

Advances in alcohol biomarker and drugs of abuse testing utilizing alternative sample matrices and high sensitivity UPLC-MS/MS

Dr. Olof Beck's ground-breaking contributions to analytical toxicology have provided the foundation for exciting innovations in exhaled breath sampling technology and blood microsampling technologies.

INNOVATIONS IN ALCOHOL BIOMARKER AND DRUGS OF ABUSE TESTING

Alcohol and drugs of abuse testing is a fast-changing field, with the emergence of new drugs that require new methods for accurate detection and quantification. Dr. Olof Beck, Professor Emeritus at the Department of Clinical Neuroscience at Karolinska Institute (KI), Stockholm, Sweden, has seen these changes firsthand throughout his career. For more than four decades at KI, he worked and conducted his research at one of the world's foremost medical universities that offers the country's broadest range of education in medicine and health sciences.

Dr. Beck's areas of interest are method development in pharmacology and toxicology with special focus on mass spectrometry (MS), alcohol biomarkers and toxicology. His most recent work is focused on new psychoactive substances, breath testing for abused drugs, dried blood spots and the alcohol biomarker phosphatidylethanol. As a renowned scientist in bioanalysis and a pioneer of alternative sampling technologies, Dr. Beck has founded or consulted for several start-up companies focused on innovations in patient sampling.



Dr. Beck has worked with Waters for two decades, and now uses the Waters Xevo TQ-XS System to support exhaled breath testing.

WORKING WITH WATERS

Dr. Beck started working with Waters™ ultra-high performance (UPLC-MS/MS) instrumentation as the drugs of abuse testing field began shifting from GC-MS techniques to LC-MS techniques about two decades ago.

Eventually he turned to the Waters Xevo™ TQ-XS System for his exhaled breath work and later research efforts. But it's the support from Waters that was particularly valuable over these many years.

Dr. Beck explains:

"The support team for Waters in Sweden is very good. Mass spectrometers are complicated instruments, and you always need service and support. The Waters service personnel are very experienced and skilled. That's important in a clinical laboratory setting because your instruments need to be running every day."

One of those innovations is Breath Explor, owned and manufactured by Munkplast AB of Uppsala, Sweden. This device uses exhaled breath to measure drug use in almost any setting. In 2016 Dr. Beck founded Capitainer with several colleagues to develop quantitative microfluidic dried blood sample devices for home use, known as the Capitainer®qDBS. He also recently began consulting for ABC Labs, a private company developing a digital infrastructure around toxicology testing that better integrates healthcare providers, laboratories, and patients to make diagnostics more affordable, accessible, and readily available.

All these innovations were made possible by the technical evolution of liquid chromatography coupled with tandem MS (LC-MS/MS) technology, which has alleviated many issues with the traditional immunoassay methods used in toxicology. Dr. Beck describes how LC-MS/MS has contributed to advancements in the field and transformed analytical methods for drugs of abuse and alcohol testing:

“In this field, the number of analytes that we need to detect is always increasing. New psychoactive substances and therapeutic drugs constantly need to be added to testing panels. With an increasing number of analytes, you would have to run many immunoassays for each sample, so it becomes costly. Then, with the development oral fluid testing, the reagents for immunoassay screening in oral fluids had cutoff limits that were too high, and they were not stable for the application. At the same time, the performance of LC-MS/MS instruments has improved significantly, making it a better choice for testing laboratories. Today, you cannot detect these new drugs with immunoassays, you must use LC-MS/MS.”

DR. OLOF BECK

Professor Emeritus at the Department of Clinical Neuroscience at Karolinska Institute



Dr. Beck is a founding member of Capitainer and also recently began consulting for ABC Labs, a private company developing a digital infrastructure around toxicology testing.

In all these endeavors, as well as his own academic research, Dr. Beck has relied on the Waters Xevo TQ-XS Tandem Quadrupole Mass Spectrometer in combination with the ACQUITY™ I Class UPLC™ System to provide a robust and suitable system that can also offer the needed sensitivity for sample limited toxicology applications.

DRUGS OF ABUSE AND ALCOHOL TESTING

Drug testing in using urine samples became the standard in clinical and forensic applications after the development of analytical technologies based on immunochemistry in the 1970s allowed for cost-effective screening of common drugs of abuse with gas chromatography-mass spectrometry (GC-MS) methods for evidential confirmation of positive outcomes. Since that time, developments in MS technology have made it possible to avoid immunoassay screening entirely and directly make an analytical investigation with evidential LC-MS/MS methods, changing the paradigm.¹

At the same time, interest in alternative matrices can be traced back to the early days of drug testing because of the limitations and potential for fraud when relying on urine samples. Today, alternative sampling technologies are becoming more common in routine applications, which can be credited to the development of more powerful technologies for bioanalysis-based LC-MS/MS instruments that have set a new standard of method performance.²

"I was a GC-MS person from the early 1970s, and I was really biased toward this technique. However, when we started using LC-MS/MS, I saw the light. In the early 2000s, when UPLC came about, we saw its potential. We started with Waters Quattro Premier instruments, which solved several problems associated with using GC-MS for confirmation analysis, including laborious sample preparation, long run times and a complicated and lengthy validation processes. Customers, of course, were frustrated with these very long reporting times. We started with a concept called dilute and shoot, which I helped to pioneer. Using the separation power of UPLC, we could replace all the complicated GC-MS methods with LC-MS/MS direct injection. It was very successful."

