FACES OF MASS SPECTROMETRY

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July 2022

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Wildcat Screeners: Part 2

Immediately animated by hearing the voices of each other as they joined our online call, David Millington and Donald Chace (Figure 1) reconnected as if they last saw each other just yesterday. A bounty of exciting material emerged as David and Don reminisced and reflected on a pivotal time in their careers. We have shared their narratives in two parts.

Part I focused on how the duo began to collaborate in the field of newborn screening. Part II transitions into David and Don's insights into how the field of genetic services has been transformed, how mass spectrometry changes and saves lives, and what has inspired them to overcome challenges and keep forging ahead.

For more than three decades David and Don collaborated on the development of mass spectrometry techniques that forever shaped their individual paths as well as the field of mass spectrometry and genetic services. Their cooperative development of tandem mass spectrometry methods in the 1990s has been recognized as a notable milestone in the advancement of newborn screening methods.

Separated only by distance, David is in North Carolina — currently Professor Emeritus of Pediatrics at Duke University— and Don is now in Massachusetts and is the application and product specialist for Capitainer. These two will forever remember being nicknamed "Wildcat Screeners" when they were renegade scientists who chased their shared vision, even when confronted with distrust or resistance to change. Teamwork, determination, and a healthy dose of optimism led to an incredible journey.

Where do most genetic screenings using mass spectrometry take place?

David: The public health laboratories have acquired the technology gradually. Some of them are very small, in terms of their population, and the affordability of the technology for them is challenging. They may form a consortium with a larger program that has the technology. There's also a company called Perkin Elmer Life Sciences that offers to do this under contract. The initial testing on the dried blood spot (DBS) is still done within the public health arena because they own the DBS and prefer not to let them go out of their jurisdiction. Traditionally, the testing is still done within the state laboratory.

Don: Consideration for the use of DBS beyond newborn screening (NBS) is taking off, and even more so because of COVID. At the height of COVID, it was not desirable to go to the hospital or clinic to have a blood sample collected. Ideally, a sample should be collected at home, which would allow a patient to avoid traveling to a clinic and being exposed to, or potentially exposing others to a disease like COVID. With home sampling and DBS, you could simply collect a drop or two of blood, apply to filter paper, and send to the laboratory via the mail. The idea of DBS is experiencing a reawakening and a reimagining. But it is not new, and I always remind colleagues of that. "Don't forget the roots of NBS," because we were doing it—how many years ago, David? At least 30?

David: Probably even more than that.

Don: I always look at mass spec and DBS as going together. In 1960, you had the first NBS of a DBS for the disease PKU. By 1980, there were six tests, none of which involved mass spec. But now, you can say that NBS is almost all mass spec. There are some diseases that aren't screened that way, obviously, and in 20 years, those in molecular biology might say it'll be all DNA. But I think mass spec has, in any event, made a huge contribution.

Have you seen growth in outreach to communities that provide information about genetic services and NBS?

David: Don and I have worked to educate public health programs about this—to provide the ability and the wherewithal to acquire the technology and to use it appropriately for this purpose. But remember, NBS is only a screen—it's not a diagnosis. Even if the initial NBS test is strongly indicative of a metabolic disease, the confirmatory diagnoses are made in the biochemical genetics laboratories. Every state has at least one regional genetic center that will either perform the testing if they have a laboratory, like Duke does (Figure 2) within the center, or they will refer diagnostic testing to one of the commercial labs, like Quest or LabCorp. But the real challenge for NBS comes in limiting the number of false positives—those are very costly to follow up on. Now we have

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Figure 1: Don stands in the stone matrix and archways of Machu Picchu. It is amazing to discover what human civilizations can do with stonework and gardening thousands of feet high—and you thought mass spectrometry was amazing! (Photo courtesy of Donald Chace.)

tools that help us with that, but it's important to remember that it's two separate systems. In the NBS laboratory, we're asking the question: Is this baby at risk for one of these inherited metabolic disorders? I would say that the expansion of testing for metabolic disorders in the newborn due to tandem mass spectrometry has put NBS firmly on the map and given much impetus to the field of biochemical genetics. Now, there are all kinds of special interest groups encouraging further expansion, because we have the opportunity to treat patients with rare diseases using new therapeutics. But it started in the early 2000s when tandem mass spectrometry became accepted within the standard practice of NBS. Right, Don? Would you agree?

