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IN THIS ISSUE:

MICROSAMPLING

Microsampling significantly lessens the volume of blood and plasma/serum that is collected and analyzed to determine circulating concentrations of therapeutic drugs, metabolites, and biomarkers in preclinical and clinical research.

In preclinical research, microsampling technology supports the 3Rs of animal research, and allows for less intrusive blood collection procedures.

By definition, clinical microsampling reduces sample volume to less than or equal to 50 microlitres (µL) compared to conventional venipuncture wherein millilitres (mL) of blood volume is collected. In Altasciences' experience, microsample volumes being analyzed are less than or equal to 20 µL, with some microsampling techniques as low as 5 µL.

Available microsampling technologies range from dried blood spot (DBS) to capillary microsampling for obtaining plasma samples. In addition, microsampling options available for human use include patient-centric, wearable, fully automated blood collection technologies.

MICROSAMPLING BENEFITS IN PRECLINICAL RESEARCH

Fewer Lab Animals (Reduction)

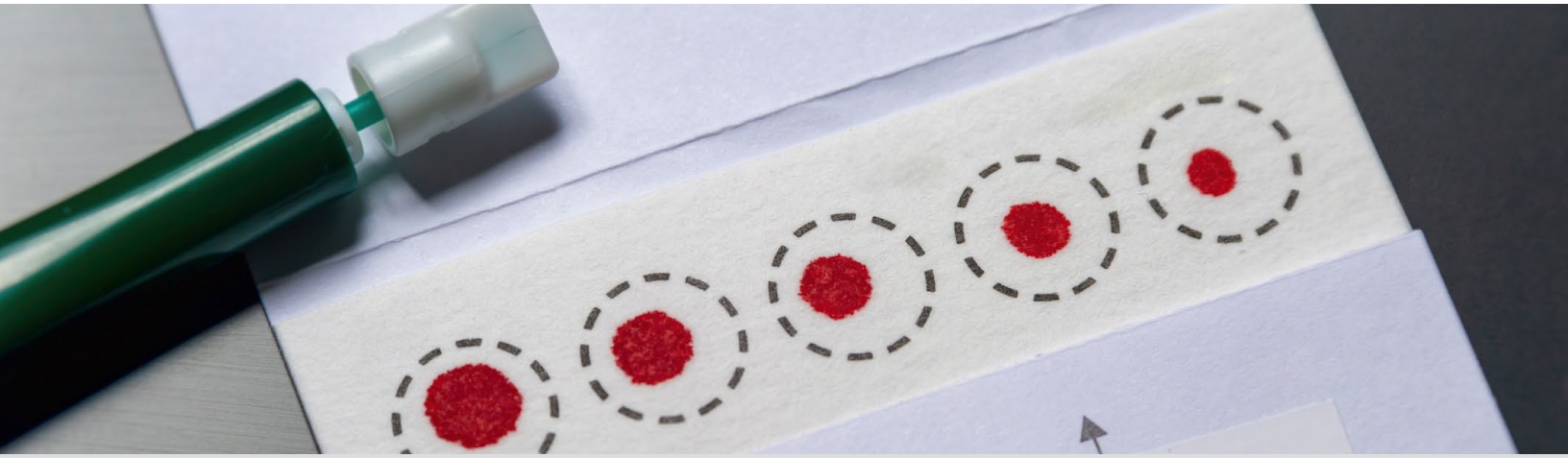
Microsampling addresses two of the 3Rs (replacement and reduction) of preclinical research. By eliminating or greatly reducing the use of satellite animals, microsampling can reduce the number of rodents in certain preclinical studies by 30 to 40 percent. Decreasing the number of animals required can have an added benefit of reducing R&D costs related to animals and housing, particularly for toxicokinetic (TK) studies that use satellite animals to minimize repeated conventional blood sampling procedures. There is less variability in pharmacokinetic (PK) profiles when derived from individual animals versus composite profiling. With microsampling, serial TK/PK sampling can be performed in rodents, rather than using composite bleeds from several cohorts of animals.

