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Preanalytical Processing and Biobanking Procedures of Biological Samples for Metabolomics Research: A White Paper, Community Perspective

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Guest: Dr. Jennifer Kirwan works at the Max Delbrück Center for Molecular Medicine as part of the Berlin Institute of Health initiative to improve translational research.

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.

Garbage in equals garbage out. That's a commonly used phrase in laboratory medicine to describe the effects of specimen quality on analytical results. This refers to the fact that proper handling during the collection and processing of specimens is crucial for producing reliable results. These pre-analytical steps are highly vulnerable to the introduction of experimental bias and variance, which may drastically degrade specimen quality. Analytes are affected differently by uncontrolled variations in specimen collection, processing, and storage protocols.

The low molecular weight molecules of a given biological system's metabolome are among those that are most sensitive to these conditions. Thus, it is necessary to consider the particular requirements for pre-analytical processes and biobanking in metabolomics research. Poor practice can create bias and have deleterious effects on the robustness and reproducibility of acquired data.

The August 2018 issue of *Clinical Chemistry* includes a Review article from the Precision Medicine and Pharmacometabolomics Task Group of the Metabolomics Society. The authors summarize the current variability among pre-analytical processing and biobanking procedures for metabolomics research and provide recommendations for best practices.

For this podcast, we are joined by the Review's lead author, Dr. Jennifer Kirwan. Dr. Kirwan works at the Max Delbrück Center for Molecular Medicine as part of the Berlin Institute of Health initiative to improve translational research. She heads the BIH Metabolomics Platform in Berlin and is also an honorary assistant professor in veterinary metabolomics at the University of Nottingham in the U.K.

So, Dr. Kirwan, first, why did you take on this Review?

Jennifer Kirwan: Well, this paper was written by a subgroup of the precision medicine and pharmametabolomics task group at the Metabolomics Society. And I should acknowledge that all the members of the task group have made some contribution to the paper, which I would like to thank them very much for their expertise.

So, this working group has been running for about two years now and under the leadership of Rima Kaddurah-Daouk at Duke in the USA. And it focuses on how metabolomics can contribute to furthering precision medicine.

So precision medicine, it's a bit of a buzzword at the moment and it's the idea that we can best customize healthcare to the individual needs of the individual patient. What that means in practice is for instance, can we subtype a patient's exact disease type and use better treatments based on what we know about that? Or, can we use knowledge of a patient's unique biochemistry to allow us to select the medicine that we know the patient is unlikely or less likely to have side effects to?

So, this is the idea behind precision medicine and metabolomics, we as a community think metabolomics has a lot to offer in advancing precision medicine. So, this task group was set up and we got together to discuss basically, how we could go about furthering the involvement of metabolomics in precision medicine. And, the discussion turned to biobanks which share these repositories of base human specimens or normally human--sometimes animal--specimens, and the data associated with them, and they use an evidence-based precision medicine. And there's been a massive rise in the number of biobanks.

But, as we were discussing this, we realized that there were a wide variety of collection protocols in use for collecting samples for biobanks, and many of them were not designed specifically for metabolomics. And, metabolomics is an extremely powerful science in determining a phenotype of an individual. But, because it's such a powerful tool, it requires reliable, repeatable, comparable, experimentation methods in order that we know that we are measuring the biological difference when we do metabolomics studies and not just technical differences in experimentation methods. And, this has to start with sample collection.

So, from this came the idea that we should review the literature and find out what protocols are being currently used, which are best validated, and where we as a community need to concentrate on to further validate and

confirm which protocols are best for use for future biobanking practices.

Bob Barrett: Well, let's get to it. What were the most important conclusions of the Review?

Jennifer Kirwan: I'd say that, the most important general conclusions, sample collection methods need to be considered carefully, very early on. I'd say, at the design stage of a biobanking initiative.

And particularly for metabolomics, I would argue that where it's a general biobank and it's collecting the other purposes, it's helpful to have a metabolomics expert giving the metabolomics perspective when deciding which samples to collect and how. We found that there's a huge variety of protocols in use even when it's for the same sample type, but sometimes, the knowledge of how well validated these protocols are, it's not always available to the public to evaluate.

And so, one of our conclusions was that spending some time on validating collection protocols is expensive, but it's important and it's worth spending the effort on. As much as anything, because once you've got that validation, you can use that protocol for future studies for the same tissue or biofluid. And, it allows you to know and understand the strengths and the weaknesses of that protocol for individual metabolytes. And, that allows you to better influence experimental decision-making in the future.

