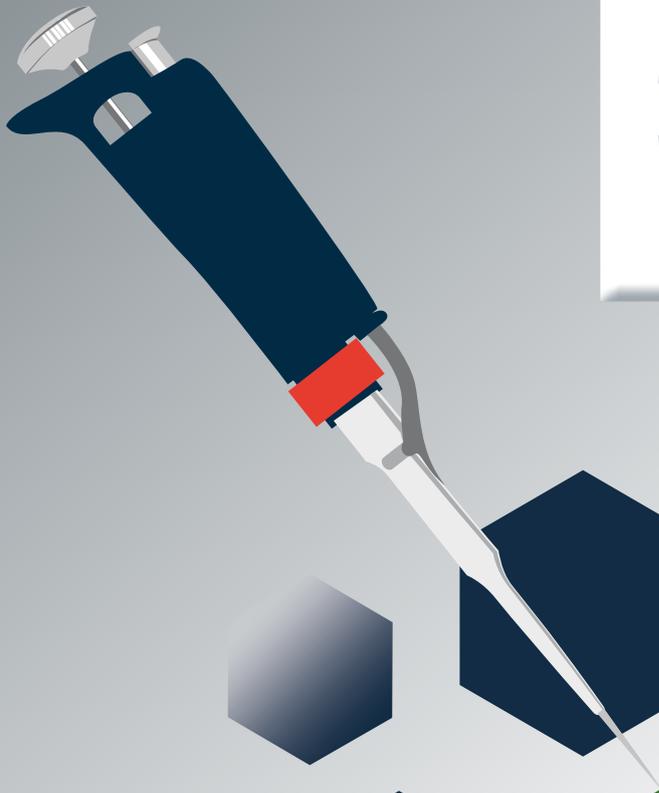




**BIOANALYSIS**  
ZONE



# Bioanalysis Zone Explores Microsampling

– bringing you the latest developments in the field



## FOREWORD

Dear colleague,

It is my pleasure to welcome you to this special Bioanalysis Zone interactive supplement, which has been created to bring you the latest developments and leading opinions from some of the key experts in the field of microsampling.

Microsampling has gained increasing attention within the bioanalytical community in recent years as its benefits have become progressively more convincing, particularly in the context of the three Rs. With the development of newer, more sensitive bioanalytical instruments and novel microsampling technologies, facilitating the application of microsampling in simpler workflows is now easier than ever before.

As I write today, obtaining and working with very small sample volume is becoming a routine reality for many bioanalysts.

In this special supplement we take a look back at how the field has developed since our last examination of the subject 2 years ago. We explore how attitudes have since changed in our recent survey and explore the various directions of microsampling with contributions from leading experts including James Rudge (Neoteryx), Timothy Sangster (Charles River), Roger Hayes (MPI Research) and Craig Aurand (SIAL).

I hope you enjoy exploring the field of microsampling further with us!

Ayan Ali,  
Editor, Bioanalysis Zone



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“ DBS takes its legacy from the science of bacterial inhibition assays. A science dating back to 1889! ”

## MICROSAMPLING – HOW THE PAST MAY BRING THE FUTURE TO DISEASE DIAGNOSIS

By Dr. James Rudge Global Microsampling Specialist, Neoteryx (UK)

In the early 1960s, a physician called Robert Guthrie from the Institute of Massachusetts (Cambridge, MA, USA) sparked a revolution in the way children are tested for inborn errors. In doing so, he and his research group were responsible for saving the lives of thousands of children year on year, often allowing them to live almost normal lives. He achieved this, in part, using a microsampling method. This was of course DBS, or dried blood spot testing. What Dr Guthrie realized was that there were a number of inborn errors of metabolism, which, if caught early and with the right intervention, could be treated effectively. One of these illnesses was phenylketonuria or PKU. It is estimated that this neurological condition affects about 1 in 10,000 babies (dependent on the population) and is caused by a genetic defect in the production of an enzyme called phenylalanine hydroxylase. Abnormally low levels of this enzyme cause an accumulation of phenylalanine (PHE), which in turn affects uptake of other large neutral amino acids in the brain thus causing abnormalities in brain development. Thankfully, there is an effective treatment for the condition, which is achieved through a diet low in phenylalanine plus medication if needed.

Scientists in the 1960s were restricted to the technology of the day. The modern LC-MS would seem like something out of Star Trek to them. Today in many hospitals, it's the modern mass spectrometer (MS) that is currently used to diagnose PKU in a matter of seconds. Ironically, it can be argued that if Dr Guthrie had available a modern MS, DBS may never have become the popular technique that it is today. The reason is that DBS was involved not only as a convenient way to collect and transport blood samples, but it was also part of the analytical assay. DBS takes its legacy from the science of bacterial inhibition assays. A science dating back to 1889!

A bacterial inhibition assay works by placing an impregnated disk of paper on an agar plate impregnated with compounds that either inhibit or promote microbial growth. Indeed, it was a variant of this technique that led Alexander Fleming to discover the antibacterial property of penicillin. Using a similar idea, Dr Guthrie took small drops of blood from newborns and spotted them onto paper, after which he allowed the paper to dry. He then punched out disks of blood and placed the disks onto his agar plates. The high concentration PHE in the dried blood of babies with PKU promoted rings of bacterial growth around the spot. Those with normal PHE levels did not promote growth. This was a neat and simple diagnostic that carries a huge legacy to present day neonatal screening. In some states in the USA, over 50 conditions are now screened by DBS.

If Dr Guthrie had the modern MS would he have been led to use paper disks? Maybe he would, or maybe he would have developed a conventional serum blood test? Who knows! However, his brilliant legacy still remains to this day. Moreover, the use of dried matrix sampling such as DBS is used in many different fields, such as therapeutic drug monitoring and diagnosis of viral diseases like HIV and hepatitis.

“ So it can be argued that there are many assays where, given the right analytical technologies, dried blood assay could be just as effective as the common wet plasma assay. ”

The success of the newborn screening initiative was its simplicity. However, one of the observations that soon became apparent was that even though DBS worked really well for semiquantitative assays like for PKU, the development of more quantitative assays became more of a challenge. Indeed, in recent years there have been efforts to address this by solving a number of fundamental quantitation issues with DBS. One such issue is volumetric hematocrit (HCT) effect, where the viscosity of the blood (due to percentage HCT) can bias the results when a sub-punch is measured from the DBS sample. This has been solved by the emergence of fixed volume sampling devices and also 'whole spot' extraction techniques. Another issue is blood plasma ratio effect. Compounds only found in the plasma fraction will be inversely proportional to the percentage HCT of that sample. This raises a fundamental debate in blood measurements as to what matrix we should measure our analytes in.

### BLOOD VS PLASMA

I've been to a number of meetings where the question of blood vs plasma has been debated. Quite simply, which is the 'better' matrix to measure the physiological concentrations of a certain biomarker / drug? For simplicity, I'm going to refer to 'plasma' as plasma or serum. It must be noted that for some assays there are differences between plasma and serum, however I'm not going to address these in this article as these differences are often subtle. The big difference between plasma and blood is that plasma is clear and blood is not and this determines the type of analytical methodology.

There are certain cases where plasma is the only option. An example of this is in the measurement of plasma potassium. Potassium is actively pumped into cells so any degree of hemolysis will skew the data and the assay will fail. Furthermore, there are some incidences where it is vital that whole blood is measured and not plasma. One example of this is the measurement of the anti-rejection drug tacrolimus. The reason is that tacrolimus partitions primarily into blood cells. There are however plenty of assays where plasma or blood could equally be analyzed. One of these assays is the detection of hydroxylated vitamin D metabolites. These biomarkers are only found in the plasma fraction and not in the cellular fraction. To compare plasma and blood concentrations of the vitamin, either a measurement of the percentage HCT is needed for each sample or an average HCT value is taken and the observed concentration is corrected with respect to the HCT fraction. Alternatively, new reference ranges must be agreed on for blood. So it can be argued that there are many assays where, given the right analytical technologies, dried blood assay could be just as effective as the common wet plasma assay.

### SO WHY PLASMA?

There are a number of arguments as to why many assays have been developed from plasma. The first and most important is the choice of detector. Many of the assays used in routine clinical analyzers today are conducted by measuring changes in the wavelength or intensity,

“ Taking a child for a blood test is one of the most stressful experiences both a parent and child can undergo. ”

or some change in electromagnetic spectrum from a sample. The methods of how this is achieved range from simple enzyme catalyzed reactions to sophisticated immunoassay experiments, but all using light to detected changes. Plasma is key for these assays because it can allow light to penetrate it. Blood, being the colour that this is, is incompatible with many of these assays. Furthermore, instrument manufacturers will often declare a maximum percentage allowable hemolysis.

Other reasons why plasma is chosen over blood is that plasma is seen as a simpler matrix to measure analytes. Another argument for plasma is based on what is more physiologically relevant, blood or plasma? And there are compelling arguments for both sides. Nevertheless, with the emergence of newer technologies like LC-MS and some immunoassays, assays developed from blood are indeed possible. So, blood does offer some distinct advantages.

### AN ARGUMENT FOR BLOOD

Taking a child for a blood test is one of the most stressful experiences both a parent and child can undergo. Moreover, the smaller the child the less free circulating blood. Blood volumes are dependent on the age and size of the individual. An average 70-kg adult will have approximately 5 l of blood, which is plenty for standard blood tests where vacutainers hold up to 10 ml of blood per tube. However, a neonate weighing 3 kg will only have approximately 250 ml of blood. So specialized blood tubes are needed to collect small volumes of blood (1 ml).