DR. OLOF BECK

Professor Emeritus at the Department of Clinical Neuroscience at Karolinska Institute

Over time, Dr. Beck began to add the Waters TQ-XS Tandem Quadrupole Mass Spectrometer to his laboratory set up, which has played a significant role in his patient sampling innovations over the years. Dr. Beck has had many opportunities to collaborate with other leaders in alternative sampling, including Dr. Michael Böttcher, Head of Drugs and Drug Analysis at MVZ Medizinische Labore Dessau Kassel GmbH. Dr. Böttcher has also developed and optimized groundbreaking methods for oral fluid testing, which is now gaining in popularity, as well as capillary blood testing, using the Waters Xevo TQ-XS Mass Spectrometer. Dr. Beck explains the impact this instrument has had on this type of work:

"I started to use the Waters Xevo TQ-XS instruments for my work on exhaled breath specimens. That's a really demanding application that requires very high sensitivity. Since then, I've continued to rely on the Waters Xevo TQ-XS, recently installing them in a new laboratory that I'm helping to start."

FINGER PRICK BLOOD SAMPLING

Dr. Beck has also worked on a new blood sampling patient sampling device. Using a combination of paper and polymer microfluidics, he and his colleagues developed the Capitainer®qDBS (currently known as Capitainer®B) Device, now owned and distributed by Capitainer of Solna, Sweden.

This device has made it possible for everyone to obtain a precise 10µL volume of blood from a finger prick, enabling patients to take a blood sample for clinical analysis anytime and anywhere, without sacrificing testing accuracy.

The technology enables blood from the finger prick to be immediately applied to a pre-prepared DBS filter paper for drying. This workflow provides significant time savings as it reduces or even eliminates the need for downstream sample preparation because the circular discs punched from the filter paper serve directly as the sample for many downstream testing applications.

Since DBS testing is often used in conjunction with sensitive analytical technology like mass spectrometry, blood volumes in the microliter range are sufficient. Dr Beck describes the origins of the technology:

"I was doing malaria research in Uganda with one of my PhD students, and they were trying to take venipuncture samples in the field, then using a pipette to store it on filter paper and bring it to the lab. That was 17th century technology, so I thought we needed something better. We figured it out, published our findings in 2015, applied for a patent, and then started Capitainer."



Capitainer has developed quantitative microfluidic dried blood sample devices for home use, known as the Capitainer®qDBS.

Due to the high volumetric accuracy and precision of the device, the sample collection is on par with industrial pipetting and just as reliable as venipuncture, which is routinely used in the health care system. Samples can be taken anywhere without the need for trained personnel, allowing self-sampling and at-home/remote testing, which greatly expands the possibilities for testing in regions where access to medical professionals is limited.

The dried samples, which can survive for up to a year at room temperature, can then be sent for various laboratory analyses. Due to the stability of the dried blood samples, the technology is also suitable for testing in developing countries where samples must be sent to a laboratory far away, perhaps even in other countries. When compared to urine sampling, it can be less intrusive and more resistant to fraud.

“From a finger prick, we can do alcohol biomarkers and drugs of abuse screening, as well as measure the exact concentration of methadone in patients who are on methadone treatment. The clinicians were very accepting of the idea to get everything in just one finger prick. If you do finger prick sampling in the correct way, there is no pain. You just put a bandage over it, and the next day it’s healed. When the sample arrives at the laboratory, it’s exactly 10 μ L. It’s more accurate than pipetting. Then you just use it for analysis directly. You just take the disc and put it into the system directly. It’s possible to automate it as well in a 96-well format for high volume applications.”

DR. OLOF BECK

Professor Emeritus at the Department of Clinical Neuroscience at Karolinska Institute

Because of its quantitative properties and ease of use, Capitainer®qDBS can successfully be used for home sampling in large populations. Monitoring could include applications such as viral infections and hormone levels, or biomarkers such as PSA (prostate cancer) or NT-proBNP (cardiovascular diseases).

Another group that could benefit greatly from the technology is immune compromised individuals, such as transplant patients. These patients can reliably be monitored from their own homes, thus avoiding pathogen exposure by going to the hospital for testing.



