Proteomic Tests
David Bunk, Ph.D.

DR. TEUTSCH: Thank you. I hate to rush you through all of that, but I want to give everybody else a fair chance.

Let's move on to Dr. Bunk, who is going to talk to us about proteomic tests. Welcome.

DR. BUNK: Thank you very much. Thanks for the invitation to come speak to you this afternoon. Now for something slightly different, some protein work that we are doing at NIST. This is a new effort in terms of helping to standardize and improve the measurement quality of proteomic clinical research.

Proteomics has not yet moved its way into the clinical diagnostic lab. I'm sure it will be entering soon enough. Right now proteomics is mostly used for medical research and medical diagnostic research. But the important thing here is that the measurements still need to be standardized. There still need to be high-quality measurements in order to make sure that the medical research is moving forward in the right directions and not leading down the wrong paths.

Just a quick definition in case we are not familiar with what proteomics is. Proteomics is the identification and quantification of all proteins of whatever sample you are talking about, whether it is the human proteome or specific tissue proteomes.

The interesting thing about proteomics, where it differs from genomics or metabolomics, is that very little research in proteomics actually measures intact proteins. You can divide proteomics into two distinct approaches: the top-down proteomics, where intact proteins are measured, but the vast majority of proteomic research is done using an approach called bottom-up proteomics, in which proteins are degraded down into peptides and peptides are measured. Then we are relating that information back to try to figure out what is going on at the protein level.

That is important when we talk about how we standardize the measurement techniques because we need to know what is going on. If things are not being done at the protein level, then we don't necessarily need reference materials at the protein level. We can actually do a lot of work by having peptide-based reference materials.

Clinical proteomics is a subcommunity of all proteomics. Really, from my understanding, the goal of clinical proteomics is to discover new diagnostic biomarkers. It is both looking at the change in the structure of the concentration and interactions with different proteins in order to improve clinical diagnostics.

If we look at the clinical biomarker pipeline, the first phase of biomarker work is the discovery phase, where we identify candidate biomarkers. That moves into the verification of these candidate protein biomarkers and finally into clinical validation. Currently, proteomics is being used in the discovery phase and the verification phase. The clinical validation is large-scale, large cohort studies in which most of the work is done using traditional techniques like amino assays.

But there is some belief that proteomic measurement technology will be used in clinical validation in the near future, and some of these technologies are being developed in order to do that. But currently, proteomics is focused on the discovery phase and the candidate verification.

The distinction here is, in the discovery phase we are only talking about a small number of samples, maybe one healthy and one disease state samples. As we move into verification, we want to try to reduce the number of candidate biomarkers down to a manageable number, and so we use a larger amount of clinical samples. Of course, with clinical validation, we are talking about thousands of patients in order to make sure that we have a true biomarker that has either diagnostic or prognostic utility.

Proteomics is still in its infancy, to a certain degree. There are a lot of problems in proteomic measurements. That is one of the reasons why NIST is involved. We want to bring a higher level measurement quality to proteomics.

Basically, I think one of the fundamental problems in proteomics now is that there are no quality metrics. There are no performance criteria. At least, there have not been in the last few years. There have been a number of studies published. The Human Proteomics Organization has published a number of studies where they are looking at interlaboratory comparisons of proteomic investigations. Unfortunately, many of the results are not very positive. There has been very little comparability in proteomics investigation from laboratory to laboratory. Obviously, if you want to develop technologies for doing clinical diagnostics, the field of proteomics had to be improved in order to get more reliability and more comparability of the measurements.

The other issue is, it is very difficult to assess truth in proteomics. No one knows what the human proteome is. It is very difficult right now to assess agreements if you don't have standards. That is one of the reasons why we are here at NIST.

Unfortunately, all of this has led to the potential of diminishing opportunities for future research funding. On that note, a few years back we partnered with the National Cancer Institute on one of their initiatives and really discussed this.

One of the fundamental approaches we take in developing reference materials and reference measurement procedures for clinical diagnostics is partnering. We at NIST are not clinical chemists. I am not a clinical chemist. What we do know at NIST is the basic fundamentals of measurements.