Care needs to be taken about the total allowable amount of blood that can be taken from an individual. There are various guidelines in the literature for what this limit is. Let's take 5% total blood taken per 30-day period as an example; this is only about 12 ml for a 3-kg neonate! This is a lot of blood, especially if regular blood draws are needed. Due to this, the smaller the blood samples the better for the patient. However, harvesting good-quality plasma as a microsample is possible but poses technical and practical challenges. Analyzing whole blood samples solves this issue; however analyzing whole blood microsamples on clinical chemistry analyzers also poses huge technical challenges. There must be a better way to conduct blood tests, and the answer could come from the emergence of the omics revolution.

### IS MICROSAMPLING AND OMICS THE ANSWER?

Omics is a broad term for the emerging science of global screening. Omics is all about finding the proverbial needle in the haystack, whether the haystack is the proteome, genome or metabolome. Omics require very sensitive instruments with high levels of selectivity. The sensitivity of some of these instruments is allowing some labs to employ microsamples in their very promising research.

“ ...imagine a future in which a universal detector could rapidly map out the phenome and the genome of an individual from one single drop of blood. ”

In conclusion, imagine a future in which a universal detector could rapidly map out the phenome and the genome of an individual from one single drop of blood. If a significant abnormality is detected, data is sent immediately to a physician via a smart device. The physician would then be able to respond in real-time with an intervention. What we are talking of course is part of the 'personalized medicine' revolution, which we are already seeing the infancy of. If this happens, then Dr Robert Guthrie's microsampling legacy will have evolved into the mainstay of clinical diagnosis.



#### About the author

James Rudge has served as the Global Microsampling Specialist at Neoteryx (UK) since January of 2015. Prior to joining Neoteryx, Dr Rudge worked for Phenomenex for 14 years and is a coinventor of the Mitra Microsampling Device and the Volumetric Absorptive Matrix Sampling (VAMS) technology. During his 14 years at Phenomenex, Dr Rudge held a number of roles including Key Account Manager, Field Service Specialist and latterly European Clinical Business Development manager. roles allowed him to collaborate with customers on a wide range of projects regularly working in

customer laboratories (globally) developing novel sample preparation and LC/LC-MS methods. Dr Rudge graduated from the University Wales, Swansea with a BSc (Hons) III in Biochemistry and a PhD in Organic Chemistry, where he worked on novel chemiluminescent probes for immunoassays.

# collect a drop anywhere, at anytime, by anyone

WHERE ARE WE NOW?



## MICROSAMPLING: WHERE ARE WE NOW?

By TIM SANGSTER (CHARLES RIVER LABORATORIES, UK)

Microsampling has been an interest of mine since the 1990s and to reflect where we are in the field today I would like to review how we got here by taking a personal look at microsampling over the last couple of decades.

I began working in this industry in 1995 and instrumentation and chromatographic advances rapidly changed over my first couple of years as a bioanalytical chemist. The first instrument I had the pleasure of using was an API300, which rapidly advanced to API365 and then API3000. While the mass spectrometers increased in sensitivity through the generations, we also improved, or more correctly, changed the chromatography to give enhanced sensitivity. The increase in sensitivity in the early years was used to reduce the complexity of the sample preparation performed. A lot of the methodology being developed moved away from the selective techniques that were commonly used, such as liquid-liquid extraction or solid-phase extraction, to use protein precipitation.

So while sensitivity increased in our assays, the volume of plasma or blood being collected from the animals was not reduced. I looked at the available techniques in the late 1990s and the only thing that appeared to be commercially available and appropriate to separate low volumes of blood to plasma was the microvettes from Sarstedt (Numbrecht, Germany). I had some success separating 150  $\mu$ l of blood to create plasma in the laboratory, but, when I tried to get support to utilize the reduced volumes to change our toxicology study designs, I was unable to find support to move this forward.

In the early years of the millennium, microsampling was not a major focus for me and there was not a lot of visible activity in the industry. We routinely reduced samples to generate our bioanalytical data and the impetus to change sampling on either toxicological or clinical studies was relatively minimal. The advantage of the sensitivity was used to really drive down the amount of sample being extracted and to reduce the impact of matrix on analysis. The bioanalytical community became highly aware of the impact of matrix effects when using electrospray ionization, and one of the easiest ways to deal with this was to reduce the amount of matrix included in the final extract. So, we utilized the enhanced sensitivity we had gained to reduce the limit of quantification in our assays, while also using significantly less matrix.

During the 1990s there were some real advances in microsampling. The team at GSK (UK) had started to use dried blood spots (DBS) for samples in both preclinical and clinical studies. While we are acutely aware of the problems that have since come to light related to DBS, I firmly believe that the microsampling cause was significantly accelerated by the work that GSK undertook.

At a similar period of time, the team at AstraZeneca (Södertälje, Sweden) was also investigating microsampling and they came up with an approach that was initially targeted at blood microsampling, but adapted to generate extremely small volumes of plasma. The teams I worked in then were very keen on both of these new techniques. Since then we have done significant amounts of research and studies with both approaches supporting preclinical development.

## Easy, Economical, and Convenient!

Volumetric absorptive microsampling (VAMS™) delivers simple, accurate 10 (or 20)  $\mu$ L specimen collection in seconds, no volumetric HCT bias as seen with DBS cards, and elimination of plasma processing steps / cold chain shipping.



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The Mitra Microsampler class 1 medical device is for direct specimen collection of blood and other biological fluids. It is not specific to any clinical test, and is not for use in diagnostic procedures. Use of the Mitra Microsampler in Laboratory Developed Tests (LDTs) requires further processing including the establishment of performance characteristics and successful validation by the laboratory in a manner consistent with CLIA requirements.

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“ *Microsampling in early development (discovery) has become very much the accepted approach.* ”

### SO WHERE ARE WE TODAY?

Microsampling in early development (discovery) has become very much the accepted approach. Many people have been using blood microsampling or a variety of techniques involving capillaries, other separation techniques and also solid sample matrices. The other separation techniques involve the current version of the microvettes I tested back in the 1990s. The acceptance into regulated workflows has been relatively slow with few of the big pharmaceutical companies really coming to the fore. GSK have worked closely with Drummond Scientific (PA, USA) to develop a novel capillary device and AstraZeneca have worked on their capillary microsampling technique; both have applied these successfully to their regulated toxicology studies.

The industry is starting to see many more companies begin to embed microsampling into their preclinical workflows. In the past couple of years, we have seen some real commitment to microsampling by several large companies and also from our regulators. In January 2016, the International Conference for Harmonization released a Q&A document on microsampling, which importantly discusses the idea of taking safety data measurements from main study animals and generating the toxicokinetic and exposure data in the same animals. There has also been a lot of discussion within the industry ensuring that we generate our safety and toxicokinetic data in the same animals. Microsampling has not only facilitated these discussions but is also enabling our ability to deliver this. The removal of toxicokinetic subgroups from studies is having and will continue to have a significant impact on reducing the number of animals used in research.

Microsampling is now part of many companies' workflows within the research and laboratory space and is becoming part of our day-to-day language in bioanalysis and clinical development. Microsampling outside of the laboratory is a key development, although here I am not meaning clinical support and the potential benefits of home sampling. Over the last few years at Charles River (Edinburgh, UK) we have been involved with investigating the use of microsampling to develop new opportunities in environmental research. Charles River has used DBS to support studies in quail, partridge and, most recently, the common vole.

With the increased interest in and advantages of microsampling, which have led to a lot of development and investment in new technology, I would like to bring into focus the newest developments to your attention as a look into the future of microsampling.

Volumetric absorptive microsampling (VAMS) is a unique approach to a solid matrix, which overcomes some of the concerns and issues that have been identified with DBS. The potential of VAMS is very high, particularly in clinical research.

The clinical use of microsampling was really driven during the development of DBS, but has really lost traction over the past couple of years. However, there is some excellent work in the pediatric space and Hitesh Pandya (University of Leicester, UK) has really shown the advantages of DBS and is now working on the new VAMS device.

“ *...microsampling is quietly vibrant and the future is looking bright.* ”

### MICROSAMPLING TOMORROW

I believe we are in a strong position as an industry where research companies, regulators and suppliers are all actively discussing the future, but also engaged in maximizing the potential of the current technology to the betterment of research programmes. I know the work of the European Bioanalytical Forum (EBF) and their current research work into microsampling, through an industry consortium, will be most significant to the continued acceptance and application of microsampling.

Currently, microsampling is quietly vibrant and the future is looking bright. As an industry we have moved into an exciting time in bioanalytical research as we utilize the gains in sensitivity, from our wide variety of analytical platforms, to change the way we conduct our in vivo research, supported by some really innovative developments in sample collection and handling.

I urge anyone who wants to know more about the current state of microsampling or to find out more about any of the techniques I have mentioned above to visit the NC3R's microsampling website – <https://www.nc3rs.org.uk/microsampling>



#### About the author

**Tim Sangster** is a well travelled bioanalytical chemist, having worked in Scotland, Italy, England, America and finally back to Scotland to head up the Bioanalysis and Immunology Department for Charles River, Edinburgh, 6 years ago. During his travels he has gained experience in both CROs and Pharma supporting drug development from a bioanalytical perspective from discovery through to market. Currently Tim's main scientific focus is in the use of microsampling to reduce and refine toxicology study designs as well as applying it to other application areas, and also the use of LC-MS in the analysis of macromolecules.

“ The 2014 Spotlight on microsampling survey revealed that laboratories and regulators were still hesitant to adopt the technique, thus revealing another key message – a deep conundrum that existed in the community between having the knowledge and appreciation of the potential value of microsampling and hesitation in applying it. ”

## MICROSAMPLING: WHERE THE ROAD AHEAD HAS SINCE LED TO

BY AYAN ALI, FUTURE SCIENCE GROUP

Two years ago Bioanalysis Zone hosted an engaging Spotlight on microsampling – Microsampling: the road ahead. We engaged with a number of leading experts within the bioanalytical community to discuss their experiences using microsampling, the current state of the field, in addition to looking at what the road ahead looked like for the field.

