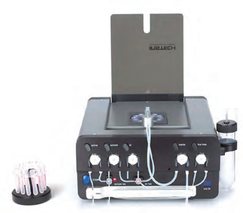
Utilization of Mouse Automated Blood Sampling (ABS) for Serial Plasma Pharmacokinetic (PK) Studies

Jacqueline Kenny; Elizabeth Mahan; Ken Anderson; James Monahan; James Marr; Neal Dube; and Andrew McLaren

Merck & Co., Inc., Kenilworth, NJ, USA

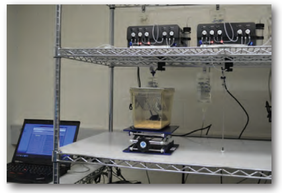


Introduction

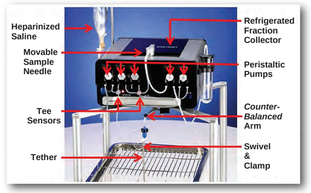
In response to the increased demand for rodent PK data in the scientific industry, automated blood sampling (ABS) has now provided the capability to serial micro-sample blood from mice. It uses minimal manpower and resources when compared with traditional manual blood sampling techniques.[1] This automated technology can obtain small amounts of plasma for routine mouse PK screenings without utilizing traditional orbital and terminal blood collection methods.

The use of the ABS instrumentation has proven to streamline drug metabolism studies verses manual blood sampling procedures. This process minimizes the amount of handling and restraint per animal and decreases animal usage per study.[2,3] Overall, ABS sampling promotes the need for refinement and reduction in animal testing by collecting multiple blood samples from the same animal over prolonged periods of time.[1,2] As a result, it supports the increased demand for quality PK data.

Example of ABS mouse study in progress



Anatomy of an ABS unit



Objective

To evaluate the Instech ABS2™ for serial micro-sampling blood collection for routine plasma PK mouse studies.

Approach

To compare data results and collection processes utilizing the Instech ABS2™ equipment verses a standard manual blood collection method in mice.

References

1. Strand C, et al. AccuSampler Standard - Automated Blood Sampling for ADME Studies. *Asian J Pharmacodyn Pharmocokinet*. 2009;9(2):156-158.
2. Deferme, S. Automated Blood Sampling in Drug Discovery and Development. *The Health*. 2011;2(4):115-116.
3. Instech Laboratories. 2012. *Automated Blood Sampler for Laboratory Animal Research Brochure*. http://www.instechlabs.com
4. Diehl KH, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol*. 2001;21:15-23.

Experimental

Experiment 1

(Manual)

A total of twelve naïve male C57BL/6 mice were dosed IV bolus via the tail vein at the 1 mg/kg (1 mg/mL) with compound A. Animals were divided into four groups that consisted of N=3 per group. Initially, three groups were orbitally bled under isoflurane at one of the following time points: 15 min, 30 min, and 1 hr. Approximately 150 µL of ocular whole blood was collected in an EDTA tube. Next, all groups were terminally bled from the vena cava. Each group was euthanized with CO_2 at one of the following time points: 5 min, 2 hr, 4 hr, and 6 hr. Approximately 150 µL of whole blood was collected into an EDTA tube at the final time point. After completing the centrifuging process, approximately 70 µL of plasma was decanted from each sample.

Experiment 2

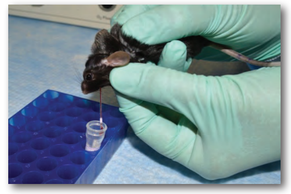
(ABS)

A total of three pre-cannulated male C57BL/6 mice were dosed IV bolus via the tail vein at 1 mg/kg (1 mg/mL) with compound A. Each mouse was sampled serially at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, and 6 hr via the Instech ABS2™ unit. Approximately 60 µl of whole blood was collected into an EDTA tube. After completing the centrifuging process, approximately 20 µL of plasma was decanted from each sample.

Plasma

First the blood was centrifuged at 10,000 rpm for 2 minutes to separate RBCs and plasma. Next the plasma was decanted into a matrix box and the red blood cells were discarded. The samples were kept frozen until they were analyzed.