Less Strain on Animals (Refinement)

Lower-volume blood draws, from both rodents and non-rodents, taken from less disruptive locations, allow lab animals to recover more quickly, and have fewer sampling-related adverse events compared to conventional sampling. Refinement of the bleeding technique also translates to less animal handling, minimizing stress.

More Reliable Data

The elimination of satellite animals improves the quality of research data, as scientists conduct all TK analysis using main study animals. This enables direct correlation of exposure with pharmacodynamic and toxicological outcomes. Even in studies in which TK satellite animals cannot be completely eliminated (e.g., in mouse studies in which multiple microsamples can impact hematology parameters), microsampling allows all TK samples to be collected from the same animals, allowing for more accurate TK parameter calculations.



MICROSAMPLING BENEFITS IN CLINICAL RESEARCH

Microsampling in clinical research reduces patient burden, improves enrollment and engagement, and reduces drop-out. Self-administration facilitates the data collection during studies related to unpredictable clinical episodes such as acute migraine or epilepsy attacks, and allows episodic sampling to relate efficacy with bioavailability. The dried blood samples can be stored and transported at ambient temperature, eliminating the costly cryopreservation and shipment that conventional wet blood samples demand.

Improved Participant Access

Microsampling provides a convenient, low-cost way to collect blood and plasma/serum from patients remotely. Individuals in remote geographic locations are able to participate in clinical trials, as they can conduct their blood draws at home. Microsampling also facilitates studies involving pediatric and critically ill patients, where blood volumes are a clinical or ethical concern, and ambulation may be an issue.

Therapeutic Drug Monitoring (TDM)

Real-time, self-collected blood samples offer additional advantages in the area of therapeutic drug monitoring for clinical trials. PK data can provide insight into whether participants are being compliant with study protocols and taking the drug on schedule, minimizing potential bias in trial results. Microsampling PK data can serve to inform decisions for optimizing and individualizing dosages, especially in participant populations where precision is critical for safety, such as pediatrics, patients, or those with co-morbidities and co-medications.

Variety of Sample Collection Devices

There are a number of microsampling devices currently used in clinical trials.

Neoteryx has developed the Mitra® VAMS® system, a self-indicating finger stick device that collects the sample on a hydrophilic porous substrate attached to a handler.

The Trajan hemaPEN® is a device designed for finger stick blood collection, transferring sample onto pre-cut cellulose disks located in the cap of the pen. Four DBS samples are collected per finger-stick event, and the entirety of the pen is shipped to the analytical laboratory. The dried blood samples are stable for shipment and storage, with the pen containing desiccant to preserve sample integrity.

The Tasso OnDemand system is applied to the body, most commonly the upper arm, and uses a lancet to nick the dermal layer and vacuum to extract the capillary blood through a microfluidic channel with deposition onto polymeric substrate. Sampling is reportedly pain-free, and the device mitigates potential error for over/under sampling. As with the hemaPEN®, there are replicate samples due to the presence of four substrate in the collection pod. The Tasso system is less cost-effective than finger stick collection, but more automatable and less painful, ideal for remote and pediatric sampling.

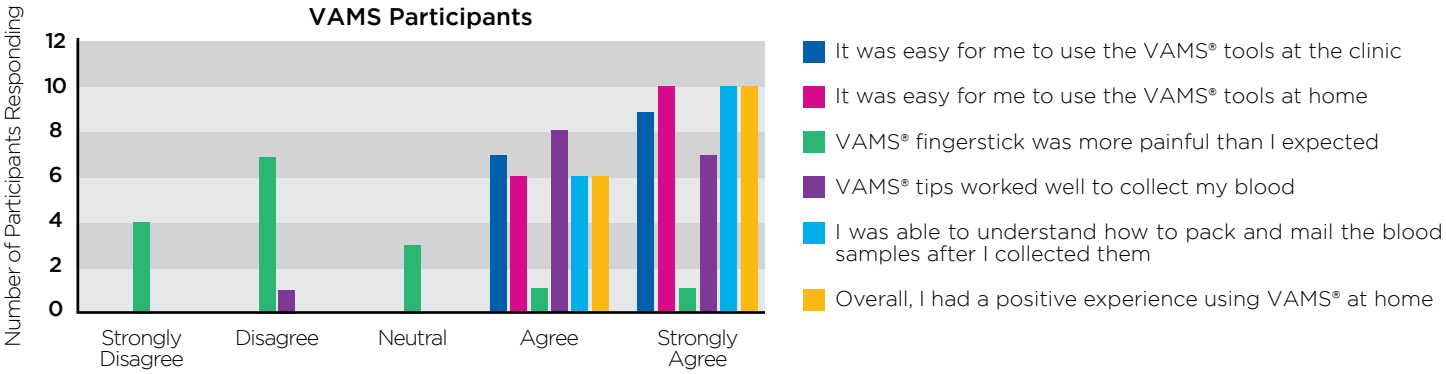
PATIENT ACCEPTANCE

In a recent survey of 16 Phase I trial participants who used Mitra® VAMS®, 100% of respondents either agreed or strongly agreed that it was easy to use the product at the clinic and at home, and that the overall experience was positive.

Figure 1.

Survey From Mitra® VAMS® Fingerstick Users

Phase I Study Conducted by Merck

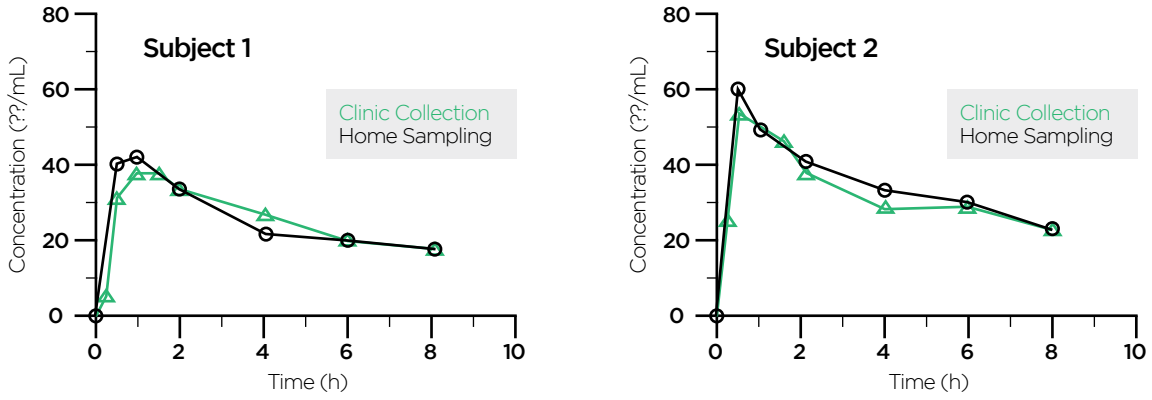


When analyzing PK profiles from at-home microsamples collected using Tasso technology versus in-clinic samples using conventional venous blood draws, scientists found a close correlation between analyte concentrations derived from each blood source; a similar correlation was noted for the Mitra® VAMS® finger stick collection.

Figure 2.

In-Clinic vs. At-Home Tasso Sampling

Seven-Day Washout Period; PK profiles based on blood concentration



BIOANALYTICAL CONSIDERATIONS

The accurate analysis of dried blood microsamples requires equipment with high sensitivity and specificity, such as that offered by triple-stage quadrupole mass spectrometry systems paired with liquid chromatography front ends to optimize signal-to-noise ratio. When additional selectivity is required, this can be addressed by accurate mass filtering (e.g., time-of-flight or orbitrap mass spectrometer) or the use of differential ion mobility spectrometry to separate analyte from interference prior to mass selection.

A drawback of traditional cellulose-based DBS microsampling is the hematocrit (Ht) effect, a phenomenon which creates a sample volume bias when using the sub-punch technique for excision. The result is a concentration bias proportional to blood Ht, which refers to the volume fraction of red blood cells in whole blood.

The Mitra® VAMS® blood collection approach offers a volumetric strategy for sampling, independent of Ht. However, blood Ht can impact analyte recovery, with recovery decreasing with increasing Ht.

Healthy Ht

Male	Female
40 - 45%	37 - 43%

Population range:

28 - 67%

To overcome the Ht-induced recovery bias from the Mitra® VAMS® substrate, bioanalytical experts have developed a universal extraction technique that ensures not only high recovery, but recovery independent of Ht. The impact-assisted extraction (IAE) technique has successfully supported over a dozen programs using Mitra® VAMS®, with no ISR failures.[1]

CASE STUDY 1

Anti-Epileptic Drug Monitoring – Sample Preparation Using Impact-Assisted Extraction

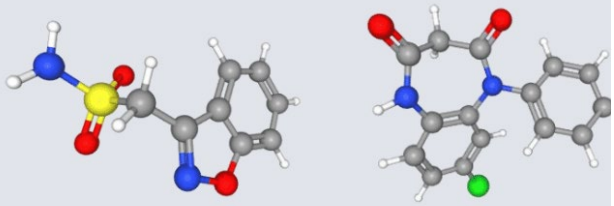
Objective: To demonstrate the applicability of Mitra® VAMS® with impact-assisted extraction for the elimination of Ht recovery bias in the determination of 16 anti-epileptic drugs (AEDs) (Table 1) using a single assay, incorporating scheduled multiple-reaction monitoring (sMRM) with continuous dynamic polarity cycling.

Methods: Human blood was sampled onto Mitra® (10µL), dried for 24 hours at RmT in the presence of desiccant, and extracted by IAE using 80% methanol. Data were acquired using a SCIEX Triple Quad 5500 operated in sMRM mode with continuous polarity cycling of the ESI source.

Table 1.

Properties of Targeted AEDs

AED	cLogP	Pos. MRM	AED	cLogP	Neg. MRM
Levatiracetam	0.59	171.1 > 126.1	Zonisamide	0.11	211.1 > 147.0
Felbamate	0.68	239.2 > 117.0	Phenobarbital	2.14	231.1 > 42.0
Lamotrigine	1.43	256.0 > 211.0	Phenytoin	3.40	251.1 > 101.9
Rufinamide	1.93	239.3 > 221.8	Valproic Acid	2.80	143.2 > 143.2
S-Licarbazepine	1.73	255.1 >194.4	Nitrazepam	2.55	280.0 > 252.2
Topiramate	0.13	357.3 > 264.1			
Carbamazepine	2.77	237.1 > 194.3			
N-Desmethylclobazam	3.42	287.0 > 245.1			
Clonazepam	3.15	316.1 > 270.2			
Clobazam	2.55	301.2 > 259.0			
Stiripentol	3.12	217.0 > 187.0			



Results: The IAE method demonstrated excellent linearity for all 16 AEDs with intra- and inter-assay precision and accuracy meeting all acceptance criteria. Recoveries of AEDs of disparate logP (0.11 - 3.42) from dried blood on Mitra® substrate ranged from 84% to 99%, obtained without Ht bias (Table 2).

Table 2.

Evaluation of Ht Effect

AED	Low QC				High QC			
	30%		66%		30%		66%	
	%CV	%Nominal	%CV	%Nominal	%CV	%Nominal	%CV	%Nominal
Levatiracetam	3.6	103.3	5.1	106.7	4.1	109.7	5.4	105.2
Zonisamide	1.9	103.2	3.9	100.8	4.0	109.7	8.4	105.7
Felbamate	2.1	110.1	4.1	96.5	6.3	118.0	8.5	94.7
Lamotrigine	5.6	103.2	8.4	101.6	2.4	107.1	6.8	101.7
Rufinamide	1.5	100.6	5.8	107.9	6.7	100.3	9,7	105.1
S-Licarbazepine	9.1	105.4	8.7	106.9	6.9	109.0	4.7	100.2
Phenobarbital	3.2	102.0	5.1	107.5	6.0	104.3	9.3	107.2
Topiramate	2.8	104.6	4.2	109.3	6.2	106.2	12.0	111.0
Carbamazepine	0.4	106.5	6.3	107.8	7.3	98.8	6.4	98.4
Phenytoin	2.2	104.5	2.2	108.4	7.4	106.6	8.8	106.1
Valproic Acid	6.9	98.9	2.6	103.2	5.9	101.0	2.1	95.4
Desmethylclobazam	2.0	108.6	5.7	104.4	4.4	102.6	8.7	103.8
Nitrazepam	0.9	101.0	8.2	101.3	3.2	96.9	10.7	97.8
Clonazepam	7.0	109.0	3.4	111.3	4.1	106.1	6.9	104.0
Clobazam	1.3	105.1	4.0	111.7	4.8	105.5	6.3	103.3
Stiripentol	2.3	105.6	6.5	106.6	10.4	104.9	3.9	102.2