In a series of stimulating webinars, engaging commentaries, and numerous other special features we explored the emergence of microsampling in bioanalysis. We aimed to provide a wide-ranging and all-inclusive understanding of the field from a variety of perspectives that would promote discussion and help move the field forward. This event culminated in a lively panel discussion consisting of seven key experts working on various applications of microsampling.

The key message echoed in this Spotlight was the bioanalytical community's undisputed understanding of the benefits of microsampling. There was no contention on the value of the method and its promises; at the time it appeared microsampling was enjoying expansion and utility among the broader bioanalytical base.

To further understand the uses of and attitudes to microsampling within the bioanalytical community, as part of this Spotlight we carried out a survey. The survey revealed great optimism and confidence in the approach – approximately 60% of survey respondents confirmed that they are regular or occasional users of microsampling. Furthermore, at the time of the survey, a larger number were planning to use the technique.

Nonetheless, as optimistic as the broader attitude of the community appeared to be, it wasn't all as straightforward as it may have seemed. The 2014 Spotlight on microsampling survey revealed that laboratories and regulators were still hesitant to adopt the technique, thus revealing another key message – a deep conundrum that existed in the community between having the knowledge and appreciation of the potential value of microsampling and hesitation in applying it.

This conundrum was further debated in the culmination of this Spotlight event – a panel discussion with key experts in microsampling. The panellists discussed the huge growth in the variety of techniques in microsampling, emphasizing how a number of these techniques are becoming well established (namely DBS) and are even being applied more routinely. Since then, these microsampling techniques have become well established with more novel technologies appearing in the market; furthermore, the development of newer, more sensitive bioanalytical instruments are also facilitating the increased application of microsampling in simpler workflows.

The success of microsampling continues to develop and remains undisputed, just as our panellists and the bioanalytical community predicted 2 years ago. It is a promising future that

will only evolve further as bioanalysis continues to advance.

It is positive to see that the optimism expressed 2 years ago has since materialized to an extent – this wasn't a case of a lackadaisical interest expressed by the community, but rather a deeply sincere and carefully measured desire to gain a better understanding of how they can do better science, and gain high-quality data in a more ethical and economical way.

Two years later we see this sincerity materializing – according to our recent survey there has been a 20% increase in the application of microsampling compared with the results generated by our microsampling Spotlight 2 years ago. Our recent survey further showed that although microsampling is still more common in nonclinical contexts, there has been a significant increase in the application of microsampling in clinical research, this is a promising finding of microsampling's high potential.

In her commentary on the 2014 survey results, Kathryn Chapman (Executive Manager at Milner Therapeutics Institute, Cambridge University; Cambridge, UK) highlighted the emphasis that companies are placing on the three Rs, with two of the five major reasons for implementing microsampling being reduction in the number of animals and refinement of procedures, reducing animal stress. It is positive to see that these same reasons continue to drive the industry to apply microsampling and in the process pave the way to achieving better science.

Perhaps it is the strong scientific spirit evident through the non-competitive collaboration across companies in this field, evidenced in recent microsampling workshops, which reflects the optimism that best captures the future of microsampling.

Below are some of the top content from our 2014 Spotlight Microsampling: the road ahead hosted on Bioanalysis Zone. Revisit this Spotlight to see how the field has developed and what issues still remain.

### HIGHLIGHTS FROM SPOTLIGHT ON MICROSAMPLING: THE ROAD AHEAD

- Microsampling Panel Discussion
- Microsampling: the road ahead Survey Infographic
- Interview with Roger Hayes (MPI Research) on microsampling in drug discovery and development
- Microsampling and the bioanalytical challenges in their routine analysis
- Microsampling: is the future bright?
- Interview with Sufyan Maqbool (MedImmune) on capillary microsampling

Microsampling is the technique for the collection and quantitative bioanalysis of blood, or any other biological matrix in very low volumes. It is utilized for certain preclinical or clinical studies, particularly with small animals or children.

## Microsampling in the lab

How often do you use microsampling in your laboratory?

### 2014

29% of respondents used microsampling regularly

31% of respondents had planned to use microsampling



Microsampling in the lab

- Regularly
- Occasionally
- Not currently but we plan to in future
- Never

49% of respondents currently use microsampling regularly

### 2016

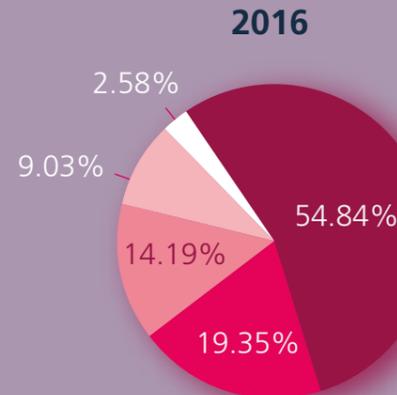
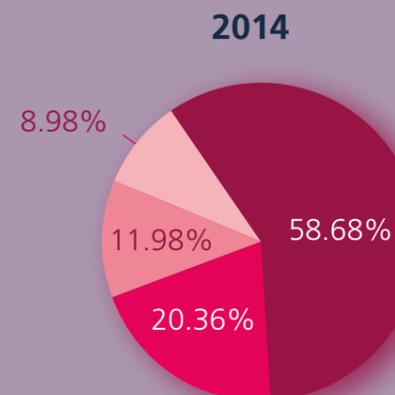
- Regularly
- Occasionally
- Not currently but we plan to in future
- Never



## Molecules analyzed

What type of molecules do you work with?

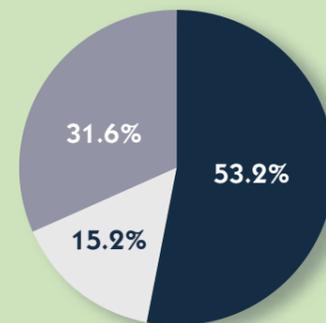
- Small molecules
- Large molecules e.g., biologics
- Efficacy markers
- Safety biomarkers
- Other



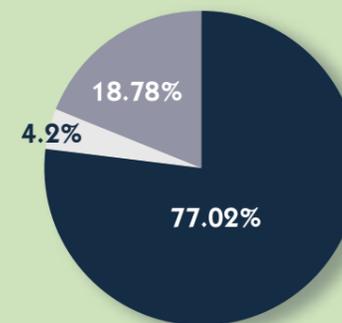
## Applications

Microsampling is still commonly applied in non-clinical research application

### 2014



### 2016



- Non-clinical research/applications
- Clinical research/applications
- Both

- Non-clinical research/applications
- Clinical research/applications
- Both

In clinical research it has increased from



Top 5 reasons for using microsampling

### 2014

- ✓ Reduction in number of animals used
- ✓ Ability to collect more samples per animal/subject
- ✓ Cost saving
- ✓ Reduced stress to animals
- ✓ Time savings

### 2016

- ✓ Reduction in number of animals used
- ✓ Ability to collect more samples per animal/subject
- ✓ Reduction in blood volume required in humans
- ✓ Access to new sampling
- ✓ Cost saving and improved data quality

## The future of microsampling

“Microsampling is the only way forward. It is the most rational solution and should increase in the future...”

“Microsampling will offer new challenges for laboratories but also several chances to improve analysis reliability.”

“Microsampling will continue to develop and be applied in a broader range of study types, beyond the more typical pharmacokinetic applications.”

“Microsampling will be adopted as a common method of collecting diagnostic blood samples in humans.”

“...microsampling is coming into the vernacular for bioanalysis.”

## INTERVIEW WITH ROGER HAYES

### MPI RESEARCH



This interview is a transcript taken from a podcast interview with Roger Hayes, Senior Vice President, DMPK, at MPI Research (Mattawan, MI, US).

In this short interview Roger shares his experience in microsampling and what drew him to the field. He discusses some of the key challenges still faced by the community.

Listen to the full audio of the interview [here](#).

### Q Could you tell us more about your current work?

I've had the fortune of managing an organization that supports predominantly nonclinical bioanalysis drug metabolism and pharmacokinetic (PK); this includes management of an ADME group doing drug metabolism biodistribution and bioanalytical work in both large and small molecules. I also have responsibility for analytical work in supporting nonclinical toxicology as formulation support.

My current works spans the whole gamut of DMPK from discovery through to Phase I and then to Phase III for the bioanalysis. So it includes a lot of the bits and pieces that go to drug development.

### Q What does microsampling mean to you?

Very simply and practically a microsample is a small aliquot that is less than 50 µl. That aliquot could be serum, plasma, blood or even cerebral spinal fluid.

### Q What began your interest in microsampling?

My interest in microsampling actually occurred in parallel with the need to improve assay sensitivity.

It really kicked off for me in the 2000s when we were doing a lot of discovery screening involving new molecular entities that medicinal chemists would synthesize. They were very keen on understanding the pharmacokinetics. You didn't have a lot of test articles to dose a lot of animals, which then led to finding techniques for even smaller volumes to reduce the amount of test articles being dosed, but also come up with good quality data.

And so serial sampling was the ultimate technique for data quality and so with those two components, small amount of test article with high quality data in a fast as possible scenario, this led to techniques and workflows that relied on small volume of sample.

“The conservatism will disappear; I think microsampling will become absolutely routine.”

### Q What are the benefits of microsampling?

Well if I didn't say the three Rs I'd be frowned upon. So really the replacement, refinement and reduction of animals, that's key. But there's the quality associated with that, the better you can do serial sampling the better the quality of the data coming out of what you are analyzing. When you're using fewer animals on study, and this is more strictly in the discovery and nonclinical space, everybody is happier. There is a cost reduction as well; in addition to getting the quality with a cheaper study.

Serial sampling has been taken as the gold standard for doing pharmacokinetic analysis, there's less variability when you're taking blood out of the same animal, it's also faster and you don't have to wait as long to get that sample. However, with recent volumetric accurate devices that are around, like volumetric absorptive microsampling (VAMS), they can take samples of 10 µl, are much faster and cause less stress on the animals.

