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Molecular Diagnostics: Going from Strength to Strength

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Guest: Dr. Carl Wittwer is Professor of Pathology at the University of Utah and Medical Director at ARUP Laboratories in Salt Lake City.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Molecular Diagnostics is clearly going from strength to strength. That's the title of the lead article by the guest editors in the January 2020 issue of *Clinical Chemistry* that is devoted to this topic. It has been five years since the last molecular diagnostics theme issue was published in *Clinical Chemistry*. In this new edition of the theme issue, the editors have highlighted some of the most exciting developments in the field including the maturation of massively parallel DNA sequencing technology allowing production of genomic data at the hospital or even population level.

Joining us in this podcast is one of the guest editors for this special issue, Dr. Carl Wittwer. He is Professor of Pathology at the University of Utah and Medical Director at ARUP Laboratories in Salt Lake City. So, Dr. Wittwer, let's get basic, first of all. What is molecular diagnostics and why is it important?

Dr. Carl Wittwer: Molecular diagnostics has a relatively recent history. It was essentially unknown before about 1960 or so. And if you think about the actual words, it just means diagnostics, looking at molecules and of course, that's nothing new. We've looked at molecules for diagnosis, glucose for diabetes, etc, for many, many years. What was different around 1960-1970 or so is that, nucleic acids were starting to be used for diagnostics.

And that's what molecular diagnostics has come to mean today, is looking at nucleic acids, the DNA and RNA in particular that have become known as the molecules of molecular diagnostics. And nucleic acids of course, are important because DNA is the blueprint of life. It defines who we are in terms of genetics, in terms of any kind of a malignancy or cancer that might arise and also in terms of infectious disease.

So, the use of DNA and protein for molecular diagnostics, that really has been in the last 50 years or so. And why is it

important? Well, it really does define who we are. When things go wrong in genetics, you can end up with cancers and it's also a great way to look at infectious disease because each organism then has its own characteristic DNA or RNA that can be tested for. So molecular diagnostics looks at nucleic acids.

Bob Barrett: What are some examples of doing more with less in molecular diagnostics?

Dr. Carl Wittwer: One of the reasons why molecular diagnostics has become so important is that you can get a lot of information from a very small amount of material. And the first crucial technology that provided an example of this was the polymerase chain reaction or PCR. And that really allowed you to look at a very small amount of material, a small amount of nucleic acid, and amplify that in a test tube in vitro so that you could then easily look at it in terms of determining its sequence or its specific genotype. And the PCR has been here now for 30 or 35 years or so, and it has gone through a number of different changes over time.

One of the more recent advances in the polymerase chain reaction is called digital PCR. So, instead of an analog signal, PCR has been converted into a technique that actually counts individual particles. So, digital PCR output is the ones and zeroes that we're all familiar with in terms of digital analysis. That allows you to look at any particular amplified target in a level of detail that hadn't been done before.

In addition to PCR, the other major advance in molecular diagnostics over the years is in terms of sequencing, particularly massively parallel sequencing. When I say massively parallel, this is sequencing that's performed not just on one or two or 10 or 100 different targets at once, but thousands or even millions of targets at once. So, this kind of massively parallel sequencing, also known as next-generation sequencing, has opened up new frontiers in molecular diagnostics. And much of the molecular diagnostics issue is dedicated to different applications of massively parallel sequencing. So, this has allowed us to go from, not just looking at specific mutations or even single genes, but to effectively interrogate sets of genes, so-called gene panels, for particular disorders.

It can allow us to look at complete exomes, or all of the expressed genes, in a genome. You can also do complete genomic analysis of three billion base pairs that we all have can be completely sequenced by massively parallel sequencing. And these days, you have the ability not only to look at individuals, but to look at populations. So, there are projects in multiple countries to massively parallel sequence

thousands of people. In the UK, there's a project called 100,000 Genome Project to completely sequence the genome of 100,000 different people. So, massively parallel sequencing is possible by the ability to look at single molecules and amplify them as clones and later sequence those clones. So, it's an amazing technology that everyone in the lay press and current scientific investigation is really taking advantage of.

It's advanced not only to look at the sequence, but also to be able to determine copy number changes in the genome and also fusions or translocations by looking at RNA. So, in summary, you've got in the background PCR, you've got the introduction of massively parallel sequencing, that really allows us looking at single molecules potentially even within single cells. A lot of this is implemented and enabled by advances in microfluidics, looking at small volumes of fluids to look at those single molecules.

