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As If Biomarker Discovery Isn't Hard Enough: The Consequences of Poorly Characterized Reagents

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Guest:
Dr. Karin Rodland is Chief Scientist for Biomedical Research at Pacific Northwest National Laboratory in Ridgeland, Washington.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I'm Bob Barrett. The discovery phase of proteomics is essential for the identification of suitable markers for exploration and validation of promising new clinical tests. But can researchers be certain if what they believe they are measuring is in fact what they are actually measuring? A report in the February 2014 issue of *Clinical Chemistry* demonstrates that this might not be the case. And we've heard from a group of Toronto researchers that brought this to light in a previous podcast.

That research article had an accompanying editorial written by Dr. Karin Rodland, Chief Scientist for Biomedical Research at Pacific Northwest National Laboratory in Ridgeland, Washington. She's our guest in today's podcast.

Dr. Rodland, what motivated you to write that editorial on, "As if Biomarker Discovery Isn't Hard Enough: The Consequences of Poorly Characterized Reagents?

Dr. Karin Rodland: Well, there's been a lot of discussion in the proteomics field about the issue of ELISAs that did not recognize what they were supposed to recognize. I was asked by *Clinical Chemistry* to review the article that was later published, "False Biomarker Discovery due to Reactivity of a Commercial ELISA for CUZD1 with Cancer Antigen CA125." And I thought the authors of that article did a tremendous job of documenting very carefully and precisely exactly what was wrong with the ELISA they had purchased, and demonstrating very conclusively that it was raised not against the supposed antigens CUZD1 but against CA125.

There was a reference in that manuscript to a similar occurrence in Gutierrez et al. in the *American Journal of Nephrology* indicating that it's not just a one-time fluke. And this topic had been discussed several times at proteomics meetings that I've attended. So I felt it time to emphasize to the community the degree of caution that's really necessary these days.

Bob Barrett: So doctor, what is the take home message of that publication, “False Biomarker Discovery due to Reactivity of a Commercial ELISA for CUZD1 with Cancer Antigen CA125?”

Dr. Karin Rodland: Well, the simple message is “buyer beware.” The more complicated message is that simply because a product is a commercial kit, you cannot assume that it was produced with the same degree of care that you would have produced in your own research laboratory. And quite simply, you need to do the same controls on a commercial ELISA kit that you would have done on a home brew ELISA or a home brew antibody.

I think there is another very large message to this, and that is the cost, both in career, people time, and dollars, of not doing due diligence early in your discovery process.

Bob Barrett: How do you think researchers could avoid encountering similar problems with their work?

Dr. Karin Rodland: Well, you have to have skepticism about your experiments. And that’s always been one of the first principles of biological research. I think you really have to ask yourself the question, “What could have gone wrong with this experiment?” And then do the control experiments to make sure that that has not gone wrong.

And one of the foundations of this approach is orthogonal measurements. If you make an observation with one technique, like in ELISA, you need to verify it with a second technique that is using a different set of reagents or a completely different biochemical approach. You could use a Western qualified antibody and verify that you saw the protein of the appropriate molecular weight in a gel without a loss of spurious bands. Or my favorite, you could use mass spectrometry to identify that you're really looking at the protein you think you're looking at, both because of its solution by liquid chromatography and you have verification at the amino acid sequence level.

Bob Barrett: You mentioned new targeted mass spectrometry methods as a possible alternative to antibody-based methods for protein quantification. Do you really think that's practical?

Dr. Karin Rodland: Yes I do. The new targeted methods, whether you call it selected reaction monitoring, multiple reaction monitoring, or parallel reaction monitoring, have got the same degree of specificity as the antibody methods. And new approaches, either immuno, MRN or the PRISM-SRM method developed at PNNL, have got sensitivity down to the same level as one can get with commercial ELISAs. We have actually done a

head to head comparison with PRISM-SRM against ELISAs to CA125, and get exactly the same lower limit of detection and lower limit of quantification with our more targeted SRM assays. So this demonstrates that you can actually achieve the sensitivity with mass spec-based methods.

At present, it's an art. It requires an experienced practitioner. But as the field evolves and gets more mature, it will become more accessible to the rest of the research community.

Bob Barrett: Well, finally, how would you compare mass spectrometry-based methods with traditional ELISAs?

Dr. Karin Rodland: Well if you have an ELISA that is well-qualified and well-characterized and it measures what it intends to measure as in an CLIA-approved ELISA, then an ELISA is less expensive, less cumbersome, takes less time and effort, it's more convenient. But the effort of getting to that stage in an ELISA experiment is intense. You have to generate an ELISA-capable pair of antibodies and verify their specificity and verify that they don't interfere with each other. And there's no guarantee that you will be able to come up with a pair of ELISA-qualified antibodies. So there's a huge upfront investment in time and effort and expense with no guarantee of a positive outcome.

These days, with the new targeted mass spec measurements, you can be sure of getting a measurement, initially. It will be more expensive, the machine is more expensive, it requires practitioners of the art; but as the field matures, it's going to become more accessible to more and more practitioners. And as the instrumentation available improves, the specificity has already gotten superior to the specificity of ELISA. And the sensitivity has gotten down into the same categories as an ELISA.

So what I suggest is a strategy where you can use mass spectrometry initially with a candidate protein that you're interested in, either a candidate biomarker or a candidate drug target or a candidate member of a pathway. You use targeted MS methods to verify your hypothesis. And once you have verified your hypothesis, then you invest the effort in developing an ELISA.

Bob Barrett: Dr. Karin Rodland is Chief Scientist for Biomedical Research at Pacific Northwest National Laboratory in Ridgeland, Washington. She has been our guest in today's podcast from *Clinical Chemistry*. Her editorial, "As if Biomarker Discovery wasn't Hard Enough," appears in the February 2014 issue of *Clinical Chemistry*. I'm Bob Barrett, thanks for listening!