So what we have to do is partner with professional organizations like the AACC, the IFCC, and the National Cancer Institute in this case, to bring their expertise into our efforts in standardization. We apply our measurement skills, our knowledge of the fundamentals of measurement, and we bring in their application knowledge to solve the problems that are relevant to them.

The National Cancer Institute, about three years ago, developed a program to assess proteomic technologies because, basically, their advisors were telling them that they are not going to be funding much future research for proteomics because there was no payoff. So NCI decided they needed to initiate a program to evaluate the technologies.

It is a very interesting program because it is not about biomarker discovery. It is about validating the technology used in clinical proteomics.

The role that NIST plays in this program is that we are advising them in some of their interlaboratory study designs and developing the materials that are being used in interlaboratory studies. We are working with them to really help assess the technology ourselves. In the

meantime, we are learning a lot about proteomics. So we are gaining the knowledge from the community by working with these partners, and that is an important aspect.

Through this initiative we are working on interlaboratory studies but we are also developing the information we need to develop our own reference material program to support proteomics.

Let me go back to the biomarker pipeline once again to draw some distinctions here. Biomarker discovery is mostly a qualitative or relative quantitative measurement. This work is mostly done these days in tissues, so we are looking at the sources of disease, like cancer would be in tumors.

The verification stage is doing more of an absolute quantification of signature peptides from whatever the candidate biomarkers are. That is being done in mostly plasma because this is leading toward a more diagnostic platform. The instruments being used are much more qualitative.

Realizing that proteomics is playing a role in both of these fields, discovery and verification, NIST is developing reference materials to support both efforts because if you are not supporting the entire pipeline you are still going to run into problems. We need to have reference materials and standard operating procedures and validation tools for the entire pipeline.

Let me just mention some terminology we use in terms of reference materials, which is horizontal versus vertical standards, or vertical reference materials.

When we are talking about a very complicated measurement technique or measurement pipeline like in proteomics, where there is sample collection, sample processing, instrumental analysis, and data analysis, there are a lot of places where problems can come in. We approach that we take at NIST is to develop horizontal standards, which are standards which support measurement quality in individual steps along the way.

The other thing we also develop is vertical standards, which are very much application-specific standards.

A horizontal standard might be a standard that can be used to validate your data analysis, whereas a vertical standard would be a more complex, application-specific standard like cholesterol in serum, where it is geared towards a much more specific measurement problem. The standard is carried through the entire measurement process.

In proteomics, that is the approach we are taking. We are developing horizontal standards and vertical standards in order to support the measurements.

In most cases, for a new measurement area it would be impossible to develop just vertical standards. The applications where proteomics is being used are very significant, so we would have to develop vertical standards for every specific application.

In clinical diagnostics, we have reference materials for cholesterol measurements, glucose measurements, creatinine measurements, and so on and so forth. That approach for proteomics just wouldn't work because there are too many areas in which it is used. So a horizontal standard is a way that we apply our resources to improve the measurement as best we can.

Currently, we have two reference materials in production. The horizontal standard is a mixture of synthetic peptides, so it is not application-specific. It is designed to improve quality in mass

spectrometry instrumentation. So all fields of proteomics that involve mass spectrometry could benefit from this reference material since this is a common point in their pipeline, making that a horizontal standard.

The other reference material we are currently developing is a yeast proteome reference material. This is a vertical standard, so this is designed for proteomic investigators to take a complex protein mixture through their entire proteomic pipeline and validate the procedures that are being used here.

We also have plans to develop more complex proteomics reference materials that are plasma-based for quantitative measurements.

In addition to those two new reference materials and the additional one that I mentioned of complex-matrix horizontal standards and vertical standards, we are also looking at developing higher-order measurement tools for assessing performance of affinity reagents in proteomic arrays, multiplex arrays, as well as developing and validating novel affinity capture reagents. So we are looking at both improving technologies, developing standard operating procedures for people doing proteomics, as well as delivering services through reference materials, which people can use to validate their technologies and their techniques in proteomics.

We hope that by having all these different areas we can support the measurements that are going on in the clinical community and improve the outcome of clinical proteomic research.

Thank you.

DR. TEUTSCH: Thank you, Dr. Bunk.