited to 100 bead types is appealing to us for long-term development. We also chose the VeraCode system because it allows for the development of two-color assays.

**i: What assays have you designed using the VeraCode system?**

**SC:** Being able to do on-site mutation detection ourselves instead of sending out samples for sequencing, which takes a couple of weeks and is obviously expensive, led us to look at hemoglobinopathies first. We wanted to start multiplexing for common hemoglobin mutations that are also clinically significant, so we decided to look first at HbC, HbE, HbSickle, HbO, and HbF. We also selected Spinal Muscular Atrophy (SMA) which is a copy number assay. SMA has a very strong advocacy group in the U.S. that is pushing for screening of SMA in the newborn screening panel. Currently, there isn't any treatment available for this disease, but there are promising research projects that suggest there may be a potential treatment. So, we want to have this assay ready. I've been designing primers for these assays to use with the Universal Capture Beads and I am now in the final optimization stage.

**i: How has the assay development process for the VeraCode system been for you?**

**SC:** It's been rather painless. The guidelines that are given in the VeraCode Assay Guide are fairly straightforward in terms of designing your assay: matching the  $T_m$ s, keeping your products in range, the cycle numbers, the PCR ASPE programs. I have some experience with the VeraCode Assay Designer, your web-based assay design tool. I think it will be a huge advantage for people who don't have time to data mine or try to find their perfect PCR primers. For me, it's my primary job, so it's part of the fun. But I definitely think that a researcher who is busy or can't have a post-doc dedicated to developing primers will benefit immensely from this resource.

The assays I am developing using the VeraCode Universal Capture Beads worked the first time I tried them. I'm still optimizing these assays, but the fact that we were able to get results and have a good starting point with a multiplex reaction the first time is outstanding. I'm now making final tweaks to ensure that these assays are

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optimized for high throughput, speed, and efficiency so that it takes minimal training for the technicians and leaves little chance of error. They're being developed so the number of unnecessary transfers is reduced and the time for doing the assay is as efficient as possible.

**i: You mentioned that efficiency and reduced handling time are important for your clinical applications. Does the VeraCode assay protocol meet these requirements?**

**SC:** The protocols that you have set up for the VeraCode assay definitely involve a lot less movement of sample, which is a fantastic thing for a molecular diagnostic lab because less movement of sample means less chances of error or contamination, which are both major issues. If contamination has happened, or if there is any doubt that something has been shifted or put in the wrong well, that whole plate has to be redone which takes more time, and we don't have that. Anything we can do to reduce handling time and variability is an important advantage for us. With the Luminex system, the way you plate out the beads, you have to take each individual vial, ultra-sonicate it, vortex it, pipette out what you need, then spin down the tube, take off the supernatant, reconstitute it, and then pipette it out individually out into the wells into a 96-well plate. There will be variability across the plate when we are taking the length of time to pipette 96 wells from start to finish. And there will be more room for handling errors. The VeraCode Bead-Kitting tool takes a minimal amount of time and eliminates a lot of repetitive pipetting. The only thing that is redundant is that you wash the plate six times. But

that is the lengthiest procedure you have. It would take me less than an hour to do a VeraCode assay with 14 or 15 bead sets, whereas it would take me two to three hours to do with the Luminex system. So it's about a third of the time.

**i: With the VeraCode technology, you can run both DNA-based and protein-based assays. Do you see this as a benefit?**

**SC:** Yes, most definitely. The fact that we will be able to multiplex for DNA- and protein-based assays with the same machinery has several great advantages for a molecular diagnostics lab. One of them is space. The space in most diagnostic labs, and ours isn't unique in this, is a critical issue. If you can use one machine to do DNA- and protein-based tests on, that's outstanding. The fact that you can multiplex it so that you actually save time rather than running an immunoassay in series is a great thing. Not having to buy a separate immunoassay instrument, which can be a fairly large machine—they are about three times the size of the BeadXpress—is a major, major benefit.

**i: What are some of the future projects you have planned?**

**SC:** We will be screening for cystic fibrosis before the new year and we are currently completing our validation studies for this assay. We are very interested in trying multiplex protein assays using VeraCode technology. We are also interested in developing a steroid panel and techniques to look at chromosomal abnormalities. Eventually we want to be able to do some onsite whole-genome screening.

#### ADDITIONAL INFORMATION

Please visit [www.illumina.com](http://www.illumina.com) to learn more about VeraCode technology.

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Pub. No. 170-2007-010 11Oct07