We've also found that there's a balance between the best scientific practice and the costs of collection and the impact on the collection. The people doing the collection are often medical staff and they often have time limitations, which can mean that very complicated protocols are difficult for them to carry out. And, this highlights the needs for good training. If every member of the collection team is well trained, and understands why certain aspects of the protocol are there, you have a much higher probability that that protocol is well carried out by everybody involved.

So, the more specific conclusions that we have, blood products are probably the best characterized and validated of all the biofluids and tissues that we've looked at. But, even with these, there is still some debate within the community about collection procedures.

And for some sample types such as, for example saliva, there appears to be very little published validation of the collection method, making them very difficult to compare. And most surprisingly, some papers are not reporting their collection methods in full and this makes it very difficult to

directly compare results and repeat the experiments. And sometimes, it's not clear when people are just not publishing the protocol or whether certain things were not included in the protocol. So to use saliva as an example again, some protocols have the people rinse their mouth out before providing the specimens and this rinses food from the mouth. And sometimes, it just wasn't mentioned. So, we don't know whether this happened or didn't in some protocols.

Bob Barrett: So, you found a huge variety of protocols in use. Were they all equally valid and are they comparable?

Jennifer Kirwan: Comparable, I would say that every protocol that is being well validated has its strengths and weaknesses, and it comes down to a good validation shows up the strengths and weaknesses of the protocol that you're using. It's a compromise between going for the very, very best scientific results and something that is usable in practice.

Bob Barrett: Do you support standardizing protocols for collection of metabolomic samples for biobanks?

Jennifer Kirwan: That's a difficult question. I would say that I support more standardization and certainly, if you know in advance that you want to be able to directly compare results such as, multicentric studies, standardization is vital. And, more standardization across the global community of biobanking would certainly help for future comparisons. However, to insist on complete standardization with all collection protocols may lead to some unintended consequences. For instance, if you want to carry out a study involving people who live in a very remote region, then access the thing such as liquid nitrogen, or minus 80 freezers, may not be very easy. And so there may need to be some compromises made depending on the particular needs of your study.

So overall, I think, what's come out of this for me is the protocol is validated and you understand the strengths and weaknesses of it. And, importantly, if you publish the full sample collection protocol and the validation work, then it allows both you and the community to understand the technical issues that may arise from using that protocol, but also the strengths of it. Things such as publishing, validation of protocols is not done as often as you may anticipate even by some biobanks.

Bob Barrett: Doctor, do you think a paper like this can change the field of metabolomics and/or biobanking?

Jennifer Kirwan: I would be honest of the limits of this Review. So, no, I don't necessarily think it, alone, will change the metabolomics and biobanking field. What I would hope for

is that it may contribute to the ongoing discussion on good protocol, design, and validation, and the importance of it in any good metabolomic setup. Where I hope we may have made life a little easier for people, and probably the main reason I hope people may read the Review is that we spent quite a long time collecting and referencing several existing protocols which form three tables in the centerpiece of the Review and I hope this may assist people who are trying to design their own collection protocols or reutilize somebody else's.

Bob Barrett: Well, finally, Dr. Kirwan, we started off by talking about the working group, who are all involved in writing this paper, did everyone in this group always agree on the best protocol to use?

Jennifer Kirwan: Well, it was a collaborative piece of work and like any collaboration, there was plenty of opportunity for friendly discussion. For example, one of the discussions centered on whether heparin or EDTA was the better anticoagulant choice for collecting and storing plasma. And, there are pros and cons for both. So, for example, EDTA has a low molecular weight and that can interfere in the detection of some metabolites of interest; while heparin isn't detected in a standard mass spectrometry analysis which is an advantage. However, heparin can cause lithium atoms to form in a mass spectrometric analysis and that adds an extra challenge to the interpretation of the spectra.

EDTA doesn't have that and in addition, it has the advantage that it's very compatible if you want to do proteomics from the same sample. So, we have two anticoagulants, they both have pros, they both have cons, and in the end, there's not necessarily a best option, but it does help to understand the limits of whichever protocol you're using. And it's obviously always important to be consistent in whichever anticoagulant and indeed, in whichever protocol you choose when you're collecting samples for use in one study.

Bob Barrett: That's Dr. Jennifer Kirwan of the Max Delbrück Center for Molecular Medicine, part of the Berlin Institute of Health initiative to improve translational research. She heads the BIH metabolomics platform in Berlin and is also an honorary assistant professor in veterinary metabolomics at the University of Nottingham in the U.K. She's been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening!