Altasciences, 6605 Merrill Creek Pkwy, Everett, WA 98203, United States
Altasciences.com | contact@altasciences.com

Preclinical Strategies in Rodent Studies Using Volumetric Absorptive Microsampling (VAMS®)

Narine Lalayeva[1], Julie Forget[1], Jeff Plomley[2]
[1]Altasciences, Everett, WA, U.S.; [2] Altasciences, Laval, Quebec, Canada

ABSTRACT

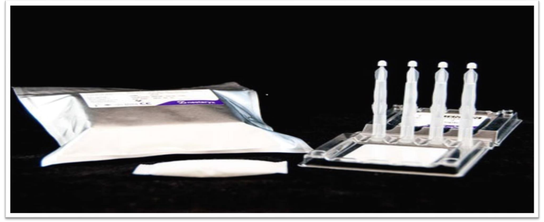
The adoption of blood microsampling as the preferred collection technique for the entirety of a drug development program has increased, particularly for those indications requiring pediatric or vulnerable and critically-ill study populations in which low-volume patient-centric sampling offers enormous benefit. Blood microsampling in the nonclinical space affords both a reduction and refinement in experimental rodent use through the collection of small volumes from less disruptive locations, the ability to correlate toxicological effects with exposure in the same individuals and circumvent the hematocrit (HCT) effect that normally occurs with higher sampling volumes. Nonetheless, microsampling in rodents has its limitations, especially in mice, where blood volume is the greatest challenge. Volumetric absorptive microsampling (VAMS®) technology, sampled onto a Mitra® microsampling device, is a suitable method for nonclinical studies. Using VAMS® (10 µL/sample), a pharmacokinetic (PK) rat study can successfully be completed with three animals/dose level (serial), and a PK mouse study, with eight animals/dose level (split in two subsets – sparse). This represents a reduction in population by 50% and 60%, respectively, compared to study designs using a standard sample volume of 0.5 mL/sample. Further refinement of study designs for regulated rodent studies includes a full or partial consolidation of satellite toxicokinetic and main study groups, which results in significant improvement in animal use. Although drug development processes and regulations are very conservative, various microsampling strategies are now available and have proven to be successful for drug development programs, including studies to support clinical trials, with significant ethical benefits.

INTRODUCTION

Microsampling strategies in preclinical research allows for consolidation of satellite toxicokinetic (TK) and main study groups in an effort to replace, refine, and reduce animal numbers. Additionally, toxicological effects can be correlated with exposure in the same individual. Capillary microsampling techniques circumvent the hematocrit (HCT) effect often reported for DBS analysis; however, processing is tedious and drugs exhibiting non-specific binding or requiring matrix stabilization are problematic. A recent alternative is volumetric absorptive microsampling (VAMS®), wherein an accurate volume of blood is absorbed onto a hydrophilic polymeric tip, thereby simplifying sample collection. The use of this technique allows for a dramatic decrease in the number of animals used in a toxicity study. A pharmacokinetic (PK) rat study can successfully be completed with three animals/dose level (serial), and a PK mouse study, with eight animals/dose level (split in two subsets – sparse). This represents a reduction in population by 50% and 60%, respectively, compared to study designs using a standard sample volume of 0.5 mL/sample, thereby addressing each aspect of the critical 3Rs of animal research.

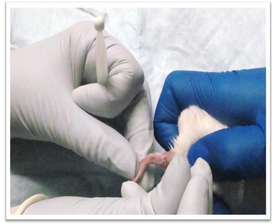
MATERIALS AND METHODS

Mitra® microsampling device

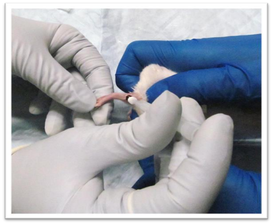


Picture 1:
The Mitra® microsampling kit includes the following:
- Four sampling tips – allows for collection of 10, 20, or 50 \\\\uL samples.
- Clamshell holding case for the tips for storage and shipment – allows for fast and convenient packaging for shipment; no drying time required, when stored in a provided bag with a desiccant.
- Shipping bag with a desiccant – can be labeled with the identity of animals, sample type, etc., and is ready for shipment.

Collection Procedure



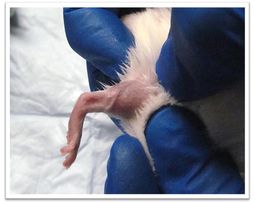
Picture 2:
Puncture the vein with a needle and wipe off the first drop of blood.



Picture 3:
Keeping the Mitra® tip vertical, bring it close to the puncture site and allow the tip to absorb the drop of blood. The tip should not be submerged in blood.

Collection Procedure (cont.)

Picture 4:
Restrain the animal and shave the collection site (saphenous vein).



Picture 5:
Apply lube to isolate and visualize the vessel.

Picture 6:
When the Mitra® tip is fully red, wait two seconds, then remove it. Apply pressure to the collection site to aid in stopping the bleeding.
Place the Mitra® tip back into the clamshell box, and when all four samples are collected, place into the transfer bag with desiccant for shipment.

BENEFITS

Study Design for CD-1 Mouse Study

Group	Terminal		Recovery		TK (Mitra)		TK (Terminal)	
	M	F	M	F	M	F	M	F
1 (control)	10	10	6	6	4	4	6	6
2 (low)	10	10	-	-	8	8	45	45
3 (mid)	10	10	-	-	8	8	45	45
4 (high)	10	10	6	6	8	8	45	45

Fifteen timepoints were required for a mouse toxicology study for TK profile assessments.
- In a routine study with terminal blood collection, a total of 102 mice would be required for a sparse collection with three animals/sex/timepoint.
- With the use of Mitra® tips, the number of animals was able to be reduced to 56 animals (sparse collections with four animals/sex/timepoint).

Study Design for Sprague Dawley Rat Study

Group	Terminal		Recovery		TK (Mitra)		TK (Serial)	
	M	F	M	F	M	F	M	F
1 (control)	15	15	5	5	0	0	3	3
2 (low)	15	15	0	0	0	0	9	9
3 (mid)	15	15	0	0	0	0	9	9
4 (high)	15	15	5	5	0	0	9	9

Fifteen timepoints were required for a rat toxicology (tox) study for TK profile assessments.
- In a routine study with serial blood collection, a total of 90 rats would be required for a serial collection with three animals/sex/timepoint.
- With the use of Mitra® tips, the TK subset of animals can be removed completely, and blood samples can be collected from the terminal tox animals using a less invasive method, thus eliminating any potential complications associated with jugular blood collections (routine for TK sample collection).

CONCLUSIONS

The use of Mitra® tips can significantly decrease the number of animals required in a routine toxicology study, thus addressing the critical 3Rs of preclinical research. The procedure, which is much less invasive than a traditional approach (jugular draw), has the added benefit of eliminating potential complications (death, histological changes, etc.). Collection of TK samples from tox animals allows for correlation between possible test article-related effects with the exposure levels, much like what is typically done in non-human primate studies.

In conclusion, the use of Mitra® tips if a valuable tool that should be considered in all applicable toxicity studies in small animals.