Capitainer®qDBS samples can be used for accurate quantitative analysis, which opens the technology to almost endless possibilities.

Beck also has consulted on studies to evaluate other ways Capitainer®qDBS samples can be used for accurate quantitative analysis, which opens the technology to almost endless possibilities. In 2021 SciLifeLab researcher Jochen Schwenk and his team published an article demonstrating that dried blood samples from the Capitainer®qDBS device could be just as accurate as existing plasma sampling used in the hospital for monitoring SARS-CoV-2 infection.³ The study demonstrated the potential of the technology, and many became interested in collaborations. For example, the Public Health Agency of Sweden (FHS) has used the qDBS technology in three big Covid-19 monitoring studies. SciLifeLab plans to work on further development of the device for proteomic profiling, as well as explore the possibilities of quantifying other molecules.

EXHALED BREATH

Dr. Beck’s research on exhaled breath resulted in another patient sampling device, Breath Explor, owned and manufactured by Munkplast AB of Uppsala, Sweden. Apart from volatiles, exhaled breath contains aerosol particles that carry non-volatile components from deeper parts of the lung.⁴ Samples can be analyzed using MS for the presence of drugs-of-abuse or clinical biomarkers. The device is intended for professional use in workplace drug testing, dependence clinics, clinical studies, and the monitoring of therapeutic drugs.

“The discovery I made back in 2010 is that you can collect aerosol particles during normal breathing. Every time you breathe, you also produce some micro particles from the deeper part of the lung as part of the breathing process. If your body is contaminated with the drug, you will see it in the particles. But you can also use these particles for other biomarkers or inflammation. For example, you can measure therapeutics drugs for asthma using MS to confirm that they are present in the right compartment of the lung.”

DR. OLOF BECK

Professor Emeritus at the Department of Clinical Neuroscience at Karolinska Institute

The Breath Explor sampling device consists of a collector unit with three separate collectors, a body, and two caps. The device is easily disassembled for both manual and automated robot sample preparation. Thanks to its three collectors, the device offers multiple test options from the same sample. This makes the device attractive for forensic, clinical and research purposes, where confirmatory testing or diverse testing may be required. Dr. Beck describes how the device can be used in the field:

“Traditionally researchers might go out to nightclubs and ask people whether they are intoxicated or not, or whether they used cocaine recently. Now they can ask to take a sample too. We published a study⁵ where 4.3% of the interviewed persons self-reported that they've been using drugs recently. When we did the analysis, 13% were intoxicated. People don't confess to it, which affects the accuracy of the research.”

A NEW APPROACH TO TOXICOLOGY TESTING

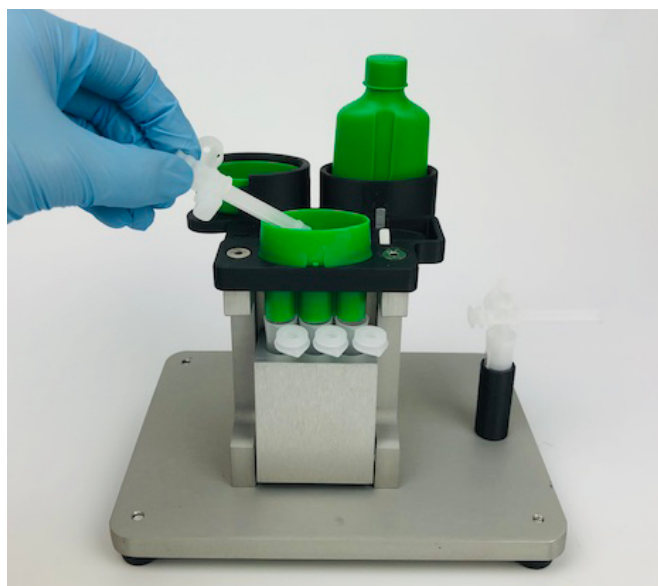
Dr. Beck's newest endeavor is consulting for ABC Labs, which was originally built and deployed over seven weeks in response to the unprecedented challenges of Covid-19. ABC worked closely with the Swedish Public Health Agency and eventually conducted up to 25% of all national testing for Covid-19. Through partnerships with academic scientists, the lab created unique products that enabled the Swedish national screening program for the Alpha, Delta, and Omicron variants as well as the first test for measuring t-cell immunity in Europe.

Dr. Beck describes his initial interest in the company:

“ABC Labs could provide 24/7 service for Covid-19 tests, and they analyzed about 30,000 samples each day with a 24-hour response time. I was really impressed how they developed operations of that size in a very short time. I want to do the same with toxicology.”

