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Microbiome Diagnostics

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Guest: Dr. Robert Schlaberg is Chief Medical Officer at IDbyDNA in San Francisco and Assistant Professor of Pathology at the University of Utah School of Medicine in Salt Lake City, Utah.

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.

Breakthroughs in sequencing technology and computational biology have provided the basis for studies of the myriad ways in which microbial communities in and on the human body influence human health and disease. In almost every medical specialty, there is now growing interest in accurate and replicable profiling of the microbiome for use in diagnostic and therapeutic application.

In the January 2020 issue of *Clinical Chemistry*, which is devoted to topics in molecular diagnostics, Dr. Robert Schlaberg reviews microbiome diagnostics. He is Chief Medical Officer at IDbyDNA in San Francisco and Assistant Professor of Pathology at the University of Utah School of Medicine in Salt Lake City. Dr. Schlaberg is our guest in this podcast. So, doctor, we hear a lot about the various microbiomes that live in and on our bodies and how they may influence our health and disease, but is microbiome profiling ready for primetime?

Dr. Schlaberg: This is a great question. The microbiome profiling uses a range of different technologies that have evolved quickly over the last decades or so, it started all using 16S-phased amplicon sequencing using traditional Sanger sequencing, and has more recently evolved to using next generation sequencing, initially also based on 16S amplicons and then shotgun sequencing. And so, the scope of this technology has evolved and it's evolving quickly.

So, with that, our knowledge has grown substantially. And there are increasing applications that are being tested. So, whether this technology is ready for primetime depends somewhat on exactly the scope and the intended use that we are talking about. So, traditionally profiling of microbial communities has been focused on bacterial populations, the bacterial microbiome.

More recently, there has also been growing interest in profiling members of the viral and fungal flora. We have

studied different sites of the human body such as the intestinal microbiome, but also other sites, in the lung or the oral microbiome, skin and placental microbiome and so on. And so, the answer to this question will depend on the intended use.

But there are lower hanging fruits, so there is already an FDA-approved or multiple FDA-approved test for bacterial vaginosis, which essentially is a test for dysbiosis or an altered bacterial flora, that are on the market. They are not using more complex sequencing-based technologies. They are based on more traditional nucleic acid amplification tests, but this is an initial foray into the broader world of microbiome profiling. And so, I think the scenario that is of growing interest to not just any more scientists, but also diagnostic laboratories.

Bob Barrett: Do diagnostic laboratories need to consider microbiome testing and what type of laboratory is ideal-suited for such testing?

Dr. Schlaberg: Yeah. So, there are a number of laboratories that are already offering these tests. Again, there is evidence that's rapidly evolving for use of microbiome profiling tests for diagnostic or prognostic uses and widely varying applications from identifying specific pathogens or strains of organisms that have been associated with outcomes in this genotype. There have been profiles, microbiome community profiles, that have been strongly associated to the risk for cancer development, for example.

There are a number of different applications and so, the intended use of test will influence which laboratory may be interested to offering such tests. And so, I fully expect that the intended uses to further evolve over the next years and I think clinical laboratories, diagnostic laboratories, will find themselves being asked to consider these kind of tests or send them out to laboratories that may be offering them.

Bob Barrett: What are the challenges for diagnostic laboratories bringing on such testing?

Dr. Schlaberg: Diagnostic laboratories have increasingly benefited from automation and standardization of testing that is routinely performed. And so in contrast, the workflows that are being used for most microbiome type of applications are more complex, they are often more manual, they are harder to standardize, and so they pose challenges in implementing those workflows.

These tests inherently deal with a large number of analytes or microorganisms that are being quantified and there have been a number of studies that are well-documented that are

multiple steps in the workflow can introduce bias, and so it is challenging to ensure reproducibility of test results, which, again makes standardization very important; the same is true for the data analysis steps. So results of research studies have shown that the data analysis tools and the databases that are being used by those tools can have substantial impact on the results. And so, it is very important that all steps of the workflow are quality controlled and are standardized as much as possible.

Another challenge is, in the end, what is the information that should be reported back if this was done in a diagnostic laboratory? How should the report look like? There hasn't really been strong consensus on how to communicate the complex and multi-dimensional data back for patient care purposes. So, in the example I gave earlier about the bacterial vaginosis test, although what is being quantified is the relative composition of the microbiome, in the end, that information is converted into score and so, there is a yes-no type of answer provided.

And so, the community has to solve this problem and I see a strong role here for diagnostic laboratories who are used to dealing with quality control, standardization, communication of test results to clinical colleagues, and this is where I see the next phase of the development in these application is going.

Bob Barrett: Are there lessons that can be learned from other next generation sequencing applications such as metagenomics testing?

Dr. Schlaberg: Yeah, I strongly believe so. So, a number of laboratories are offering metagenomics-based pathogen detection test. The number of labs that are doing that is growing. And with that, there has been lessons learned, the need to standardize is very similar here and many of the challenges are shared. So, I believe there are many lessons that can be applied to microbiome profiling applications as well. The clinical metagenomics tests are often not interpreted as quantitative results. There are some differences. But many of the challenges are shared and so, I think it's very useful for laboratories to consider those lessons. There have been a number of publications about validation studies of clinical metagenomics tests. All of that information can be useful in making the next step for microbiome profiling tests.

Bob Barrett: Well, finally, Doctor, how can quality and reproducibility of microbiome testing be ensured?

Dr. Schlaberg: I believe it's very important to introduce quality control steps that diagnostic laboratories are very familiarize with throughout the entire workflow. That includes the use of

positive and negative controls, spike in controls or internal standards, their calibrators that help calibrate and standardize the results that help identify failures along the complex workflows. These are some of the lessons that laboratories have learned in implementing clinical metagenomics tests.

I believe that protocols both for the sample processing in the laboratory as well as for the data analysis need to be standardized. It will be beneficial to also standardize the data analysis, databases used, the way results are interpreted and then communicated on reports. Those are critical steps. There are a number of studies emerging that have performed proficiency tests or reproducibility studies across laboratories. So, with that, we have learned which steps are most vulnerable or most prone t introduce bias. And so, those are the first ones to focus efforts on to standardize workflows.

There have been control materials that has become commercially available that can be used for quality control and proficiency testing for comparing results across laboratories and across runs. So, it is becoming easier to standardize and quality control these workflows. But there are definitely, there is more work to be done to really bring these tests, to make them easy to use and standardized and make it possible for laboratories to interpret results consistently and apply lessons and evidence generated as part of research studies in the diagnostic laboratory to benefit patients.

Bob Barrett: That was Dr. Robert Schlaberg from IDbyDNA in San Francisco and the University of Utah School of Medicine in Salt Lake City. He has been our guest in this podcast on Microbiome Diagnostics. His article on that topic is one that appears in the January 2020 issue of *Clinical Chemistry* focusing on molecular diagnostics. I'm Bob Barrett. Thanks for listening.