Certainly the accuracy there not only just improves the overall quality but also PK timing, trying to get a blood sample every 15, 30 and 45 min when you're trying to warm an animal up to get the normal approach to bleeding can often leads to problems, for example if the animal squirms and you just can't get the blood flow. So with new microsampling devices like VAMS, it just improves the overall quality of data and less animal stress.

### Q How has the demand for microsampling increased in recent years?

Well, I would argue microsampling has always been there – any time that you can do bioanalysis with a little as possible sample and push detection limits, this has always been there. I think the industry and the buzzword of microsampling have been confused a little bit with the dried sample. In recent years the dried blood spots (DBS) seem to be the real push and at least microsampling is coming into the vernacular for bioanalysis. And really I think that DBS has its place with simplifying the sample shipments, the opportunity for home base sampling, pediatric trials and all of those kinds of things.

But microsamples have always been there. What has happened is that instead of doing 0.5 ml plasma aliquots of bioanalysis and fairly extensive concentration workflows to get the sensitivity we need, as the instruments got more and more sensitive this became 50 µl and now it is 5 µl.

Currently we're doing nanoliters on platforms like the Gyrus workstation – so it's evident that microsampling has always been there, the technology has just improved and has just gotten easier.

*“...it won't cost more, in fact it will cost less and that's always a good thing when you're trying to keep your budget in check.”*

**Q** What is the biggest challenge microsampling faces today?

I would say getting regulatory approval – certainly this is true in the USA. The US FDA is taking a very conservative approach. For regulator studies, and this is both in GLP toxicology and the clinical space, you need to do complementary assays, both liquid. It's just the understanding that 25 µl is more than adequate and that this actually qualifies as a microsample.

When it comes to the overall perception, it is the regulatory approval of these DBS that has slowed the real explosion in that technology, but there are ways around that, again the VAMS technology is I think the way that will become very routine. Companies and sponsors are very conservative and if you said you were doing something new or let's say out of the ordinary they get very nervous and that's understandable, if I'm spending \$1 million on a toxicology study, I don't want any doubt.

And so there is that conservatism in the USA, but when we get European sponsors they are a little bit more amenable to focus on the three Rs. But what we've seen is a conservatism that shows doubt – “are you sure it's going to work”? We've obviously done all of our due diligence within our laboratory and as I said we've been doing small volumes of samples for 20 years. There is no real issue. But it's the DBS that gets all the press, the issue with hematocrit and things like that creates doubts in peoples mind when you say microsamples. So we just say we do small volumes and avoid that issue altogether.

**Q** Despite the countless benefits known of microsampling why do you think it is still taking time for it to be adopted?

I think it is the regulatory approval within the USA, I think in Europe that's not really there – the regulators are much more amenable to accepting it, and again I'm really talking about dried matrices, and that's where I think there's opportunities for clinical studies where you can do home-based sampling. That will force the situation where people will have to do it that way, and as that body of data builds, and certainly there are a lot of large pharmas currently doing a lot of pioneering work in this, regulators will start to get comfortable. It will take a little bit of time to get there; I think in the USA by 2020 there will be enough data for the regulators to say “you know what this is really routine”.

Certainly in my space, a lot of the work in the nonclinical GLP toxicology is just advancing as instrument sensitivity improves. As we do more and more in biologics, just the assay formats were already in the microsampling paradigm. So that's been accepted for many years – but it's the vocabulary around microsampling that is being associated with dried matrices that may really be what's slowing down the full implementation. That will disappear as people get more and more educated about what microsampling is, there is no mystery around it, it will get comfortable and will become as routine in the clinical space as it is in the nonclinical.

*“There's nothing to be scared about. There's no need for conservatism. It works.”*

**Q** Where do you see the field in the next 5–10 years?

The conservatism will disappear; I think microsampling will become absolutely routine. Instrument sensitivity will continue to push the vendors for more and more sensitive equipment. In parallel that will reduce the amount of volume that is needed in the bioanalysis. Regulators will start approving, new drug applications (NDAs) or biologics licence applications (BLAs) in the biologics, the concern will vanish and I think that will happen more in the next 5 years.

The tools that become available for handling small volumes, the accuracy of the sampling devices, the automation all of these will continue to improve. It is not trivial to do 384 well plates, we currently do them. It is not yet routine, 5 years from now we would like to say “well when we are going to move up to the next platform – 384 is easy”. So I think just the ease of that and the workflows, the accuracy of how we go about doing the bioanalysis will improve and with that the regulators will become very comfortable, it will just become part of the normal course of business.

**Q** If you could give a piece of advice to fellow colleagues looking to implement microsampling as part of their biosampling strategy in their research, what would you say to them?

First you have got to assure them that the study will be valid. There has been enough out there about regulators not approving it and they have to do all this extra works and so forth. I think the first piece of advice, after doing it for 20 years, would be that it is just the DBS that are the issue at the moment, but even that is being resolved.

Secondly it won't cost more, in fact it will cost less and that's always a good thing when you're trying to keep your budget in check. As soon as we have discussion with sponsors and say “look it's not a bad thing, the data is valid and it will cost you less”. Also it is not more difficult – again we have been doing it for a long time and devices like the volumetric absorption are making it even easier, and it's easier for the animals too.

At the end of the day you need an internal champion within the organization to drive it, to be that resource to say “Can we do this?” You've got to get over that activation barrier and that's true of any incremental changes – you still need someone to push to say let's give it a go, let's do the internal due diligence to make sure we can actually take a 10-µl sample out of a mouse, you need that!

And always you have to communicate, you have to communicate with your sponsors letting them know the studies are going along well, that the data quality is there, and then just continue to reinforce those advantages. That's really the advice you give to anybody about any

new workflows. It does work, it's routine. There's nothing to be scared about. There's no need for conservatism. It works.

It is an exciting time to be doing bioanalysis, in 20 years going from 0.5 ml to now thinking of 2  $\mu$ l as routine that's exciting. I'd like to see what the next 10 years looks like, it's almost unimaginable to think that we've dropped multiple logs in volume and the sensitivity has enhanced greatly; picograms have become routine and we used to think micrograms was tough, so it is an exciting time!



#### More about Roger Hayes

Roger Hayes, PhD, is Senior Vice President, DMPK, at MPI Research (Mattawan, MI, USA). Dr Hayes has held numerous leadership positions in the global life sciences industry and academia, leading teams in the development of state-of-the-art bioanalytical and analytical techniques, including mass spectrometry, chromatography, and automation in bringing medical and chemical products to market. For nearly two decades, he has led strategic and research initiatives for large pharmaceutical companies that included both GLP and non-GLP preclinical studies as well as clinical trials. Most recently, he served as President of

Bioanalytical Operations, at Cetero Research (ND, USA) where he focused on establishing overall corporate direction for bioanalytical and analytical services. Dr Hayes has published extensively and has taught numerous aspects of LC/MS method development. He is an active member of the American Society for Mass Spectrometry and the American Association of Pharmaceutical Scientists.



# TAKE A CLOSER LOOK AT MPI RESEARCH



## ASK THE EXPERT ON MICROSAMPLING

CRAIG AURAND (SUPELCO/SIGMA-ALDRICH)

Earlier this year Bioanalysis Zone hosted an 'Ask the Expert' feature on Microsampling. In this feature we explored recent developments in microsampling with leading experts in the field. Discussions included the various microsampling techniques, as well as the advantages of these methods and the challenges faced by bioanalysts when applying these methods in their work.

In this instalment, Craig Aurand, Innovation Manager at Supelco/Sigma-Aldrich, a part of MilliporeSigma (Bellefonte, PA, US) shares with us his thoughts on advances in the field and some of the recent developments.

**Q** Microsampling techniques have gained growing interest over the last few years – do you think advances in these techniques will continue to draw interest?

The ability to obtain high-quality data from a minimal amount of sample is a goal for all bioanalytical assays. Simplifying the process to achieve accurate data downstream will greatly improve the ability to rapidly diagnose and treat a subject to improve overall quality of life. One such improvement is reducing impact on the test subject; this is an area where microsampling can greatly improve current practices. Also, as the acceptance of LC-MS-based assays become more widespread, the need for collection of large volume samples becomes unnecessary.

**Q** What have been the most exciting developments in microsampling techniques in recent years?

The development of solid-phase microextraction (BioSPME) devices with sorbent materials that enable the capturing of free fraction analytes has been an exciting development. This technique allows for the differentiation of bound analytes versus free circulating components within the test subject. Some of the BioSPME devices are intended for direct insertion into test subjects, eliminating the need to draw samples from these subjects all together. A group at GSK has published studies where solid-phase microextraction devices were inserted directly into mice tails as part of pharmacokinetic (PK) evaluations. This approach is quite exciting in that an entire PK study can be completed on a single mouse, eliminating the need for terminal serial blood draws.

Other exciting areas have been the development of devices to overcome the impact of blood hematocrit; this has been addressed using various techniques such as: fixed volume absorbent materials, membranes over absorbent media to prevent absorption of whole blood cells and capillary collection devices.

**Q** What are some advantages of the various microsampling techniques currently available?

Capillary collection and DBS media are intended to reduce the complexity of the sampling process

*“ One exciting area of development is direct MS analysis from DBS and solid-phase microextraction devices by use of direct MS interfaces such as DESI and DART. ”*

and allow for minimum required sample to be acquired remotely. This reduces the need for onsite collection laboratories, and can allow patients to sample themselves with proper training.

Advantages of solid-phase microextraction include isolation of unstable metabolites, differentiation of free fraction versus protein bound analytes and in situ sample clean up.

One exciting area of development is direct MS analysis from DBS and solid-phase microextraction devices by use of direct MS interfaces such as DESI and DART. In some cases, these techniques entirely eliminate the LC portion of the assay, allowing for rapid direct analysis and real-time monitoring.

