Evaluation of Serial Sampling Procedures to Enable Clinical Pathology Evaluations in Mice

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INTRODUCTION/OBJECTIVES

Toxicology studies conducted in CD-1 mice that include clinical pathology assessments are often designed utilizing separate cohorts for hematology and clinical chemistry evaluations in order to yield adequate blood volumes suitable to generating a complete panel of endpoints. The objective of this study was to determine the feasibility of using a temporally staggered serial sampling technique to obtain both hematology and clinical chemistry data from the same animal. Further, the intent of this study was to determine to what extent it may be possible to reduce the number of mice needed for standard toxicology studies involving clinical pathology endpoints while enhancing statistical power, resulting in a more robust dataset for the experimental design.

A successful outcome was determined by volume obtained per sample, incidence of missing parameters for hematology or clinical chemistry panels and potential for procedure-related changes in chemistry values. Data were compared to (Mattawan) test facility historical control values.

METHODOLOGY

Animals

The study was conducted in two phases. Animals were selected from an in-house naive mouse colony (Phase I) or ordered from a vendor (Phase II) to have body weights generally representative of mice which might be assigned to either a 4-week or 13-week toxicology study design. A total of 44 Crl:CD1® (ICR) mice were used for this study (supplier: Charles River Laboratories) as indicated in the table below.

Group/ Phase	Body Weight Range at arrival/transfer (g)	Age at study initiation	Number of Animals	
			M	F
1	31.0–41.6	21 or 58 weeks	10	10
2	26.9–37.9	10 weeks	12	12

Diet

The basal diet, block Lab Diet® Certified Rodent Diet #5002, PMI Nutrition International, Inc. was available *ad libitum*. Lot numbers of diet used were identified in the study records.

Body Weight

Measured and recorded at arrival/transfer to study and/or on Day -1.

Clinical Pathology

Blood samples (0.25 mL) for hematology were collected from all animals via the maxillary vein on Day 1 and a standard hematology panel was evaluated. No redraws were permitted due to IACUC guideline blood volume restrictions. On Day 2, the animals were submitted to necropsy for terminal blood collection, drawn from the vena cava after CO_2 inhalation (maximum obtainable volume), for clinical chemistry testing. A standard clinical chemistry panel was evaluated, with the exception of electrolytes (insufficient volume). Sample volumes were recorded. At study termination, all animals were euthanized and the carcasses were discarded without further evaluation.

RESULTS

Hematology

Quality issues affected 2/44 of samples (4.5%) as indicated below. There were no other quality issues or changes in hematology parameters related to the sampling procedures employed.

- Volume/quality not sufficient for analysis (n=1)
- Sample was clotted (n=1)

Clinical Chemistry

Quality issues affected 3/44 of samples (6.8%). There were no significant changes in clinical chemistry parameters in either group due specifically to the serial bleeding procedure, as compared to historical control.

- Volume/quality not sufficient for analysis of a portion of (calcium, phosphorus, triglyceride, cholesterol, n=1), or all (n=1) parameters
- Sample not collected due to unexplained moribund condition, early euthanasia (n=1)

Analysis of Sample Volumes

Sample volume ranges were similar for both groups; however, average volume was higher in heavier/Phase I animals (males: 0.73 mL and females 0.71 mL) compared to Phase II (males: 0.66 mL and females: 0.57 mL). Animals with higher body weight did not necessarily always yield more blood volume when considered on an individual basis. One potential explanation is the known inter-animal variability associated with sampling from the vena cava. More consistent sampling outcomes, potentially afforded by different/refined euthanasia/collection technique (e.g., isoflurane induction/euthanasia) than employed within this initial pilot study, may be needed to reduce this variability.

Figure 1. (left, upper). Mean growth and weight chart for Crl:CD1® (ICR) mouse.
Table 2. (left lower). Range and average of blood volumes sampled, by phase/body weight cohort.
Table 3. (right, upper). Listing of determined hematology and clinical chemistry parameters.

