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Mass Spectrometry Selectivity, Specifically

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Guest:
Brian Rapplod is the Scientific Director for Essential Testing in Collinsville, Illinois.

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.

The January 2016 issue of *Clinical Chemistry* is a special issue devoted to applications of mass spectrometry to medical laboratory applications. In an opinion piece in that issue, Brian Rappold gave his views on the benefits of the technology, specifically on its selectivity.

He is the Scientific Director for Essential Testing in Collinsville, Illinois, and his work is focused on mass spec assay development and implementation for evaluations of inborn errors of metabolism, therapeutic drug monitoring, and endocrine dysfunction.

First of all Brian, what is a mass spectrometer and what does it do?

Brian Rappold: So a mass spectrometer measures compounds, and the way it does, is that every compound has a molecular weight which is called its mass, and a mass spectrometer can separate individual compounds based on their mass, and we use it to identify compounds, and in some more advanced techniques we can actually measure the amount of a particular compound in a sample.

Bob Barrett: Has anyone been credited for inventing the mass spectrometer, and when they did they first enter analytical clinical laboratories?

Brian Rappold: J. J. Thomson and Francis Aston are generally credited with inventing the mass spectrometer back in the beginning of the 21st century, and it won them both Nobel Prizes.

Up until a few decades ago, mass specs were largely used only by physicists and fundamental chemists. The first analytical laboratories were the petroleum industry, but it took more than half a century after the invention of the mass spec to be utilized in labs and looking for things associated with human biology.

Bob Barrett: So it seems mass spectrometry has been around for quite a while. Why only recently has it seemingly had such an impact on medical laboratories?

Brian Rappold: Mass spectrometry was historically a very complex device and it required a team of physics PhDs to really operate. Advances in computers and hardware have really streamlined a lot of the underlying science, so it's virtually accessible to everyone.

And about a generation ago mass specs were relatively unreliable and it could take a lot of work to process just a single sample, but now reliability and efficiency are a necessity when we are talking about the medical field, so we have finally been able to develop the appropriate technologies to bring it into the medical laboratory.

Bob Barrett: If you can, talk about a few examples of how mass spectrometry has impacted society and particularly healthcare?

Brian Rappold: So the technology is a lot more prevalent than many people realize. Mass specs are in airport security screening lines, where you get a bag swabbed and they get into a thing called the ion sense. They put mass spectrometers on the bottom of oceans; they have mass spectrometers on Mars.

In medicine though, probably the most impactful contribution to medicine and healthcare for mass spectrometry has been newborn screening, where children have drops of blood from their heel pricked, put on to a little dried blood spot card, which is then tested by mass spectrometry to look for congenital disorders. And it's one of the very first tests that a human ever goes through after they are born.

Bob Barrett: So what information do mass spectrometers provide for laboratorians and patients that they couldn't get before mass spectrometry became more established?

Brian Rappold: Well, and that's really a two-part answer, and the first part is, we now have better access to new biomarkers. So to use the previous example of newborn screening, newborn screening was invented as a functional test for fetal phenylketonuria, which is known as PKU, and it's an overproduction of phenylalanine, and this kind of functional test will try to grow bacteria in a petri dish in the presence of a newborn's blood. So if the bacteria grew, then the infant would have elevated phenylalanine and a high probability of having PKU. So it's kind of an indirect analysis.

Mass spectrometry however directly measures the levels of phenylalanine as well as a host of other compounds

associated with different disorders, many of which would be very difficult to diagnose with any other technology.

And then the second part, is that we now have better information. So mass spec has the ability to deliver highly accurate results, sort of unquestionable results, and having accurate results reduces the numbers of false positives and false negatives reported back to physicians.

Bob Barrett: Many tests performed in clinical laboratories of course are performed by immunoassay. Do those tests also suffer from selectivity issues similar to mass spectrometry, and what's the difference between immunoassay and mass spectrometry in regards to selectivity?

Brian Rappold: So, broadly, there is no perfect laboratory test from any particular technology and the underlying problem with that is really biology itself.

Biology can be really tricky, and the way in which immunoassays and mass spectrometry both are tricked is very similar. If a compound looks like the thing you are trying to measure, that compound could possibly interfere with the measurement, which most often causes a false positive, but the big difference between immunoassays and mass spectrometry is the ability to react to the error.

So with immunoassay interferences you don't know that the assay reported the wrong result until you hear back from the doctor saying that the test result did not match the clinical presentation.

