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Sebastian Lunke and Zornitza Stark.
Can Rapid Nanopore Sequencing Bring Genomic Testing to the Bedside?
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Guest: Dr. Zornitza Stark, a clinical geneticist at the Royal Children's Hospital in Melbourne, Australia.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. Genomic sequencing is quickly transitioning from the research to the clinical setting, with its implementation accelerated by substantial government investments across multiple healthcare systems, particularly at areas such are rare disease, cancer, infectious disease, and screening. For all the progress of the past decade, clinical genomic testing remains far from being a routine diagnostic test. The barriers to widespread adoption are many; high cost, limited reimbursement, long wait times to get results, expensive and cumbersome sequencing and bioinformatics infrastructure, and complex data interpretation. But can rapid nanopore sequencing bring genomic testing to the bedside? That is the question posed by Sebastian Lunke and Zornitza Stark in a perspective article appearing in the December 2022 issue of *Clinical Chemistry*.

We are pleased to have Dr. Stark join us in this podcast. She is a Clinical Geneticist at the Royal Children's Hospital in Melbourne, Australia, and involved in the integration of genomic testing into healthcare, particularly as a first-tier test in children with rare disease. So first of all, Dr. Stark, what prompted you to write this perspective article for *Clinical Chemistry*?

Zornitza Stark: So, I'm a clinical geneticist working at the Royal Children's Hospital in Melbourne and most of my work involves children and families affected by rare disease. So, I lead a national study here in Australia that's trying to provide rapid genomic testing in that clinical situation. So, we were very excited to see the work from the US using nanopore sequencing in this setting, which provided much faster turnaround times than we are generally able to achieve.

Bob Barrett: You cite a paper by a group at Stanford that reported accelerated identification of disease-carrying variants with the nanopore technology. Can you tell us what they did that you found particularly novel?

Zornitza Stark: So, I guess to set the scene a little bit, rare diseases are generally very difficult to diagnose and generally speaking, take around five years on average to reach an accurate diagnosis. So, genomic testing is now increasingly adopted within the clinical setting and it has transformed the process of rare disease diagnosis. But still, most genomic tests produce a result in around three to six months, which is still quite a lengthy period of time to be waiting. Unfortunately, many children affected by rare disease can become critically unwell and be hospitalized in intensive care units. In that sort of setting, you need results much, much faster, much more on par with other pathology tests that are used in that setting, so a test result in hours or even a day or two, is what is useful in that clinical setting.

So, despite all the advances of genomic testing in the last 10 years or so, generally, even when we try and produce a result rather quickly, the best programs in the world are doing that with turnaround times more in the region of three days, and before this study was published, the world record stood at 21 hours to have a result. What was really exciting about this study, one thing is the speed with which the result was produced, so the fastest result that they were able to achieve was 7.5 hours, so a three-fold reduction on the world record, which is really impressive.

The second thing that is really interesting and exciting about the study is the type of technology that was used. Standard genomic testing at the moment uses a short-read technology which means that considerable bioinformatics processing of the data that is produced to try and piece it together, to see exactly what is happening at genome level. So long read sequencing holds the promise of us being able to identify many more disease causing variants, therefore increasing the diagnostic yield of this type of testing, or being able to provide more answers to more families. The third thing about this study that's really exciting is that the technology it uses, the machines that it uses, are quite different to the standard machines that we use.

The standard machines that we use in the laboratory are generally very large, so they're laboratory-bound, whereas this new type of technology uses much, much smaller sequencers. So there are several different examples of those, but the smallest one is, for example, handheld. So, really raises the prospect of me, one day as a clinical geneticist being able to have a machine like this in my office when I see patients or if I'm called to the intensive care unit to see a sick baby with a rare condition, being able to have a sequencer with me so that the blood sample is taken there and we start getting a readout of the genomic information immediately.

Bob Barrett: Those authors use the phrase "ultrarapid nanopore genome sequencing," yet their best case as you said was about 7.5-hour turnaround time. While that may be rapid, it doesn't sound like ultrarapid. Does it to you? As a clinician, what would you consider ultrarapid?