Calibration curve 40% Ht; 3 replicates / QC / Ht level

Between-run precision and accuracy from three batches with six QC samples per batch met all acceptance criteria. To the best of our knowledge, this is the largest panel of analytes to be co-extracted from the Mitra® substrate, supporting the potential universality of the IAE approach.

CASE STUDY 2

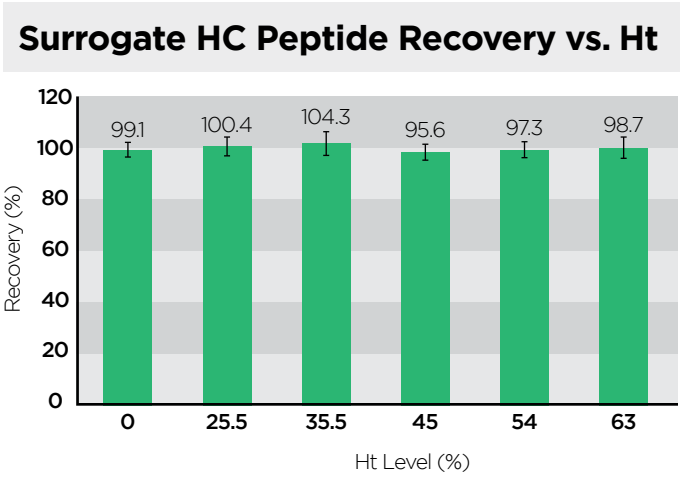
Determination of Rituximab Using a Surrogate Peptide Approach

Objective: To determine the applicability of IAE coupled to a bottom-up quantitation approach for the monoclonal antibody rituximab using a tryptic surrogate peptide derived from the heavy chain.

Method:

Load 20 µL Mitra® sampler into 96-well plate
Add grinding steel ball and extraction buffer
Seal plate and mix aggressively
Reduce (TCEP) and alkylate (IAM)
Digest with trypsin (2 hours, 37 °C)
Quench with formic acid
Analyze by LC-MRM on SCIEX Triple Quad 6500+

Figure 3:



Results: When the Mitra® tip was left in situ during reduction, alkylation and tryptic digestion, near quantitative recovery was achieved without Ht bias when using IAE. Recovery was linear across the concentration range, with regression analysis characterized by a correlation coefficient of 0.9984. The method was both accurate and precise with all acceptance criteria being met.

REGULATORY ASPECT

The method validation standard operating procedure (SOP) and validation plan for microsampling should reflect the most recent requirements of the regulatory bioanalytical method validation guidance documents. While many assay parameters requiring validation are common between wet matrix (e.g., plasma) and dried blood, there are several unique determinations required for the latter.

Assay Development/Validation for Mitra® VAMS®

For plasma or serum methods, analyte stability in whole blood is often validated up to two hours, to cover the potential time course for matrix harvesting. In the case of VAMS®, analyte stability in whole blood is validated for longer periods (up to eight hours) to allow ample time for preparation of all required microsample calibrants and QCs. For both whole blood stability determination and the preparation of blanks, calibrants and QCs, blood is sampled in-house and used within 48 hours of collection.