**Q** Are current microsampling techniques easily amenable to automation?

One area to address for this topic is post acquisition of sample, or how easily microsampling devices can be transferred into an automated workflow. In all cases, the sampling devices need to allow for sample identity to be retained during transfer into the sample preparation workflow. In some cases, the sample preparation can be executed using robotic liquid handling systems or other automated workflows. In the case of DBS media, this necessitates the selection of a spot either using a punching system or online desorption. In each case, optical systems that can recognize the optimum location to punch or desorb are necessary.

There are devices that are designed to work with liquid robotic systems. Solid-phase microextraction along with a few absorptive media devices have been developed on 96-well tip platforms. This allows for automated post collection processes, such as sample preparation and desorption.

**Q** What do you think remains the greatest challenge to microsampling?

Because there is such a long and well known history of data collected using plasma derived assays, introduction of any new sampling technique will necessitate thorough validation and statistical comparison to this historical technique. This may require replication of sample collection during the validation and early-stage studies.

**Q** Do you believe microsampling techniques will ever be universally accepted in the bioanalytical community or do you see them becoming reserved for more niche applications?

Microsampling, as with other sampling techniques, certainly has its utility for specific applications. To say that one sampling technique is acceptable for all assays would be quite limiting. There will

“ There will be continued advancements in the specificity of microsampling techniques. ”

be continued growth in this area, but microsampling techniques will be complimentary and in some cases displace existing accepted methodologies.

**Q** How do you see microsampling techniques evolving over the next 5–10 years?

Advancements to provide real time patient diagnostics are becoming closer through the innovations in microsampling. The development of MS-based platforms with simplified user interfaces has the potential to make bedside therapeutics more common in the near future. The advancements in direct MS analysis using devices such as DESI and DART have shown the potential for accurate and rapid assessments with minimal sample. Microsampling will continue to play an important role in the advancement of direct analysis.

There will be continued advancements in the specificity of microsampling techniques. Microsampling devices containing antibodies for highly specific assays are within the foreseeable future. Evolution of microsampling with biosensors has the potential to allow rapid patient assessment in under developed countries that do not have access to advanced laboratory techniques.

To learn more about solid phase microextraction for use in bioanalytical assays, please visit <http://www.sigmaaldrich.com/biospme>



#### About the Expert

Craig Aurand is the Innovation Manager for the Advanced Analytical division of MilliporeSigma (Bellefonte, PA, USA). Craig's primary role is identification and development of innovative analytical techniques for life science applications. He is responsible for the development of innovative chromatographic and sample preparation devices for life science applications, and has published numerous presentations and publications focusing on novel chromatographic separations and sample preparation techniques. Craig has more than 15 years experience in product and application

development with emphasis in liquid chromatography/mass spectrometry techniques. His most recent research has focused on the field of microsampling techniques in bioanalysis.

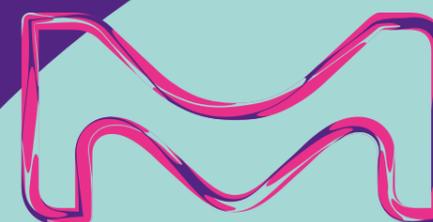
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“...subaliquoting can become even more challenging due to changes in the consistency of the matrix.”

## MICROSAMPLING: THERE IS NO SUCH THING AS THE BEST TECHNIQUE

By HANS STIELTJES AND TOM VERHAEGHE, JANSSEN RESEARCH AND DEVELOPMENT, BELGIUM

In a previous commentary in early 2015, I discussed the use of a non-capillary microsampling technique for good laboratory practice (GLP) in non-clinical studies <sup>[1]</sup>. One of the messages I received was that the choice of sampling materials is important to attain good quality data, because of the smaller sampling volumes. In this commentary, additional data relevant for a capillary microsampling (CMS) technique is presented. Furthermore, the benefit of having multiple microsampling techniques in the lab is discussed; however the focus remains on 'wet plasma' sampling procedures.

An important limitation to non-capillary microsampling as previously described is that accurate pipetting of small volumes (10 µL) is conducted by the animal technicians at the sample collection site and outside of the control of the bioanalyst. Although this was tested to be feasible, it is difficult to let go of the control over this pipetting in the in-vivo group. Adding QC samples to monitor the pipetting performance of the biotechnicians, on a daily basis or per study, was not considered practical in the hectic environment of sampling multiple time points from multiple animals. Performing accurate pipetting at the bioanalysis lab, along with QC samples, is more in line with generally accepted bioanalytical procedures for regulated studies.

Moving the entire sample aliquoting phase to the bioanalytical lab was however not considered feasible either. Although it would be possible to collect 'all' plasma and take an accurate aliquot from this at a later stage, this was rejected as well. Accurate pipetting of a small sub aliquot from an already small plasma volume is also challenging. Once a small plasma volume, especially from rodents, has undergone a freeze-thaw cycle, sub aliquoting can become even more challenging due to changes in the consistency of the matrix.

The above does not mean that the non-capillary microsampling technique cannot be applied with sufficient quality. While still suitable for early rodent studies, it is a matter of 'proof of quality' that may make it less suitable for supporting GLP studies. This was one of the reasons to move to CMS, which by now is considered to be a far easier technique than the non-capillary microsampling approach.

Also with CMS one must be prudent of material use. There are two approaches for CMS, as explained in several publications <sup>[2,3]</sup> and presentations <sup>[4,5,6]</sup>. In the approach of Johnsson O <sup>[2]</sup>, or Vitrex technique, a 32 µL volume of blood is collected into a capillary coated with an anti-coagulant, mostly K2EDTA. Vitrex or Hirschmann, to name two, provide these hematocrit capillaries.

After collection, one end of the capillary (the end that has been in contact with the blood drop) is sealed with a wax plug. After centrifugation, the capillary is cut just above the layer between blood cells and plasma. An accurate plasma volume is transferred to a small volume, non-coated end-to-end capillary.

“In our lab, we have opted for the Vitrex technique.”

Depending on the collected blood volume this can be multiple capillaries, with volumes as low as 1 µL each. This capillary is transferred to a suitable container and stored then shipped to the bioanalytical facility for further processing. This capillary can be processed in its entirety, or the plasma can be washed-out with a suitable solvent to allow taking multiple aliquots for further processing.

A second technique, the Drummond technique, uses a capillary that contains a gas-open plug on one end, and a thixotropic gel <sup>[3]</sup>. After collecting the blood (usually 70 µL), the capillary is centrifuged. The gel helps separating the blood cells from the plasma, while the gas-open plug prevents leakage of blood from the capillary. However, compounds may migrate into the gel, resulting in a lower recovery of the compound from the plasma. With a piston, through the plug, the entire blood and plasma layer are pushed upwards, until the plasma can be collected into a suitable storage container. From this plasma, accurate aliquots can be taken for further processing. This requires accurate pipetting of volumes of a few microliters from an already small volume.

As aforementioned, after freeze-thawing this may be challenging due to clot formation, which can be an issue with rat and even more so, mouse plasma. Also, this technique requires more than two times the volume of blood compared to the technique with hematocrit capillaries (70 vs 32 µL) to enable a large enough plasma volume from which a series of accurate aliquots (5 µL or more, for smaller volumes the accuracy is reduced considerably) can be taken.

In our lab, we have opted for the Vitrex technique. From 32 µL of blood from rats we easily obtain an accurate 10 µL plasma sample in a capillary. In case of insufficient blood volume, which has happened very rarely, we use a 4 µL capillary to collect the resulting plasma sample. With mice it is also possible to take 10 µL plasma from 32 µL blood, but it may be required to switch to 4 µL plasma more often due to the higher hematocrit.

For early phase non-GLP studies, the sample is entirely processed, mostly by the addition of acetonitrile followed by vortex mixing. Clogging of the end-to-end capillary has not been observed and the procedure is quite straightforward. For GLP studies, the sample is not entirely processed as a sufficient sample should be available for eventual re-analysis, incurred sample reproducibility testing or metabolite identification and profiling.

For that reason the sample is washed out with a 2%BSA solution (10 volumes) followed by horizontal shaking for 10 minutes. From the resulting sample, multiple aliquots can then be taken for analysis. With this process, it is important to notice that, unlike the use of a 'traditional' plasma sample, there is quite some contact between the cap of the tube and the plasma/buffer mixture during the horizontal shaking while the protein content of the diluted sample is lowered by a factor of ten.

Depending on the analyte and the type of cap, this might introduce the risk of lowered recovery due to adsorption. Using the same set of compounds that we tested for our non-capillary procedure <sup>[1]</sup>, we evaluated the recovery with this CMS procedure, using various caps

“ In the case of instability of the analyte and/or metabolites, extra precautions may be needed. ”

and tubes. It was observed that the use of soft push caps may reduce the recovery of some analytes, while the use of polypropylene closures (screw caps) did not have an influence at all (Table 1). It is therefore advisable to stay away from soft closures, unless testing has proven that adsorption is not an issue for the analyte being evaluated.

**Table 1: Recovery (%) compared to freshly spiked plasma: collection after 5 min and 2h incubation in the tube at room temperature and cooled with melting ice.**

Tube & cap	Temperature	Time (min)	Cpd 1	Cpd 2	Cpd 3	Cpd 4
Micronic™ tube with TPE cap (blue)	RT	5	103	90	94	90
	RT	120	101	61	74	54
	Ice	120	102	85	89	80
Micronic™ tube with EVA cap	RT	5	96	95	95	97
	RT	120	103	85	85	84
	Ice	120	107	94	97	99
FluidX screw cap (no liner)	RT	5	103	100	98	99
	RT	120	103	104	100	96
	Ice	120	110	100	103	107

Although the above described techniques will work in the majority of cases, there are situations that adaptation of the method might be necessary. These include but are not limited to instability, sensitivity and metabolites.

