A Single-Handed Jugular Venipuncture Blood Collection Technique in Unanesthetized Mice

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Introduction:

ITR is continuously striving towards refining techniques used in preclinical testing and conforming to the Three Rs (3Rs; Replacement, Reduction, and Refinement) guiding principles for the more ethical use of animals in safety testing. The most recent improvement results in a considerable reduction in the total number of mice used in toxicology studies, specifically for toxicokinetic evaluation subsets, through the development of a single-handed blood collection technique via the jugular vein. Until now in pre-clinical toxicology studies, blood collection in mice, including small volumes, was standardly performed terminally via the abdominal aorta or cardiac puncture, necessitating a new subset of animals assigned to each blood collection occasion. Utilizing this technique allows for the sub groups of mice bled on Day 1 to be maintained throughout the course of a study for use on multiple blood collection occasions.

Materials and Methods:

The study was conducted at ITR Canada[1]. Twenty (20) Crl:CD1 (ICR) mice (10 males and 10 females) assigned to the study were obtained from Charles River Canada Inc, St-Constant, Québec, Canada. Animals were weighed and assigned to groups based on their weight range.

Mice of varying body weight ranges were selected in order to observe the effects when different percentages (%) of circulating blood volumes were collected, with the objective of not exceeding a maximum of 15%. The actual range of body weights of the mice reflect those generally used in toxicology studies.

Target Weight (g)	Blood Volume (mL)	% Circulating Blood Volume	Number of Animals	
			Males	Females
25 to 30	0.2	9	0	7
30 to 35	0.3	11 to 13	8	3
35 to 40	0.3	11	2	0

Materials and Methods (Cont.):

Three (3) groups of mice, 10 males and 10 females, were group-assigned based on body weight, and were subjected to the blood sampling procedure and restrain method. Each animal was restrained by the technician collecting the blood sample by grasping the mouse by its scruff. The forelimbs were then slightly pulled towards the back with the thumb and middle finger, gently exposing the ventral thoracic area. In order to limit the animal's head movement, the back of the animal's neck was held in place with slight pressure using the index finger. In this hyperextended position, the jugular veins were easily accessible.

The blood samples were collected into tubes containing EDTA, which were subsequently assessed for quality (presence of clots).

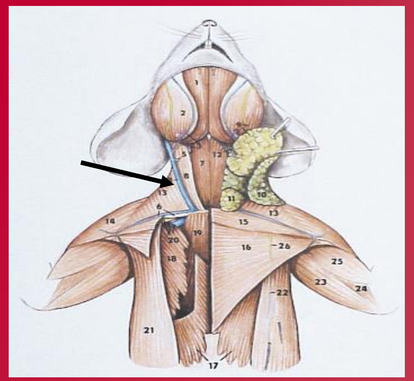

Figure 1. Location of the jugular vein in the mouse

Figure 2. Restraint for jugular blood collection

Results:

There was no mortality during the study and no clinical signs observed during the restraint and following the blood collection procedure. The lack of clinical signs observed demonstrates that this technique is not excessively stressful to the mice. Furthermore, this method of restraint is similar to that for oral gavage administrations.

There was no difference resulting from the varying % of blood collected, nor were there any sex differences noted in males and females.

The sample quality of the blood was not affected by the jugular collection technique; all samples were considered of acceptable quality, as noted by the absence of clots.

Conclusion:

The single-handed jugular venipuncture technique can be considered a contribution to the reduction of the total population of mice required to meet the objectives of a pre-clinical toxicology study. This technique is a beneficial method that eliminates the requirement for anesthetics, permits the collection of high quality samples on multiple occasions, and includes the continued protection of the health and wellbeing of the animals.

Acknowledgments:

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References:

1. S. Cinquino (2017). A Method Development Study for Jugular Blood Collection Technique in Mice