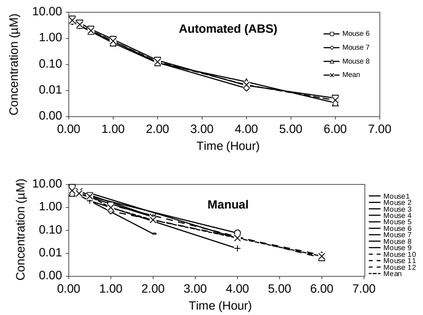
Naive mouse being orbitally bled



Pre-cannulated mouse tethered to ABS unit

Comparison ABS vs Manual Data

Time Hours	Subject Mouse 6 (Male) (µM)	Subject Mouse 7 (Male) (µM)	Subject Mouse 8 (Male) (µM)	Mean	SD	N
0.08	5.55	4.34	4.81	4.90	0.610	3.00
0.25	3.89	3.01	3.18	3.36	0.467	3.00
0.50	2.26	1.85	1.85	1.99	0.237	3.00
1.00	0.913	0.775	0.663	0.784	0.125	3.00
2.00	0.144	0.119	0.115	0.126	0.0157	3.00
4.00	0.0157	0.0122	0.0216	0.0165	0.00475	3.00
6.00	0.00509	BLQ<(0.0025)	0.00335	0.00422	-	2.00



Time Hours	Subject Mouse 1 (Male) (µM)	Subject Mouse 2 (Male) (µM)	Subject Mouse 3 (Male) (µM)	Subject Mouse 4 (Male) (µM)	Subject Mouse 5 (Male) (µM)	Subject Mouse 6 (Male) (µM)	Subject Mouse 7 (Male) (µM)	Subject Mouse 8 (Male) (µM)	Subject Mouse 9 (Male) (µM)	Subject Mouse 10 (Male) (µM)	Subject Mouse 11 (Male) (µM)	Subject Mouse 12 (Male) (µM)	Mean	SD	N
0.08	7.44	4.65	4.10										5.40	1.79	3.00
0.25				5.02	3.86	3.37							4.08	0.847	3.00
0.50							4.19	3.02	1.96				3.06	1.12	3.00
1.00										1.28	0.699	0.949	0.976	0.291	3.00
2.00				0.400	0.384	0.0715							0.285	0.185	3.00
4.00							0.0457	0.0768	0.0163				0.0463	0.0303	3.00
6.00										0.00642	0.00844	BLQ<(0.0025)	0.00743	-	2.00

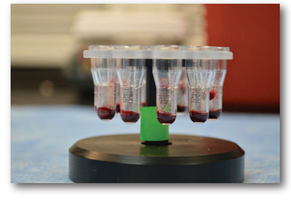
Comparison ABS vs Manual PK Data

Parameter	ABS Value	Manual Value	% Difference
AUC (µM*Hours)	3.111	4.176	-25.5
T1/2 (Hours)	0.815	0.76	7.2
MRT (Hours)	0.564	0.694	-18.7
CL minutes (mL/min/kg)	28.33	21.17	33.8
Vdss (L/kg)	0.96	0.88	9.1

Summary

The mean values of the drug concentration levels between ABS and manual collection methods were comparable and acceptable to Merck's analytical standards. Throughout the study, the ABS data did exhibit a tight PK profile. However, this automated process was very successful in mouse micro serial sampling, which eliminated the need for traditional blood collection methods. It also eradicated the requirement to sacrifice animals during an experiment for blood collection.

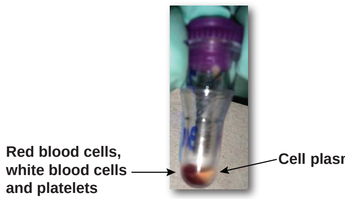
Whole blood samples collected directly into the collection tubes in a carousel from an ABS unit.



As a result, the ABS collection method significantly decreased the amount of animals used per study and provided a more comfortable environment for the animal compared to the manual sampling process. This advancement promotes a positive impact to animal welfare for reduction and refinement as well as generates accurate PK results.

Limitations

- Achieve better results with outbred strains
- Weight range: 26-30 g
- Patency of the catheter
- Amount of blood withdrawn within 24-hr period



Good Practices

- To avoid an impact on animal welfare as well as on sample parameters, it is considered good practice not to deplete the animal of more than 20% of its blood volume, eg, approximately 300-400 µL within 24 hr for mice[4]
- It is possible to analyze a number of essential parameters on very small amounts of blood, eg, 10 µL or even less, therefore reducing the number of animals by using the same subject during the entire test period

However, it is crucial to obtain a high-quality sample to avoid increased data variation and thereby increased group sizes.