A Center of Excellence for Precision Medicine

Waters Xevo TQ-XS provides the ultimate analytical sensitivity for biomarker development

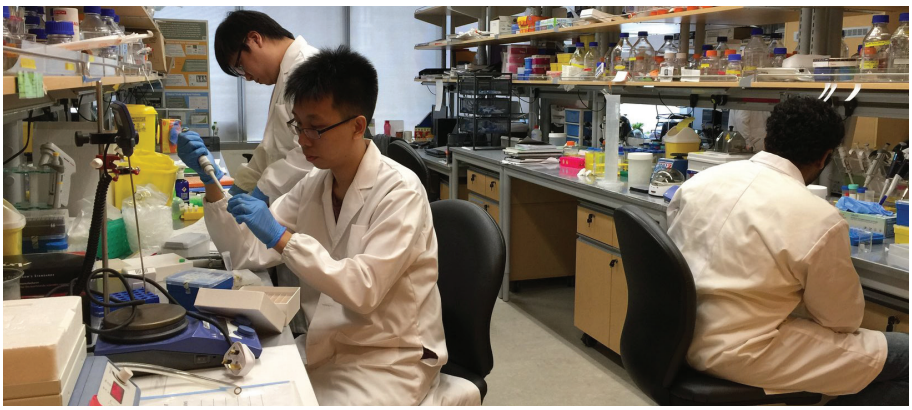
TECHNOLOGY: LC-MS/MS including **WATERS XEVO TQ-XS MASS SPECTROMETER** WITH THE **ACQUITY UPLC I-CLASS SYSTEM,** utilizing **Waters Oasis MAX Solid Phase Extraction (SPE) micro-elution** sorbent sample preparation method

AN INTEGRATED HEALTHCARE AND CLINICAL RESEARCH SYSTEM

The National University Hospital (NUH) in Singapore is a JCI accredited, tertiary hospital and major referral center for a comprehensive range of medical, surgical, and dental specialties. The hospital also provides organ transplant programs for adults (in kidney, liver, and pancreas) and is the only public hospital in Singapore to offer a pediatric kidney and liver transplant program. With 1280 beds and nearly 8000 staff, NUH provides a focus on clinical outcomes. It is the first and only hospital in Singapore to achieve triple ISO certification simultaneously – ISO 9001:2000 (Quality Management System); ISO 14001:1996 (Environmental Management System); and OHSAS 18001:1999 (Occupational Health Safety Management System) – in December 2002.

As part of the National University Health System (NUHS), the NUH participates in national research as part of an interdisciplinary network of clinical, basic science, and public health programs. The overriding goal is to ensure efficient translation of new therapies and new technologies from bench to bedside.

Dr. Chester Drum is an assistant professor at the National University of Singapore (NUS), a consultant cardiologist at the National University Hospital and director of the clinical trial innovation laboratory at the Translational Laboratory in Genetic Medicine (TLGM), a joint initiative with A*STAR (Agency for Science, Technology and Research) and the NUS, that is housed at Biopolis, Asia's biomedical sciences R&D hub.



*The clinical trial innovation laboratory at TLGM, A*STAR.*

WORKING WITH WATERS

Dr. Drum's laboratory supports its work on biomarker method development through grant funding. They realized that for the specific work they wanted to do on oxidative stress, they were at the edge of their capabilities which was hindering progress with the project. Personal relationships are often key to successful partnerships. In this case, a local technical sales representative established a great connection to the work of the laboratory. With this comes a level of trust and an openness that can result in the kind of groundbreaking work being done in Singapore with the Waters systems.

Dr. Drum comments: "*The local Waters team was key, and now, having had several conversations with senior managers at Waters, both in the region and at global HQ, I feel we are on the same wavelength. Waters understands the significance of the test and the challenges from a biological and an analytical standpoint. This collaboration has enabled us to achieve our goals. I'm looking forward to a very productive relationship in the coming years.*"

He graduated from the University of Chicago with a degree in philosophy before completing a Ph.D. in protein crystallography. Medical study followed, and Dr. Drum completed his specialist training in cardiology at Brigham and Women's Hospital (Harvard Medical School) in Boston. He moved to Massachusetts Institute of Technology (MIT) where he was director of translational medicine for the regulatory innovation group, MIT-NEWDIGS. While at MIT, he received the Burroughs Wellcome Career Award for Medical Scientists.

"*Having completed my training and made the move to Singapore, the question for me was how to combine my biochemistry background with my clinical goals?*" outlines consultant cardiologist Chester Drum, MD, Ph.D.

Dr. Chester Drum, MD, Ph.D.

Drum explains: "*With access to an outstanding research community and a well-characterized patient population, the answer was mass spectrometry (MS). Using clinical samples, informatics, and the latest MS technology, my team can approach two distinct objectives – first, developing methods for improved prediction of disease progression and therapeutic efficacy means we can better match drugs to individual patient phenotypes; secondly, I can explore large-scale informatic approaches that allow us to say something new about the underlying biology of a disease. It's a very satisfying environment for my work.*"

Dr. Drum established the clinical trial innovation laboratory at TLGM to support these objectives. The laboratory is affiliated with NUH and works on discovering new materials for advanced clinical trials, new techniques for predicting adverse drug reactions, engineering protein substrates to perform unique biological tasks and providing the integrative culture to make something greater than its parts.