Although the use of dried blood microsamples obviates the need for freeze-thaw, short-term bench top, and long-term freezer stabilities, it is essential to validate microsample stability under conditions to which study samples might be subjected in consideration of the nonclinical or clinical logistics and risk assessments for sample integrity. These include the absence of desiccant in sample pouches and the possibility for shipment at high temperatures and elevated humidity. The measurement of analyte concentration in stability QCs is determined against calibrant and QC microsamples prepared on Day 0 that are allowed to dry in the presence of desiccant prior to extraction.

Since study samples are often only collected in duplicate from a finger stick, it becomes critical to establish the stabilities that mitigate unnecessary use of the second microsample. In addition to the injection medium stability assessment covering a timeframe over which extracts can be re-injected, the IAE workflow allows an additional assessment involving the primary extraction plate. In the direct extraction workflow of IAE, an aliquot from the primary extraction plate is diluted to mobile phase compatible conditions. Should analyte response in the final extract be greater than the upper limit of quantification, another aliquot from the primary extraction plate can be taken, and analyte response brought within the assay range using a greater dilution factor, an approach preferred to re-extracting the second study sample. Logistically, the primary extraction plate includes additional extracted blanks containing internal standard to facilitate study sample dilution, with the stability of both analyte and internal standard established for at least 48 hours under typical storage conditions (e.g., 4 °C).

As indicated in previous sections, the Mitra® VAMS® blood collection approach offers a volumetric strategy for sampling, independent of Ht. However, blood Ht can impact analyte recovery, with recovery decreasing with increasing Ht. Therefore, the Ht effect is validated by performing a precision and accuracy batch at low and high hematocrit levels (LLOQ, low, medium, and high QCs). The two sets of QCs, low and high HCT levels, will be read against a standard curve prepared in a healthy adult range of blood Ht, previously tested. Low and high Ht level are defined as:

- Low HCT: 20% ± 2%
- High HCT: 50% ± 2%

SCIENTIFIC GUIDANCE

Microsampling is not appropriate for all studies, and should be considered on a case-by-case basis. Altasciences' scientific acumen and experience with different microsampling devices, supporting numerous programs, has led to unparalleled expertise in the development of assays and processes for all your drug candidates, from the straightforward to the most complex.

PHARMACOKINETIC/STATISTICAL ANALYSIS

In a microsampling drug development program, it is critical to establish the appropriate pharmacokinetic/statistical considerations to enable correlation/concordance between microsampling and standard blood sampling techniques.

Altasciences' pharmacokinetic and statistical research experts have experience in supporting the correlation analysis, to establish that the microsampling method is comparable to the standard sampling method, and can be used in future trials. The team evaluates an applicable strategy, and provides pharmacokinetic and statistical solutions to ensure success.

ALTASCIENCES' EXPERIENCE

Please consult our library of educational assets below, and contact us for more information on how we can facilitate your drug development programs and how/if they can benefit from a microsampling approach.

Webinar

Patient Centricity and the Evolving Role of Microsampling

Marketing Brochure

Microsampling in Early Phase Drug Development

Blog

Microsampling - Making a Difference in Drug Development

Posters

Towards a Universal Bioanalytical Workflow for VAMS®

Quantitation of the Monoclonal Antibody Rituximab Using VAMS®, Impact-Assisted Extraction and Trypsin Digestion

Video

Microsampling Services

REFERENCES

1. Volumetric Absorptive Microsampling Combined with Impact-Assisted Extraction for Hematocrit Effect Free Assays. Youhnovski N, Mayrand-Provencher L, Bérubé ER, et al. Bioanalysis, 16 Nov 2017, 9(22):1761-1769. https://europepmc.org/article/med/29148829 Accessed Dec. 3 2020.

ABOUT ALTASCIENCES

Altasciences is a forward-thinking, mid-size contract research organization offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 25 years, Altasciences has been partnering with sponsors to help support educated, faster, and more complete early drug development decisions. Altasciences' integrated, full-service solutions include preclinical safety testing, clinical pharmacology and proof of concept, bioanalysis, program management, medical writing, biostatistics, clinical monitoring, and data management, all customizable to specific sponsor requirements.

Altasciences helps sponsors get better drugs to the people who need them, faster.