## INSTABILITY

In the case of instability of the analyte and/or metabolites, extra precautions may be needed. By default, blood is cooled on melting ice to allow centrifugation without any risk of losing the wax plug. However, because of the small volumes and manual actions to transfer the plasma to another capillary, the sample may warm too fast to ensure sufficient analyte stability. Subsequent cooling is not immediately effective as the capillary is separated from melting ice by an air volume in the tube. In those situations a non-CMS approach is more effective. It allows for immediate addition of a stabilizer, while with the Vitrex technique this would require an immediate wash-out after sampling. Both Vitrex and Drummond techniques have the disadvantage that warming of the sample may be too quick to be controlled, because of the high surface/volume ratio of capillaries compared to a volume in a small tube.

“ Having gained experience with multiple techniques has provided us with the opportunity to adapt procedures to the needs of the project... ”

If the instability is not so critical that some minutes at room temperature would not influence the concentrations dramatically, there are other options to keep working with CMS:

- Add a stabilizer in the diluent, immediately after plasma sampling, e.g., formic acid, sodium fluoride, or others. In this case the wash-out procedure is done at the in-vivo facility instead of in the bioanalytical lab;
- Immediately process the plasma sample with precipitant, e.g., acetonitrile, after sampling. This is similar to the first situation except that in principle the entire sample preparation is already done at the in-vivo facility. In theory, no further sample processing would then be required and the sample as such is amenable to LC-MS/MS analysis;
- Split samples: instead of collecting plasma into one 10 µL capillary, collect two 4 or 5 µL capillaries and process the samples differently depending on the requirements. This may also be an option in case samples need to be shipped and a back-up sample is required as a precaution in the event of a shipment going wrong. This is not our preferred strategy, as we have never experienced samples being lost during shipment, but it might be common practice in other companies;
- Move to blood CMS, and use one of the options above. Two 15 µL blood capillaries provide the same possibilities as with the plasma CMS procedure without increasing the total blood collection volume.

The above mentioned options are of course also possible with non-capillary microsampling.

## SENSITIVITY

While with current LC-MS/MS instruments sensitivity is most of the times not an issue for bioanalysis in toxicology studies, there might be situations when for example the compound is administered topically or at low doses and large sample volumes are required to attain the required lower limit of quantification. The above described second option would be possible to avoid extra dilution. Also, one could collect a larger sample, e.g., 64 µL, and modify procedures to end up with a less diluted sample, or take a larger sub aliquot for sample analysis. For non-capillary microsampling, tube volumes of 80 or 100 µL are available, which can also help to resolve the challenges with sensitivity. A volume of 100 µL blood may not sound as a microsample, but often still is a much smaller volume than would be collected via traditional sampling (typically 300 µL of blood from a rat).

## METABOLITES

Samples may need to be used for metabolite identification and profiling. This may put higher demands on the sample volumes. However, by selective pooling of samples, the required volume may still be sufficient for this purpose. Option three also provides the possibility to treat a subsample differently from the main sample in case metabolite stability requires this. Taking a slightly larger sample with CMS (and collecting multiple sub samples) or with non-capillary microsampling also offers a way to enable metabolite evaluations if required, while still greatly reducing the impact on animals.

“...the ultimate, best suited microsampling technique for clinical use will be different from the best practices for nonclinical studies.”

## CONCLUSION AND THE (CLINICAL) FUTURE

At Janssen an evolution from regular sampling via non-CMS sampling to CMS sampling has taken place for non-GLP rodent studies over many years. Since last year CMS has become the method of choice for in GLP toxicology studies in the rat. Having gained experience with multiple techniques has provided us with the opportunity to adapt procedures to the needs of the project, while still having a clear preference for CMS. This has helped in discussions with project teams to adopt microsampling techniques in regulated studies, keeping in mind those samples may be needed for other purposes as well.

Current practices continue to be evaluated, revised or changed if needed, based on new developments within and outside our company. Introduction of CMS in mouse and rabbit GLP studies is ongoing. Maybe this technique will be applied for studies with other species as well. Reasons for this are faster and easier sampling and/or reduced stress for the animal, e.g., for minipigs.

Except for some special studies, there are no concrete plans (yet) to apply CMS in clinical studies on a routine basis. There are two main reasons for this.

One is that the maximum allowed blood volume for humans is rather high compared to most of the pharmacokinetic needs. Therefore, the driver to reduce sample volumes is limited to a few situations in which there might be conflicts with required samples for biomarkers, safety parameters and interacting drugs, for neonatal or pediatric studies or for studies with patients that cannot afford to lose a lot of blood. Rather than restricting individual sample volumes, a compromise of parameters that have priority is often negotiated.

The second reason is that, although easy to learn, the procedure still requires some practice to be correctly applied. While that might be possible for phase-1 studies, arranging adequate training for all clinical sites in multi-site studies is quite challenging. Blood CMS is used for studies for which classical plasma sampling is not an option. Refinement of CMS procedures for plasma is required to pave the road to a general adoption of this technique in the clinic.

Some companies, e.g. Shimadzu Techno-Research, are working on this, but it may take quite some years before the use of microsampling in clinical trials reaches the level of practice and acceptance as is currently the case for non-clinical studies, while for those there certainly is room for broader acceptance as well.

In the end, it might well be that the ultimate, best suitable, microsampling technique for clinical use will be different from the best practices for non-clinical studies.

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### About the authors

Hans Stieltjes graduated in Organic and Analytical Chemistry at the Free University of Amsterdam, The Netherlands. After working in bioanalysis in a university hospital laboratory (4 years) and contract laboratory (CRO) for 6 years, he joined Janssen Research and Development (Beerse, Belgium) in 2004. At Janssen Research and Development Hans is working in the bioanalytical department giving support for projects in both the nonclinical and clinical stages of compound development. This also includes method development and trouble shooting for bioanalytical methods, and giving scientific and regulatory support to colleagues and trainees. He also has an active role in outsourcing methods for new compounds, monitoring method validations and sample analysis. Besides this, he is active in implementing various microsampling procedures for nonclinical studies and scientific validation procedures for clinical studies internally and at CROs.

Tom Verhaeghe is scientific director of the Development Bioanalysis Group of Janssen Research and Development in Beerse, Belgium. In this role, Tom has global responsibility for the full range of activities in support of preclinical and clinical bioanalysis for small molecule new molecular entities ranging from method development, method validation, sample analysis, project interface support, outsourcing, and maintenance of quality and compliance standards for the group. Tom joined Janssen in 1996 with a specialty in GC-MS. Tom earned his PhD in Analytical Chemistry from KU Leuven (Belgium) in 1993.



## INTERVIEWS WITH NEIL SPOONER & JAMES RUDGE



Click on video to play

NS: Hello, I'm Neil Spooner of Spooner bioanalytical solutions and I'm also the senior editor of Bioanalysis.

JR: Hello, I'm James Rudge, I'm the global microsampling specialist for Neoteryx.

**Q** What is your greatest career achievement?

NS: My greatest achievement so far has been helping – hopefully in some small way – to bring microsampling forward into the public agenda, and start to change how people think about doing animal studies, how we collect samples in clinical studies, and how we actually process those samples.

**Q** Could you provide a summary of your career to date?

JR: I did my degree and PhD at the University of Wales, Swansea. Having completed my PhD, which was in organic chemistry synthesizing chemiluminescent probes for immunoassays. I then went on to work at Phenomenex in 2000, where I had a number of roles. In 2014 I moved to Neoteryx to be their global microsampling specialist.



Click on video to play

Could you summarize what microsampling means to you?

**Q** Could you summarize what microsampling means to you?

NS: It's a variety of techniques for taking small biological samples, typically blood or plasma, or other biofluids, to generate high quality quantitative data that is reflective of the subject. Taking smaller samples is a more ethical approach for both the animal and for clinical subjects and enables us to get samples we just wouldn't be able to get in other circumstances.

**Q** What began your interest in microsampling?

NS: My interest in microsampling started 9 or 10 years ago when we were trying to figure out how to collect samples in paediatric studies. Obviously when dealing with very small children, conventional methods of collecting blood samples were just not adequate, so we did some research and that's where we started to get involved with dried blood spot sampling.

**Q** Do you see a shift in interest in implementing microsampling?

NS: I think there is a growing interest of users from different companies and other areas wanting to implement microsampling. They recognise the benefits of the technology – it's obviously a big change for them, and that is making it go slowly but you do see it growing. There are an increasing number of publications in Bioanalysis Zone and Bioanalysis journal and an increasing amount of interest in those publications, and lot of talks at major conferences.

### Q How important is education to increase application of microsampling?

NS: I believe that with any new technique, change is difficult and something as fundamental as microsampling is a big change for an organisation. Running workshops where you can not only educate people on what the state of the art is and where it might be going, but you can also listen to people's concerns, get feedback, and get their ideas on how to move the agenda forward – I think that's the more constructive way of moving to a new reality.



What is your  
greatest career  
achievement?



Click on video to play

### Q What is your greatest career achievement?

JR: My greatest career achievement so far is conceiving the idea of volumetric absorptivity of microsampling and then watching what was essentially a concept idea be transformed into a fully-fledged product. I've been very lucky to work with some really talented people, both within what was Phenomenex and now Neoteryx and I have to say a big 'thank you' to Neil and Phil Denniff, who provided a lot of encouragement and enthusiasm throughout the project.

### Q Could you tell us about the Mitra VAMS technology?

JR: VAMS stands for volumetric absorptivity of microsampling, and is a technique for obtaining a small amount of a biological fluid. It's based on the best bits of dried blood spot and low

volume pipetting. The idea behind it is that you've got a small, absorptive hydrophilic polymer on the end of what looks like a pipette tip; when you apply the tip at a positive angle at a blood pool, whether that's a finger prick or taken from an animal's tail, and then very rapidly the blood (in this case) is absorbed onto the tip, allowed to dry. It's quantitative so it usually takes up 10 or 20 microlitres which solves one of the fundamental issues of DBS. Then in very much the same way as dried blood spotting (DBS), the blood on the tips are dried and they can be sent via regular mail to a laboratory, so it allows for remote sampling and analysis at a laboratory many thousands of miles away. Within the laboratory this is where the pipette tip comes into play – having the pipette tip means you have the option to automate a product onto standard lab liquid handling systems.