Dr. Drum elaborates: "*We run a translational lab in the classical sense of the word, working on around 10 clinical protocols.*"

> "The team consists of myself as a licensed physician, plus an expert with targeted assay development and validation experience, six nurses collecting samples, and 15 people in the laboratory working on informatics and mass spectrometry data interpretation."
>
> DR. CHESTER DRUM, MD, PH.D.
> *Consultant Cardiologist*

WORKING FOR A DEEPER UNDERSTANDING – AND IMPROVED CLINICAL OUTCOMES

Clinical scientists around the world have long been calling for more sensitive assays for the markers of important diseases in order to guide pharmaceutical development. One example is Troponin, a marker that helps with the diagnosis of myocardial infarction (MI). New high-sensitivity Troponin assays have become available, bringing the limit of quantification down from 100 pg/mL to near 1 pg/mL. The new assays are now in use in most regions of the world (US FDA being one of the last to approve its use – in January 2017). The benefits of the additional sensitivity are clear - in the setting of acute myocardial infarction 'conventional' assays are frequently negative at first draw and require hours before they become abnormal. High-sensitivity Troponin on the other hand allows rule-out of acute MI at the first blood draw if the values are very low and are accompanied by other reassuring clinical characteristics. For many clinicians, the new assays have totally changed how they evaluate patients for MI.

Now, Dr. Drum and his 15-strong team are adopting similar methodology to develop biomarkers of oxidative stress.

Importantly, Dr. Drum's approach is always to look for a hard clinical endpoint rather than simply a biomarker correlation, hypothesizing that: "*If I treat a patient to the defined endpoint, I want to see a successful clinical outcome for that patient – for example, I know that if I lower cholesterol to a specific point I can reliably reduce the incidence of cardiac complications.*"

He continues: "*If I ask myself 'What's the big biomarker that I would love to order on my patients but that is not available right now?' I'd say it would be a validated marker of oxidative stress and damage. I see this as the 'holy grail' for biomarkers, and it is where I see MS as being ideal to help us develop the most relevant tests.*"

A FOCUS ON OXIDATIVE STRESS

Oxidative stress is related to biological outcomes comprising tissue damage and diseases in humans such as hypertension, stroke, diabetes, cardiovascular disease and ischemia, for example. Dr. Drum comments: "*There are currently a lot of claims about antioxidants: acai berries, vitamin E, vitamin C, for example. Some may work, they may be important, BUT they may be hogwash! We need clinical trials to show if and how oxidative stress is being reduced. These have not been done and, as I mentioned before, the ideal test doesn't exist yet.*"

Isoprostanes are a series of prostaglandin-like compounds that are a marker of systemic oxidative stress over time. One group – F2-isoprostanes – is considered the 'gold standard' test for quantifying lipid peroxidation/oxidative stress.

Dr. Drum is now turning the spotlight onto F2-isoprostane with his work as part of NUHS. Quantitation of F2-isoprostanes in a random urine specimen is the most accurate and robust measurement of circulating isoprostanes. GC-MS/MS, LC-MS/MS and ELISA methodologies can all be used to measure F2-isoprostane. Dr. Drum explains the problem they faced: "*Current GC-MS/MS protocols are relatively sensitive (limit of quantitation around 200 pg/ml), however they are time consuming as a derivatization step is required before the separation. In contrast, ELISA assays have operational advantages, but the limit of quantitation is significantly higher – at between 1 and 2 ng/mL.*"

PUSHING THE BOUNDARIES WITH LC-MS/MS

Against this background, Dr. Drum initiated a project to develop and validate a new sample preparation method with high recovery, ease of use and avoiding evaporation and reconstitution steps. Using this as part of a new LC-MS/MS protocol would provide a simple, ultra-sensitive and high-throughput analysis for 8 Isoprostane F2α in human plasma. An early realization was that the incumbent mass spectrometer in the laboratory could not achieve the level of sensitivity required, even when using 500 µL of plasma sample. This challenge proved a bottleneck to the project which was only overcome when local technical sales contacts at Waters in Singapore suggested an approach and instrumentation that could help achieve Dr. Drum's aims.

These conversations led to the launch of a development partnership between NUHS and Waters, with the objective to develop a clinically relevant marker of oxidative stress and a streamlined assay that is suitable both for research on samples from biobanks and potentially routine clinical use. The key principals of the partnership are Dr. Drum, Barry Halliwell, and Mark Richards.

Dr. Drum elaborates: "*Professor Barry Halliwell is one of the most cited authors in the world, he has made an outstanding contribution to our understanding of the biology of oxidative stress. I am privileged to have him as a collaborator in this work.*"

Subsequently, a Xevo® TQ-XS equipped with the ACQUITY® UPLC® I-Class System was installed in the laboratory in the first quarter of 2017. Oasis® MAX chemistry was selected for the sample preparation step.