Bob Barrett: Let's talk now about gathering the information from those cells. How much molecular information can you obtain from groups of cells, individual cells, even molecules outside of cells?

Dr. Carl Wittwer: Historically, molecular diagnostics was often performed in the case of cancer in terms of surgical excisions. So, biopsies that were taken during the time of tumor excision, over time smaller and smaller amounts of tissue were necessary for molecular analysis. So, instead of a complete surgical excision, localized biopsies and needle biopsies could be taken. One of the more important advances is not even needing to sample the actual tumor for cancer diagnostics.

The liquid biopsy allows biopsy not of the tumor, but of whole blood taken by regular venipuncture, and this can inform about the state of tumors because those tumors actually shed tumor cells into the circulation. These are called circulating tumor cells and had been one focus of enabling molecular diagnostics in cancer that allows you to determine the prognosis of a tumor and to follow its response to therapy by looking at circulating tumor cells rather than biopsies of the actual tumor itself.

Not only do tumors shed cells, but they also shed circulating tumor DNA into the bloodstream, so that DNA circulates. Not only DNA but RNA from tumors and small RNA molecules called, micro-RNAs, are also shed into the bloodstream and carried by small extra-cellular vesicles. So, the liquid biopsy enables monitoring the status of tumors not by sampling the tumor, but by looking at circulating entities like circulating tumor cells and circulating tumor DNA.

Being able to look at circulating tumor DNA has become very important clinically in terms of identifying circulating fetal DNA in terms of non-invasive pre-natal testing. So, disorders such as Trisomy 21 and micro-insertions or deletions can actually be identified. Some of the more recent advances use circulating fetal DNA to detect even single gene defects like cystic fibrosis.

Bob Barrett: How can molecular diagnostics help to diagnose infectious diseases?

Dr. Carl Wittwer: Infectious disease of course is one of the more important causes of human morbidity and mortality. And before the advent of molecular diagnostics, diagnosis of infectious disease involved plating and growing the micro-organisms on media. And what molecular diagnostics has enabled in terms of molecular diagnostics, now we can look at the DNA or RNA specific to the infectious agents for diagnosis. Each organism of course, is identified just as we are, by a unique set of DNA sequence and this allows individual organisms to be identified and diagnosed including things like HIV and hepatitis C and other hepatitis viruses.

Since, often individuals present with a syndrome that looks like some sort of infectious disorder, one of the advances recently has been syndromic diagnosis in infectious disease. So, given a syndrome, can the molecular technique identify which particular organism, which virus or which bacteria is causing say flu-like illness or gastrointestinal disorders or meningitis. So syndromic diagnosis has become important recently and there are now sample-to-answer techniques that provide and identify the causative agent and then enable specific therapy.

Bob Barrett: While detecting molecular changes for disease diagnosis, that's one thing, therapeutic intervention is a whole another thing. Can molecular techniques be used therapeutically?

Dr. Carl Wittwer: Very interesting question in terms of therapeutic use of molecular technologies. Molecular diagnostics itself, of course, just diagnosis, but there's been recent advances in molecular technology that allow alteration of the genome, editing the genome, changing the sequence of the genome from something that may be disease-causing to revert it back to normal sequence. A lot of this has been enabled by CRISPR-Cas9 genome editing that makes practical this kind of replacement or editing of base changes. It is the beginning of a paradigm shift in molecular medicine where active molecular intervention of diseases caused by genetic defects can be modified.

So, there's strong hope not only for diagnostics, but also for therapeutic intervention. At the same time, all of these technologies, these very powerful technologies in terms of the depth of information, also potentially can be misused. So, as we step into this new age, there are concerns about discrimination, use of genomic information, for instance, for insurance stratification, also concerns about privacy. One additional concern on this information is its repeatability. Massively parallel sequencing is extraordinarily powerful, but you do get different answers depending on which cells you analyze and quality control mechanisms to ensure reproducibility between laboratories is becoming more and more important. With the massive information, the interpretation of the data often becomes the limiting resource in terms of utilizing genomic information and there are advances in using artificial intelligence to aid in this process with different kinds of machine learning to analyze the extent of information.

Bob Barrett: That was the Dr. Carl Wittwer, Professor of Pathology at the University of Utah and Medical Director at ARUP Laboratories in Salt Lake City. He has been our guest in this podcast on Molecular Diagnostics, the focus of the January 2020 special issue of *Clinical Chemistry*. I'm Bob Barrett, thanks for listening!