### Q What are the uses of the technology?

JR: the uses of the technology are quite wide, from drug clinical trials in which the blood of individuals needs monitoring through to research in low-resourced regions, through to analyzing even larger molecules such as proteins and peptides – even RNA and DNA!

### Q What are the limitations of the technology?

JR: The limitations of the technology are based upon what it is the lab really wants to measure and how it's going to measure it. If one takes, for example, an immunosuppressive like (Tacrolimus), it's a perfect candidate for volumetric absorptive microsampling because that molecule will partition mainly into the hematocrit, and in actual fact, in the lab it is analyzed from blood and not a portion of blood such as plasma or serum. On the flip side, if one wanted to measure potassium then it wouldn't be a good candidate and the reason for that is because plasma-potassium is tested quite a bit in terms of measuring electrolytes. The body will selectively pump potassium into cells so when you collect blood and you let that blood dry onto a surface like a DBS card then the drying action causes a release of cellular potassium into the plasma massively biasing the results and making them interpretable. If you've got compounds which only exist in the plasma fraction such as vitamin D then you can measure vitamin D levels using VAMS but then one has to be aware that the plasma concentration. This is because for the same volume is always going to be higher for the blood concentration because the blood cells take up some of that volume so one has to think about different reference ranges or adjusting the data to match the plasma data. So it really depends on what it is you want to do.



What benefits of microsampling have you seen in your own work?



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To what extent is lack of training/education accountable for the slow transition to microsampling?

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**Q** What benefits of microsampling have you seen in your own work?

NS: In the pre-clinical area, we've seen some big reductions in the number of animals used, by being able to take the toxicokinetic samples from main study animals. Previously they had been satellite animals because the volume taken would affect the outcome of the toxicology study. By taking only smaller volumes, we now know in many cases that will not affect the outcome of the study, so we can take them from main study animals, so that uses fewer animals and also gives better quality data because we can now correlate the concentration data directly to that of the toxicology outcomes in those same animals. Obviously there's also the benefit of being able to sample from children and very small children – samples which we just wouldn't have been able to take in the past. We're also able to take samples in remote locations, particularly with the dried samples that we just wouldn't have been able to collect because a lot of these places don't have access to centrifuges, freezers or dry ice.

JR: In the clinical environment, it's all about the patient experience, so some of the drugs that patients are expected to take have a very narrow therapeutic index and so monitoring is important. Immunosuppressants are the perfect example, when dosed too high you get necrosis of an organ; a too low a dosing and then you risk organ rejection. One of the side effects of these drugs is that some of the people on these drug regimens can become immunocompromised; going to clinic is disruptive for the patient, as hospital could potentially be exposed to pathogens. So, to be able to offer a microsampling solution where they can test at home, is really good and is less disruptive to their lives, it's safer, and it means you can potentially take more time points – one could argue for and against why you would want to do that, but it gives the opportunity to do this rather than go back into the clinic for multiple blood draws. Another great area is drug compliance and there's been some really interesting statistics out, which show that drug non-compliance can be anywhere between 30 and 80%. One of the ways in which we can try and improve drug compliance is to monitor at home by people testing themselves, and that provides some huge benefits.

**Q** To what extent is lack of training/education accountable for the slow transition to microsampling?

NS: I think that's part of the picture, but I think more important is peoples' concern over the quality of data that they're generating and the acceptance of that data. I think that's the bigger problem and training is then part of that to help people understand how they might be able to perform experiments to show them that they are generating good quality data, and for people to publish and talk about how they have shared their data with regulators and how it is accepted. I guess that does come in to training and education, but I think the main problem is people just being concerned about the quality of the data and it being accepted by others.

JR: The key thing for me is that there has to be a body of good peer-reviewed data out there – both in analytical chemistry, but also in clinical chemistry, so that late adopters of technology feel confident that the technology is going to work for them, and that's what's really important.

**Q** How can we overcome this?

NS: We can overcome this reluctance by continuing to listen to peoples' concerns and acting on those concerns, and as James said, building up a good compendium of data in peer-reviewed journals where people have shared their experiences, shared their negative data as well as their positive data because things don't always go right with new technology and people need to share that so that those who are adopting it don't have false expectations of

what the technology is going to do for them. Good peer-review, written articles, workshops – anything we can do to get the word out, people thinking about it, asking questions and performing high-quality experiments for themselves to understand the benefits and the limitations.

JR: It's all about education, all about learning, training, reading and being engaged with the technology. Once one feels more comfortable about using the technology, you know what the potential drawbacks and challenges are, you can build upon that and make good, robust methods that can be very relevant for what it is you're trying to do.

NS: One thing that's been very successful is companies coming together in a non-competitive way to design experiments, share data and publish that data. We've seen the IQ Consortium and the European Bioanalysis Forum doing that and I'm sure there'll be others in the future and I think that's a very powerful way – more powerful than just an individual or an individual company saying they believe in this and have good data. If there's 10 or 20 companies all coming forward and saying the same thing, then it tends to convince the slow adopters and the people who will, in the end, accept the data and have to make decisions based upon it.

and they've never been able to before and maybe now you can help them. Don't use it for everything, but use it where it's appropriate and where it's going to give you good quality data.

JR: For me it would be to learn as much as you can – go to conferences, read journals. It's a new technology – yes, it's been around for a while but back in the 1960s those blood spots were essentially analysed by semi-quantitative methods. There are a lot more challenges now as we're really trying to tighten up the quantitation. So don't give up!

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If you could give a piece of advice to researchers/labs looking to implement microsampling what would you say to them?



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Where do you see the field in the next 5-10 years?

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**Q** What advice would you give to researchers/labs looking to implement microsampling?

NS: I'd say 'try and come at it with an open mind'. It is new; it is different; it is a change and it's not perfect. While it's not going to work for everything, it's a useful tool to have in the toolbox to use in places where maybe you've got project teams who want to collect samples

**Q** Where do you see the field in the next 5-10 years?

NS: Hopefully in 5-10 years we'll see microsampling being a standard technique in areas where it provides a real benefit over standard sampling – hopefully in discovery and development toxicology studies and PK studies it will be standard practice, and hopefully in paediatric clinical studies and areas where we need to collect extra samples in remote areas or at-home sampling it will be standard practice.

JR: In the next 5-10 years, because of the massive increase in computing power and instrument sensitivity and specificity, I see a whole raft of biomarkers coming out for various clinical reasons, so there'll be a whole pile of new tests compatible with microsampling that's going to be clinically relevant and clinically available. Perhaps in the next 20 years I hope that with just one drop of blood we'll be able to analyze the genome, phenome and metabolome, and then detect and personalize the treatment of a disease based on one blood spot.

NS: I agree with that James, and building on that I think we're going to see more patient-centric sampling where the patients are going to start taking a little bit more control of taking samples for themselves and finding out data about themselves that they will then take to the doctor to be interpreted for treatment and we can start diagnosing disease earlier, which gives us a better chance of curing disease.

JR: and also the fact that now – take cancer for example – cancer isn't a disease, it's a collection of many different types of diseases, and scientists and medical scientists within the field are actually realising there are so many different forms of a disease and the really exciting thing about the technology moving forwards is that we can identify the specific versions of disease and then medicine can be personalized for that.

NS: one last thing I'd like to see as well, is as we get more adoption of these techniques, is looking at a more simplified, integrated workflow. At the moment, the bioanalyst is possibly at the wrong end of this technology – they find it hard and don't really see the benefits. Hopefully we'll see the technologies move along and we'll see some better integrated analytical approaches that will help give good quality data in a more simple way.

JR: as technology is miniaturizing at a rapid pace, then doctors' surgeries will have generic analysers on the benchtop where a sample can be analysed there and then, or at least given an idea and then be sent off – so it's a very exciting future.

“ In our laboratory, we work closely also with nonacademic institutions just in order to deeply understand what the actual needs are in real settings. ”

## MICROSAMPLING IN SPORTS DRUG TESTING – AN INTERVIEW WITH LAURA MERCOLINI

(UNIVERSITY OF BOLOGNA)

In this interview we caught up with Laura Mercolini from the University of Bologna (Italy) to discuss some of the novel and exciting applications of microsampling in sports drug testing.

**Q** Could you please provide a brief summary of your career to date?

I am Assistant Professor in Medicinal Chemistry at the Department of Pharmacy and Biotechnology of the School of Pharmacy, Biotechnology and Sport Science at Alma Mater Studiorum – University of Bologna (Italy). Since 2013 I have been Head of the Laboratory of Pharmaco-Toxicological Analysis, where I coordinate the research and didactic activities of the group.

**Q** Could you tell us more about your current work and what began your interest in microsampling?

Currently my main scientific focus is in the development and application of novel bioanalytical approaches to reduce and refine pharmacotoxicological study designs, as well as applying it to other settings. This interest has arisen in the framework of therapeutic drug monitoring of patients treated with CNS drugs, with the main purpose to improve compliance of critical patients and assist clinicians with therapy personalization.

My research work centers on the implementation of effective sampling and pretreatment methods to hyphenated analytical systems based on LC–MS/MS, for the analysis of small molecules in complex biological matrices. The interest of the bioanalytical scientific community today is twofold: on the one hand, we have the continuous development and engineering of cutting-edge analytical technologies, able to push the boundaries in terms of sensitivity and specificity.

On the other hand, those who get their hands dirty in bioanalysis know that a trustworthy quantitative result starts with effective and reproducible sampling, followed by the preanalytical steps.