With Dr. Beck's help, ABC Labs is turning towards other services, including toxicology testing and preventive health screening. In his consulting position, Dr. Beck is working on establishing a toxicology laboratory that uses LC-MS/MS to enable a 24-hour reporting time on multi-panels for abused drugs and alcohol biomarkers for workplace and clinical testing. In addition to urine and oral fluid, the laboratory will use some of Dr. Beck's own innovations in this process, including devices for dried blood spots and exhaled breath. He explains the rationale:

“I got the opportunity to set up an analytical toxicology unit according to my own ideas and build something new that we can make profitable. In drugs of abuse testing, you usually do immunoassay screening and then you do confirmation by MS. My idea to only use MS for screening and confirmation, and that's what we are establishing in this new laboratory. We don't do any immunoassay at all. We will also use LC-MS/MS for alcohol biomarkers. Right now, we have about 40 substances in our testing panel, and we're setting up special panels for stimulants, opioids, synthetic cannabinoids and more. We're estimating we can run about 2,000 samples per day. When I began to set up the testing laboratory at ABC, I decided to only use the Waters Xevo TQ-XS instruments as standard. Even if the instrument is more expensive, it's more productive.”



Breath Explor uses exhaled breath to measure drug use in almost any setting.

When the laboratory is up and running, ABC Labs will fill a gap in workplace drug testing for employers across Sweden. Dr. Beck describes the impact:

"We have a population of 10 million people in Sweden and carry out around 300,000 workplace drug testing samples per year. I've been working very closely with the dependency clinic here in Stockholm, so I know them very well. I'm running clinical studies together with them, and I understand what they are demanding regarding alternative matrices and drug panels. I've been also very active in the field of workplace drug testing in Sweden. The concept of workplace drug testing has been well accepted here in Sweden, including by the employee unions, because we feel we have a very safe system."

ABC Labs will incorporate some of Dr. Beck's patient sampling inventions as well. The option of testing for different sample types is another key component of the new laboratory.

"The idea is offering all these different specimens as part of the service. When oral fluid came about 20 years ago, people were expecting oral fluid to replace urine, but it didn't. It has created some new markets where urine is not possible to use. So, I think in the future we will use all of them."

DR. OLOF BECK

Professor Emeritus at the Department of Clinical Neuroscience at Karolinska Institute

NEXT STEPS

Although technically retired from academia, Dr. Beck sees opportunities for his consulting work to continue as companies develop new patient sampling products that evolved from his research. He also sees opportunities where these devices can be used to support patients in new ways.

"You can use this technology to determine if a candidate is at risk of alcohol abuse, for example, and provide the support to help the person reduce his/her drinking. Or maybe they are waiting for liver transplantation, and they must stop drinking to qualify. The concept is not designed to kick them out, but to get them the help they need. In some cases, they may not even need a transplant once they've stopped. So, it can support the patient, not just punish."

DR. OLOF BECK

Professor Emeritus at the Department of Clinical Neuroscience at Karolinska Institute

References

1. Rosano TG, Ohouo PY, LeQue JJ, Freeto SM, Wood M. Definitive Drug and Metabolite Screening in Urine by UPLC-MS-MS Using a Novel Calibration Technique. *J Anal Toxicol.* 2016;40(8):628-638. doi:10.1093/jat/bkw050.
2. Takyi-Williams J, Liu CF, Tang K. Ambient ionization MS for Bioanalysis: Recent Developments and Challenges. *Bioanalysis.* 2015;7(15):1901-1923. doi:10.4155/bio.15.116.
3. Roxhed, N., Bendes, A., Dale, M., Mattsson, C., Hanke, L., Dodig-Crnković, T., Christian, M., Meineke, B., Elsässer, S., Andréll, J., Havervall, S., Thålin, C., Eklund, C., Dillner, J., Beck, O., Thomas, C. E., McInerney, G., Hong, M. G., Murrell, B., Fredolini, C., Schwenk, J. M. (2021). Multianalyte serology in home-sampled blood enables an unbiased assessment of the immune response against SARS-CoV-2. *Nature communications*, 12(1), 3695. <https://doi.org/10.1038/s41467-021-23893-4>
4. Beck O, Olin AC, Mirgorodskaya E. Potential of Mass Spectrometry in Developing Clinical Laboratory Biomarkers of Nonvolatiles in Exhaled Breath. *Clin Chem.* 2016 Jan;62(1):84-91. doi: 10.1373/clinchem.2015.239285. Epub 2015 Nov 17. PMID: 26578691.
5. Feltmann, K., Elgán, T. H., Böttcher, M., Lierheimer, S., Hermansson, S., Beck, O., & Gripenberg, J. (2022). Feasibility of using breath sampling of non-volatiles to estimate the prevalence of illicit drug use among nightlife attendees. *Scientific reports*, 12(1), 20283. <https://doi.org/10.1038/s41598-022-24741-1>

Waters™

Waters, The Science of What's Possible, ACQUITY, UPLC, and Xevo are trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2023 Waters Corporation. Produced in the U.S.A. April 2023 720007919EN PH-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
waters.com