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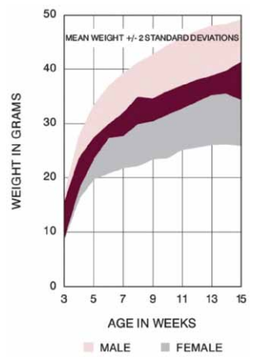

Table 2. Summary of Blood Volumes for Clinical Chemistry (maximum obtainable)

		Range (mL)	Mean sample Volume (mL)
Phase I	Male	0.4–0.9	0.73
	Female	0.5–1	0.71
Phase II	Male	0.4–0.9	0.66
	Female	0.4–0.7	0.57

Table 3. CBC Parameters

Hematology Parameters
• Leukocyte Count (total and absolute differential)
• Erythrocyte Count
• Hemoglobin
• Hematocrit
• Mean Corpuscular Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin Concentration (calculated)
• Absolute Reticulocytes
• Platelet Count
• RDW
• Blood Smear Preserve and Stain
Clinical Chemistry Parameters
• Alkaline Phosphatase
• Total Bilirubin/Direct Bilirubin
• Aspartate Aminotransferase
• Alanine Aminotransferase
• Urea Nitrogen
• Creatinine
• Total Protein
• Albumin
• Globulin and A/G (albumin/globulin) Ratio (calculated)
• Glucose
• Total Cholesterol
• Triglycerides
• Calcium
• Phosphorus

RESULTS CONTINUED

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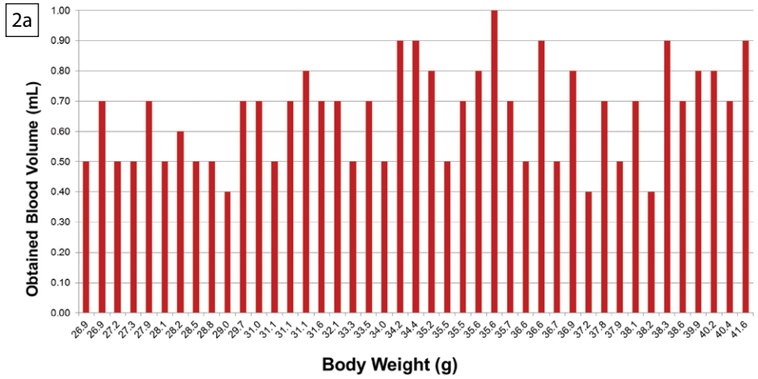

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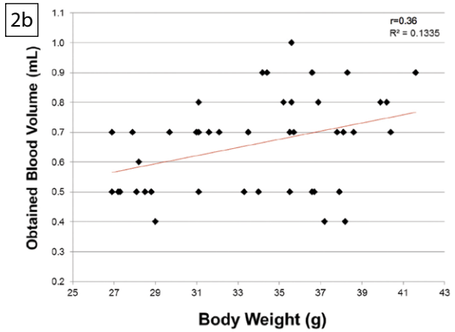

Figure 2a. Absolute blood volumes obtained from mice according to body weight. **Figure 2b.** Correlation between body weight and obtained blood volume. On average, mice have a blood volume equivalent to at least 58.5 mL/kg1,2, or according to the range of body weights obtained in the current sample, approximately 1.57-2.43 mL. On average, based on observed body weights and actual sample volumes obtained, between 32.0% and 35.4% of theoretical total body blood volume was collected for male and female mice, respectively.

CONCLUSIONS

In conclusion, it is possible to collect both hematology and clinical chemistry samples from the same mouse without using cohorts when serial sampling is used, for CD-1 mice that are 10 weeks or older, provided that the extended range of sampling employed to capture hematology and clinical chemistry data is able to be accommodated from a scientific/ experimental design perspective. This technique enhances 3Rs compliance while preserving robustness of experimental design.

Sampling techniques employed did not result in any significant deviations in expected values, in particular, for clinical chemistry parameters.

REFERENCES & ACKNOWLEDGEMENTS

1 *https://www.nc3rs.org.uk/mouse-decision-tree-blood-sampling*

2 Diehl , K-H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J-M., van de Vorstenbosch, C. (2001). "A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes." *J. Appl. Toxicol.* 21:15-23.

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