A series of experiments explored the conditions for sample extraction and optimized the method. Figure 1 shows the final protocol for standardized sample preparation. Note that the final elute can be analyzed directly by LC-MS/MS.

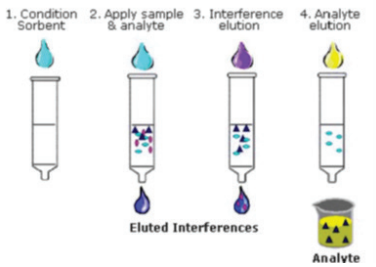


Figure 1: SPE method using Waters Oasis MAX sample preparation system.

With this in place, the first set of samples could be analyzed and a comparison made between the old and new mass spectrometer instruments. Figure 2 shows the significantly improved resolution achievable with the Xevo TQ-XS on an analysis of a 50 pg/mL sample. Dr. Drum commented: "*The most recent data from the Waters system clearly gives us a new limit of quantitation for Isoprostane of 50 pg/mL. In our experience, only the Xevo TQ-XS can reach this level of performance. The sensitivity is an absolute necessity. This sets a new benchmark.*"

Importantly for the next larger-scale stages of the project, it was also shown that a sample of just 20 µL was required for each measurement run, compared with at least 100 µL for the comparison instrument.

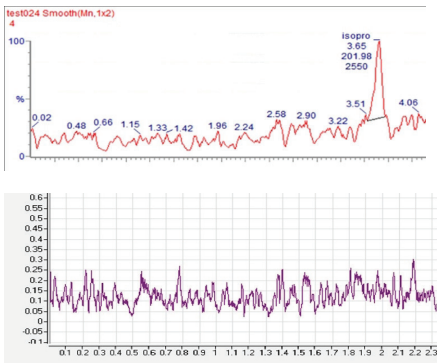


Figure 2: Comparison of the Waters Xevo TQ-XS (top) vs. an existing equivalent high sensitivity tandem quadrupole instrument from different manufacturer (bottom) measuring a sample with 50 pg/mL 8 Isoprostane F2α.

> "The TQ-XS is a great machine. What is its unique quality? That it could solve my specific problem where other targeted instruments couldn't."
>
> DR. CHESTER DRUM, MD, PH.D.
> *Consultant Cardiologist*

Dr. Drum continues: "*We tried alternative instruments and, even with a 500 µL sample, had difficulty achieving an acceptable LLOQ. With the Waters instrument, we can measure 50 µL and get a wonderful signal, we may even be able to go down to 20 µL...*"

LOOKING AHEAD

With robust methodology in place, work is now underway to validate the approach on a large number of patient samples. Dr. Drum is impatient to complete this next phase, explaining: "*What you need when validating a complex biomarker is significant statistical power in the trial, this means a lot of patients. If we were to approach this by recruiting new subjects and starting at time zero, then it would take 5–7 years for us to be able to say something, simply because it takes that long to reach the outcomes you are looking for...*"

He continues: "*...so the alternative is to go to a biobank, and in Singapore we have a wide range of high-quality frozen patient samples (each of ~500 µL) together with history and outcomes data on each, to use as our study material.*"

Dr. Drum explains that the limitations of time and sample size are a major pressure: "*We can do LC-MS/MS analysis on small volumes, so we can interrogate this pre-established outcomes-based database more quickly. Using the biobank, we can add not just a discovery data set, but a validation data set, and go really fast in terms of more complex characterization of patient phenotypes and their fuller validation.*"

By taking this approach, Dr. Drum can accelerate the development and validation of a biomarker, he comments: "*This combination of circumstances is why high sensitivity LC-MS/MS is so essential – alternative assay approaches, such as ELISA, for example, would take much, much longer. Just developing the antibody can take years. With mass spec we can often have a method developed within a few weeks of getting a relevant internal standard... and have an indication of our results in the first month.*"

Dr. Drum sums up the status of the project, and shares his view of the significance of this work: "*We are currently investigating a small clinical cohort of around 150 bariatric surgery patients where, post-surgery, they have lost weight, their oxidative stress has come down and conditions such as diabetes have improved. Our studies will soon be expanded into a 1,200-person cohort where we have mortality outcomes. I am optimistic that we are going to get a strong signal on 'all-cause mortality' out of this work. If we do, then we have a great platform for doing an interventional trial where half the cohort is titrated to the Waters assay and the other half simply gets standard of care.*"

> "We will look to demonstrate improved outcomes in the first group... that would be very exciting!"
>
> DR. CHESTER DRUM, MD, PH.D.
> *Consultant Cardiologist*

Ultimately, the project would not have been viable without the development partnership between NUHS and Waters. Dr. Drum summarizes: "*From the aspect of biomarker discovery, I can only do it with LC-MS/MS: we have shown that we can do it with a small enough volume, it is sensitive enough and I can engage large established biobanks for discovery and validation, and I can do the necessary experiments within a timeframe of 6 months to a year! The Xevo TQ-XS is suited to true high-end research, or to clinical research laboratories that want to match bleeding edge biomarker development with the best technology.*"

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To find out more about the Xevo TQ-XS go to: waters.com/xevotqxs

Waters

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