Don: I would say that NBS programs are part of public health, which has ownership of that space. Public health programs in the various states in the United States mandate what is screened by law. When we first started, one of the biggest problems with state public health labs was the differences in screening based on where you were born. If you were born in State A, you might get 6 screens, or if it was just across the state line in State B, you might get 10 or 20, depending on the laws and mandates. That's why a private lab in Pittsburgh offered supplemental screening to hospitals. So, if you were in a state that only did a handful, like Pennsylvania, in the early 1990s, and you wanted to screen for many new diseases, there would be a private lab where you could get this test done.

Dave and I did a pilot project to demonstrate feasibility of NBS by MS/MS and in just 10,000 babies detected 4 rare diseases. Obviously, for other disorders, where the incidence is perhaps 1 in 400,000, you would need to screen at least a million babies. That's what happened in the first private lab in Pittsburgh, where their supplemental screening lab started to achieve those numbers and demonstrate that it could detect new diseases that are quite rare. The numbers flowed in showing that if you combined all the diseases in NBS by tandem MS, the incidence was not so rare at all. Everything sort of started to change yet again around the year 2000, because that's when NBS using MS/MS started to be accepted and used by individual states. It was recognized that tandem MS could indeed detect these rare diseases, while also replacing other older analyses for a disease like PKU with a mass spec analysis that could justify use/cost, etc. Interestingly, with such a large panel, committees were set up to lead to more uniform panels across the United States, where the disparity between what state you were born in and what was screened no longer existed.

In terms of disease detection and diagnosis, at some point in a disorder, a diagnosis is made. Without screening, it's made after a baby suffers a life-threatening illness, and often the treatment can no longer improve the health of an affected infant. Some may die or have died, while some have a permanent disability that is physical, mental or both. Screening is so important that it cannot fail, and therefore the best analysis must be used to afford early detection, a timely diagnosis, and more effective treatment. Even though we don't call NBS newborn diagnostics, you never get to a "diagnostic" until a disease is clearly expressed, and damage is done. I have never accepted that screening was just "screening." I always believed we had to be as close to a diagnostic as we could be. Mass spectrometry got us there in terms of accuracy.

False results were a real problem with older technology. One goal of using tandem MS was to reduce false positives while never having a false negative if possible. Mass spec really did reduce false positives; with PKU, we improved it 10 to 100 times better than with the old technology. I think the way to summarize this is: We've gone from the time people said we were "Wildcat Screeners," and that this would never happen to a time when literally every baby born in the United States, and probably most of the world, is getting a mass spec profile. And that's why, from my perspective, I was lucky to end up in Dave's lab, because it was obviously a career-changer.

Figure 2: David (front) with Dr. Charlie Roe, shortly after they began their collaboration at Duke. (Photo courtesy of David Millington.)

How has your work helped to save newborns' lives?

David: Imagine that your own child is born in the hospital, and that DBS sample is collected and goes to the state lab. Tests are done that can reveal up to 35 inherited metabolic conditions. You cannot predict where a disease-causing gene mutation is going to strike in a family—it's just chance. Both you and your partner may have such a mutation in the same gene that could cause that disorder to arise in the child. And if it does, you probably will not know for several months, or even years in some cases when there is an underlying, potentially devastating condition. By doing NBS, you're avoiding that possibility—you're averting the chances that this child could be lost through a metabolic crisis occurring in the early neonatal period or later in childhood. We're absolutely certain, in fact, that this was one of the justifications used by other programs to bring tandem mass spectrometry into NBS, despite its cost and the resistance factors all over the world—what's the cost of this technology compared with misdiagnosing or missing a diagnosis?

Have you had any meaningful or rewarding experiences working directly with newborns/children and their families?