Procedure miniaturization is designed to respond to scenarios where there is the need for on-field or at-home sampling, in remote areas or at least out of the laboratory when there is the need for a higher subject compliance, whenever sample amounts are necessarily reduced, when there is the need for automatable high-throughput procedures, when rapid execution and low costs are advisable, while ensuring accuracy and reproducibility of results. In our laboratory, we work closely also with nonacademic institutions just in order to deeply understand what the actual needs are in real settings.

*“ From my point of view, any biofluid is potentially suitable to be microsampled. ”*

**Q** You recently also began applying microsampling in sports drug testing, could you briefly describe this research and what encouraged you to apply microsampling in this study?

In recent years, a few timid attempts have been made by the scientific community to propose alternative biological matrices for antidoping purposes. However, the vast majority of sports drug testing is still based predominantly on reference methods involving the use of large volumes of biological fluids (mainly urine), with some disadvantages in terms of sample handling, compound stability, analysis time and costs. For this reason, it was decided to tackle this issue by developing, validating and comparing a series of innovative microsampling approaches, in fluid and dried samples, to be applied for the monitoring of athletes both in- and out-of-competition, both at professional and amateur level.

Our recent work is carried out within a project supported by the Section for the supervision and control of doping and for the protection of health in sport, part of the Italian Ministry of Health's Technical Committee. It was also a study carried out in strict collaboration with Neoteryx®. The idea was to test the Mitra™ device, marketed for accurate whole blood microsampling, also on other biomatrices such as plasma, urine and oral fluid. I can say it worked flawlessly!

With a look to applied research focused on microsampling, with the aim of designing sound and solid sampling and pretreatment protocols, the combination of such collaborative intents has paved the way for this fascinating and promising work.

**Q** You are a member of the collaborative network of the Section for the supervision and control of doping and for the protection of health in sport of the Italian Ministry of Health's Technical Committee, how do you think the application of microsampling will have an impact on sports drug testing?

When I approached the concept of accurate microsampling, I immediately envisaged potential applications for sports drug testing, where in situ sampling and the ability to store and transport the samples at room temperature could really be a turning point in athlete monitoring. Moreover, more feasible and high-throughput methods could lead to a growth of anti-doping controls worldwide. These factors could lay the foundation for expanding and refining the anti-doping analysis and the safeguard of the athletes themselves.

**Q** Microsampling application has been mainly emphasized in blood; do you think microsampling has the potential to work equally successfully in other

*“ ...we should now lead parallel comparative studies between novel microsampling methods and the reference ones accepted by the scientific community. ”*

applications? And what impact do you think this will have for laboratories and clinics?

From my point of view, any biofluid is potentially suitable to be microsampled. Obviously, derived matrices, such as plasma and serum, require additional steps that do not allow direct sampling, thus limiting their use in a laboratory context. However, evident advantages are maintained as regards procedure automation, workflow streamlining, high-throughput sample handling and the use of limited volumes of solvents and reagents, in view of an increasing demand for more environment-friendly applications.

Research in the field of bioanalysis is definitely witnessing a progressive miniaturization of overall procedures and we await impatiently the massive spread of portable analytical technologies able to provide reliable results directly at the sampling location. In the meantime, implementation of microsampling protocols into control and monitoring frameworks could allow a refinement of current practices and their applicative expansion. If we think about contexts such as therapeutic drug monitoring, roadside DUI controls, workplace testing and sport drug testing, microsampling implementation could represent a win-win situation for patients/subjects, clinical personnel, regulatory bodies and society at large.

**Q** Where do you see the application of microsampling in your research area in the next 5–10 years, additionally what advice would you give to fellow colleagues looking to implement microsampling as part of their biosampling strategy in their research?

The major obstacles with adoption of microsampling as common practice is often inherent to those settings, where the dedicated staff are usually reluctant to undertake procedural changes and are generally devoted to classical blood withdrawal by venepuncture. Similarly, I believe the bioanalytical community is affected by decades of plasmatic reference levels, as a convenience and because methodologies did not allow much more, at least until now.

Neil Spooner (Spooner Bioanalytical Solutions, UK), once said: “we are living in bridging times and there is the need for young willing scientists rolling up their sleeves”. I strongly believe we should now lead parallel comparative studies between novel microsampling methods and the reference ones accepted by the scientific community. There is the need to flawlessly demonstrate sampling accuracy and result precision, as well as to compare plasma levels with those of capillary and venous whole blood. We have to build trust in the novel methodologies we propose through solid and overlapping results. This would pave the way to the bridging from the current and often obsolete routine methods towards more effective alternatives. Simultaneously, we should put all of our efforts into communicating these results and populating the scientific literature with findings, although preliminary. This, on the one hand, would be an incentive for other research realities in undertaking microsampling

“...we should put all of our efforts into communicating these results and populating the scientific literature with findings...”

approaches and, on the other hand, it would help to provide the regulatory and legislative authorities all the necessary elements for the implementation of the developed methods.

There is still plenty of work to do!

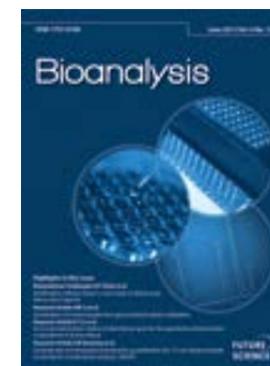


#### More about Laura Mercolini

Laura Mercolini is Assistant Professor of Analytical and Medicinal Chemistry at the University of Bologna (Italy) since 2012 and Head of the Laboratory of Pharmacotoxicological Analysis at the Department of Pharmacy and Biotechnology. Her research activity deals with the development of innovative strategies for the analysis of psychotropic compounds in biological and nonbiological samples. She has published more than 50 peer-reviewed papers and about 150 communications to national and international events, meetings and schools. Dr Mercolini graduated with a Master's Degree in Chemistry and Pharmaceutical Technologies and a PhD in Pharmaceutical Sciences at the University of Bologna.

and Pharmaceutical Technologies and a PhD in Pharmaceutical Sciences at the University of Bologna.

## BIOANALYSIS ARTICLE ABSTRACTS



### Effect of time on recovery of plasma microsamples for the quantitative determination of vancomycin

The reliability of extraction recovery of an analyte in bioanalysis is fundamentally important for downstream analytical testing. For dried format microsamples, if the recovery changes with time the concentration in clinical samples, derived from calibration standards and alongside quality control samples prepared following different drying protocols, may not reflect the true result. The purpose of this paper was therefore to evaluate changes to extraction recovery across time for one analyte, the glycopeptide antibiotic vancomycin, in plasma using two dried microsampling formats, dried plasma spots and volumetric absorptive microsampling.

8(21), 2235–2242; [www.future-science.com/doi/full/10.4155/bio-2016-0159](http://www.future-science.com/doi/full/10.4155/bio-2016-0159)

### Automated DBS microsampling, microscale automation and microflow LC–MS for therapeutic protein PK

**Aim:** Reduce animal usage for discovery-stage PK studies for biologics programs using microsampling-based approaches and microscale LC–MS. **Methods & results:** We report the development of an automated DBS-based serial microsampling approach for studying the PK of therapeutic proteins in mice. Automated sample preparation and microflow LC–MS were used to enable assay miniaturization and improve overall assay throughput. Serial sampling of mice was possible over the full 21-day study period with the first six time points over 24 h being collected using automated DBS sample collection. Overall, this approach demonstrated comparable data to a previous study using single mice per time point liquid samples while reducing animal and compound requirements by 14-fold. **Conclusion:** Reduction in animals and drug material is enabled by the use of automated serial DBS microsampling for mice studies in discovery-stage studies of protein therapeutics.

8(7), 649–659; [www.future-science.com/doi/full/10.4155/bio-2015-0006](http://www.future-science.com/doi/full/10.4155/bio-2015-0006)

### Development of a novel noncapillary plasma microsampling device for ultra-low volume of blood collection

The desire for serial microsampling in mice has led to extensive research in this field within the pharmaceutical industry. The ability to profile a compound's in vivo properties with less material and fewer mice has obvious advantages. A new device and workflow was developed at the Takeda Oncology site to allow scientists to isolate plasma from very low volumes of mouse blood (as low as 20 µl) collected using standard microsampling techniques. A side-by-side in vitro comparison of plasma concentrations was performed using this new device

and conventional sampling methods with commercial and in-house molecules. The plasma concentrations of the molecules tested were very consistent between the conventional sampling techniques and this new device/workflow. In addition, several in-life studies have also been conducted to validate this new technique as a primary PK screening tool at the Takeda Boston. The new device is simple to use and very cost effective with the added benefit that no additional training is needed for the animal technicians and the same centrifuge equipment can be employed. This device can be used for blood volumes ranging from 20 to 100  $\mu$ l enabling studies not just in rat and dog but more importantly in mice.

8(9), 871–880; [www.future-science.com/doi/full/10.4155/bio.16.26](http://www.future-science.com/doi/full/10.4155/bio.16.26)

#### Comparison of blood microsampling with DBS and conventional blood collection techniques used in a midazolam biostudy

**Background:** Quantitative DBS LC–MS/MS assay for midazolam was used to compare two sample collection techniques (venipuncture and finger prick) and the midazolam concentrations measured in plasma samples, DBS and dried plasma spots. **Methodology:** Midazolam was extracted from DBS cards and compared with whole blood collected from usual venipuncture. Dried plasma spots were also compared with plasma. The blood volume used as well as the temperature impact during the blood and plasma deposits was evaluated. Midazolam was administered to six healthy subjects during a clinical trial to obtain blood and plasma samples for the statistical comparison. **Conclusion:** The method for midazolam using DBS was validated and showed an excellent performance. Excellent correlations were observed when the same collection procedures were used.

8(8), 741–751; [www.future-science.com/doi/full/10.4155/bio-2015-0031](http://www.future-science.com/doi/full/10.4155/bio-2015-0031)

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