Zornitza Stark: It really depends on your perspective, I guess. So, 20 years ago when I graduated from medical school, it's important to remember that that was the time when the first human genome was sequenced, and that took over 10 years. Again, we've made huge strides towards making that faster with next-generation sequencing technologies, but our standard turnout times at the moment are three to six months. Even when we try really, really fast, usually most people can do it in three days. So, seven hours for me as a clinical geneticist is indeed ultrarapid, but I do absolutely appreciate the point that when I speak to intensive care physicians, seven hours to them is actually quite slow. So, some of the other types of pathology tests that are used in the intensive care environment produce results in a few seconds.

So, for example, a blood gas machine would give you a result in a few seconds. So, it does depend a little bit on your perspective. So, for me as a clinical geneticist, usually where it's three to six months for most of my results, seven hours is pretty awesome. But I think for this type of testing, it really takes off and becomes an everyday test in the intensive care environment, I think you're right. We need to match a little bit more the expectations of our intensive care colleagues and probably something that is with a one to two-hour turnaround time to them, that would be pretty awesome.

Bob Barrett: There's an old adage; fast, cheap, good and you could only pick two, so what --

Zornitza Stark: That's right.

Bob Barrett: What are the tradeoffs for faster since error rates for nanopore sequencing have been reported to be somewhat high, and at what cost can we have that speed?

Zornitza Stark: I mean, this is a problem that we grapple with all the time with this type of testing. Obviously, speed is very important for particular types of clinical scenarios, especially when patients are critically unwell. I would absolutely hate to trade off against accuracy or good quality. It's important to remember that the information that this test provides is informing critical care decisions, and some of those decisions are irreversible, or kind of life and death decisions, so you definitely would not want to trade off against accuracy. So, unfortunately, often what this means is that this type of genomic testing comes at considerable expense.

I can't really comment on the costs of nanopore sequencing and I don't think those are well understood at the moment. But just to put this into perspective, the program that we run here in Australia that provides three-day turnaround times for these patients, the testing costs five or six times more than the average genomic test and it is also important to put this into context that genomic tests are probably some of the most expensive pathology tests that we have at the moment. So, you know, usually they average thousands of dollars per test.

Bob Barrett: Doctor, you and your group have published extensively on rapid genomic testing for critically ill children. I think readers of *Clinical Chemistry* would be interested in your own work. Can you tell us a bit about that?

Zornitza Stark: We have been very privileged to lead a national study here in Australia to provide rapid genomic testing to critically ill babies and children. So, this idea has been around for 10 years and it has been championed particularly by Stephen Kingsmore in the US. But what we really wanted to do was to come up with a program that ensures that this type of testing is not just available in one or two children's hospitals in the country, but it is available to every critically ill baby or child with rare disease that would benefit from it, regardless of where they are geographically.

Australia is quite a large country by land mass, but the population is very unevenly distributed. So, you know, what's available in Melbourne, we also wanted it to be available in Darwin or in Perth. So we built a national network to facilitate this type of testing. So, our study has just finished recruitment. All out, we've tested 450 critically ill babies and children from all around Australia, so we did recruit from all of our states and territories including all children's hospitals. So, we have been able to show that this type of testing is deliverable at scale nationally.

I think that really important. I mean, it's really exciting to demonstrate that you can do something once or twice or to break the world record, but the next challenge after that is actually bringing it into clinical practice and ensuring equity of access.

Bob Barrett: Well, finally, in your perspective article, you posed this question. So, are we ready for genomics at the bedside yet? Well, are we?

Zornitza Stark: I think not quite, but I'm really excited by the prospect. There's a few hurdles I guess in front of us, and we would be keen to explore this locally. But first and foremost, this type of testing needs to be clinically accredited. So, as I said, I think that's really important because very high-stakes medical decisions are made on the basis of these results. So,

we really need to have robust reproducible systems around this test. Secondly, I guess for this to truly become a bedside test, we need the medical workforce to become as comfortable looking at genomic data as they are, for example, looking at x-rays or MRIs. So, we really need many, many more people to be upskilled in the use of genomics as a pathology test, much in the way they are already upskilled in using all sorts of other investigations. But that will require a huge amount of effort.

Bob Barrett: That was Professor Zornitza Stark from the Royal Children's Hospital in Melbourne, Australia. She has been our guest in this podcast on bringing rapid genomic sequencing to the bedside. She is an author of a prospective article with that title that appears on the December 2022 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.