With a mass spectrometer however, you can actually dive a lot deeper into the data to be able to proactively react to a possible interference or selectivity issue. So amino acids are reactive while mass spectrometry is largely proactive.

Bob Barrett: In your article in *Clinical Chemistry,* you highlight the challenges that scientists face when using mass spectrometry. For example, you talk about something you refer to as "the biological matrix" and you explain how plasticity and promiscuity can present challenges when designing new tests in a mass spectrometer.

So briefly talk us through those three terms: biological matrix, plasticity, and promiscuity, and how they relate to testing by mass spectrometry?

Brian Rappold: A biological matrix is nothing more than a sample from the patient. It could be tissue or blood or urine, whatever.

Plasticity and promiscuity are two biological concepts that make the biological matrix so tough to measure from.

So plasticity first is the change in organism as a response to a stimulus. So for example, if a person gets a cold, the cold virus stimulates an immune response which causes sneezes and runny noses and coughing and fever, and those sorts of responses can change the make up of the biological matrix, and that's where we start to have possible interference generation.

The second part is promiscuity, and that's related entirely to enzymes. And enzymes are briefly, just responsible for turning compounds in our body into more useful things. But we generally think of enzymes as having only one task, like converting one steroid to another, and that's all that enzymes will do, but that's an overly simplified model.

Enzymatic promiscuity results in one enzyme being able to interact with all sorts of initial compounds and turn them into something else entirely different. And generally, it's that something else that we are really worried about when it comes to medical diagnostic testing and selectivity, especially if whatever we are looking for has the exact same mass as whatever has been generated by the enzymatic promiscuity. That's where we have a false positive developed from.

So when we are developing a test on a mass spec, we have to be aware that those two principles of biology are really fighting us the entire time. Plasticity and promiscuity are making our lives more difficult.

And we also have to recognize that in trying to fight biological plasticity and promiscuity, that biology itself has about 4 billion year head start, so we are still trying to develop tools to get around some of these challenges.

Bob Barrett: Many specialists in mass spectrometry say they admire the selectivity of the technology, often considering it to be a "truth machine." What's your take on that belief?

Brian Rappold: Well, a brief response to that is it has the capability of being highly selective and it has the capability of providing the truth, but certainly not on its own.

The complexity of human samples and some of the underlying chemistry involved in mass spec analysis can make a liar out of your most favorite mass spectrometer, and that's not really a new problem.

We have known about this for more than half a century and scientists have developed a lot of adjunct tools to help mass spec do a better job of telling us the truth.

Bob Barrett: Could you talk about some examples where the benefit of better selectivity has been demonstrated in clinical testing?

Brian Rappold: So probably the most impactful example is in the analysis of testosterone in women and children. In 2002, two scientists out in California, Dave Herold and Rob Fitzgerald, wrote a paper for *Clinical Chemistry* that discussed how a doctor would be better off guessing a female patient's testosterone value rather than getting the test performed by an immunoassay.

And that's because the immunoassays at the time had incredibly poor selectivity, and this has enormous implications when you are looking at the diagnosis of diseases like polycystic ovarian syndrome or other sex steroid dysregulation.

And that commentary was part of an enormous effort to bring mass spec into the field of endocrinology to help provide more accurate test results back to our physicians.

So the concept of more accurate tests lies within the recent buzzwords of things like personalized medicine. If we can get to better selective and more accurate tests over time, we can really start to influence the pathways by which we treat and prognose disease.

Bob Barrett: Well finally, let's look ahead, do you ever see the day when the most commonly performed clinical chemistry tests like cholesterol and glucose will be routinely assayed by mass spec?

Brian Rappold: I don't think that we are ever going to see something like a tricorder mass spec. It's far too esoteric a technology to apply to molecules like cholesterol and glucose. The technology is great for some things, but it's not really a hammer in search of more nails, especially in routine testing.

So for cholesterol and glucose, the established mechanisms give us a very accurate and reliable result over time, but mass spec will definitely play a role in getting better diagnostic tests and getting them all harmonized between labs by being the international reference method for a lot of biomarkers.

So while mass spec might not be utilized for a glucose reading at a hospital, it might be responsible for making sure that you get the same glucose results if you are in any hospital anywhere in the world.

Bob Barrett: Brian Rapplod is the Scientific Director for Essential Testing in Collinsville, Illinois, and his opinion piece on the

selectivity of mass spectrometry appears in the January 2016 issue of *Clinical Chemistry* devoted to mass spectrometry.

I am Bob Barrett. Thanks for listening!