David: Did you ever hear of the Stallings case in Missouri that occurred in the early 1990s?[1] I was involved in that. A baby was born to a mother who happened to have an inborn error of metabolism. But doctors convinced themselves that because she kept bringing this baby back that she was poisoning the child. They did forensic lab tests, but they misread the signal for methylmalonic acid as diethylene glycol, which of course is in antifreeze. So, when the baby died during a crisis, the mother was tried and convicted of murder. By chance, she was pregnant when she went to jail, and that child was also affected. Even though she was in jail, and only had limited access to the baby, they convinced themselves that she was poisoning that child as well! So samples were sent to my lab and others to make a diagnosis on the second baby. The results were positive for a

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condition called methylmalonic acidemia, the effects of which, untreated, resemble poisoning. I asked for a DBS sample from the first baby and sure enough, it was also positive for methylmalonic acidemia. That evidence was in part used to eventually overturn the conviction and release of this woman. The cost of litigation before and after the trial far exceeded the cost of tandem mass spectrometry. I use that as an example to convince states that it would be much better to buy the tandem mass spectrometers and put them into the state labs to prevent this very thing.

Don: Being a forensic scientist myself, when I was in Pittsburgh, we developed a postpartum screening program. We started screening the medical examiners' cases for sudden infant death syndrome (SIDS). We found that 1 percent of SIDS cases were actually metabolic in origin. In deaths of shaken baby syndrome we must also rule out metabolic disease, because one of the metabolic diseases mimics shaken baby syndrome. At one hospital, there was a family with twin girls and another family with a boy born around the same time. All three of these children, from quite different family origins, had the same rare inborn error of metabolism. The twin girls were fortunate to have been screened by tandem MS, and early detection of the disease led to early treatment and prevention of the worst consequences and improved health. The boy, unfortunately, did not get the

Figure 3: David and his wife Lin enjoying holiday time near Salzburg, Austria. (Photo courtesy of David Millington.)

additional screen, the disease was not detected early, and he suffered severe disabilities throughout his life. There was a photo of the three of them together, and I have to say that photograph speaks more about the need for screening than thousands of words in a policy statement. Screening matters and, as I said, all newborns now are screened in the United States for the diseases that affected these kids.

What have been some of the biggest challenges of your career?

David: One of the main problems with NBS is getting the specimen from the birthing center to the NBS lab—you can't put mass spectrometers into the birthing centers. So, another interest of mine coming out of this NBS is a technology called digital microfluidics, where you can do some testing on a limited number of babies in the birthing centers and see whether there's a risk before the baby leaves the hospital or birthing center. They can be kept back for observation and further testing before the rest of the tests are done in the NBS lab. So that's another possible future application of technology. This would be different technology, and although it would not replace mass spectrometry, it would augment it by doing some time-critical tests on the newborns in the birthing centers, with the rest of them performed in the NBS lab. That application is new, and it's gaining some traction. But I don't think mass spectrometry is going to be replaced anytime soon

What are your interests outside of the lab?

David: I have six grandchildren, so I enjoy spending time with them. I'm also interested in cooking, reading, and listening to classical music. I love to go to the symphony and the opera, and so does my wife (Figure 3). We both enjoy traveling. I like watching sports, especially soccer, ACC basketball, rugby, cricket, and golf. Retiring a few years ago has greatly assisted me in those pursuits.

Don: I've always been into painting and arts and crafts—my whole family is crafty. I like doing science illustrations and have some fun with that. I also do a lot of gardening; we've grown some vegetables and quite a few flowers. And I enjoy playing strategy-based computer games—it's fun to see if I can run the world better than it's being run right now. I also like going to meetings. I went to the ASMS meeting last year, and there were fewer people in attendance because of COVID. But it was fun because I got to meet everybody on a more personal level. I will say this: I have always been treated well and with great respect by ASMS, and they have always understood the value of what we are doing—they have been part of my science family.

References
1. "Patricia Stallings," Unsolved Mysteries Wiki. https://unsolvedmysteries.fandom.com/wiki/Patricia_Stallings (accessed May 22, 2022).