HKSTP IACUC

04 – Blood Collection of Laboratory Animals Guidelines

Version History

Version	Effective Date
1	28/02/2023

1. Purpose

The guidelines are intended to provide guidance to reviewers, veterinarians, PIs and researchers on the limit volumes for blood sampling. The volumes for blood sampling should rely on accurate data on circulating blood volumes.

2. General Guidelines

2.1 Circulating blood volume of the typical laboratory rodent

The limit for blood sampling without volume replacement is 10% of circulating blood volume. The circulating blood volume of the typical mouse and rat at various time points are provided as reference in the tables below.

Table 1: Circulating Blood Volume of the Typical Laboratory Rodent

	Mouse	Rat
Circulating blood volume (ml/kg)	72	64
Circulating blood volume (ml/100g)	/	6.4
Circulating blood volume (ml/10g)	0.72	/
e.g. 25g mouse	1.8	/
e.g. 250g rat	/	16

Table 2: Volume of Blood Available for Sample in Relation to the Sampling Recovery Period for the Typical Laboratory Rodent

	Mouse 25g	Rat 250g	Single sampling recovery period	Multiple sampling recovery periods
Blood volume (ml)	1.8	16	/	/
7.5% blood volume (ml)	0.1	1.2	1 week	1 week
10% blood volume (ml)	0.2	1.6	2 weeks	2 weeks
15% blood volume (ml)	0.3	2.4	4 weeks	2 weeks
20% blood volume	0.4	3.2	/	3 weeks

Table 3: Approximate Blood Sample Volumes for a Range of Body Weights in Mice and Rats

Body weight (g)	CBV* (ml)	1% CBV (ml) every 24 hours**	7.5% CBV (ml) every 7 hours**	10% CBV (ml) every 2 weeks**
20	1.1-1.4	0.011-0.014	0.082-0.105	0.11-0.14
25	1.37-1.75	0.014-0.018	0.10-0.13	0.14-0.18
30	1.65-2.10	0.017-0.021	0.12-0.16	0.17-0.21
35	1.93-2.45	0.019-0.025	0.14-0.18	0.19-0.25
40	2.20-2.80	0.022-0.028	0.16-0.21	0.22-0.28
150	8.25-10.50	0.082-0.105	0.62-0.79	0.82-1.0
200	11.00-14.00	0.11-0.14	0.82-1.05	1.1-1.4
250	13.75-17.50	0.14-0.18	1.0-1.3	1.4-1.8
300	16.50-21.00	0.17-0.21	1.2-1.6	1.7-2.1
350	19.25-24.50	0.19-0.25	1.4-1.8	1.9-2.5

* Circulating Blood Volume
** Maximum sample volume for that sampling frequency

2.2 Restraint

Physical restraint is required during blood collection as movement may cause damage to blood vessels or other complications. Please refer to *10- Restraint of Laboratory Animals Guidelines* of the handbook for guidelines on restraint of laboratory animals.

2.3 Anaesthesia

Anaesthesia is required for blood collection techniques such as retro-orbital sinus and cardiac puncture. These techniques are likely to cause pain and distress to the animals and can cause serious complications without anaesthesia.

Refer to *07- General Anesthesia, Analgesics and Sedatives of Laboratory Animals Guidelines* of the handbook for guidelines on restraint of laboratory animals for additional information on anaesthesia.

2.4 Fluid replacement

Animals with over 10% of circulating blood volume removed should receive fluid replacement. Isotonic solution of either Lactated Ringer's Solution or 0.9% Sodium Chloride solution can be used. These fluids can be warmed and given intraperitoneally or subcutaneously. Mice can receive 1ml IP or SC and rats can receive 5-10ml fluid half via IP and half via SC route.

2.5 Nutritional supplementation

When large volumes of blood are sampled, especially with repeated sampling, rodents will benefit from dietary supplement such as Nutrical or diet gel.

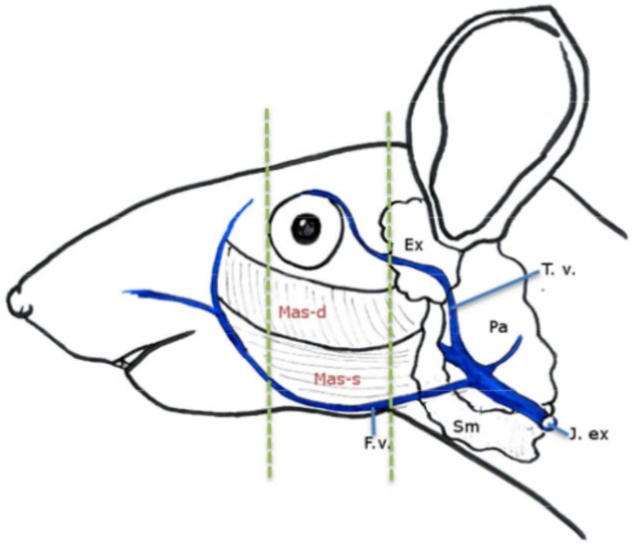
3. Recommended Methods [Rodent Specific]

Recommended methods for blood collection in mouse and rat are discussed below.

3.1 Facial vein (F.v.) sampling

This method collects blood from the maxillary facial vein. It is a safe and fast technique in mice that require short restraint and allows collection of ~200ul blood from a healthy adult mouse. See **Figure 1** for anatomical features required for this technique. Materials needed include a 20g or 22g hypodermic needle or lancet manufactured for blood collection, blood collection tubes and gauze.

Figure 1: Anatomical overview for blood sampling in laboratory mice



3.2 Lateral tail vein or ventral tail artery sampling

Depending on the selection of blood sampling location, from a vein or artery, the sampling volume can vary from small, and medium to large respectively. Blood collection from the artery yields a larger volume but special care is required to ensure adequate hemostasis.

For both mice and rats, blood can be collected by cannulating (using a needle) or by superficially nicking the vessel perpendicular to the tail. The downside of nicking the vessel is the high probability of poorer sample quality. Sample collection using a needle can minimize contamination thus improve quality but can be more difficult to perform in the mouse. Blood collect using a needle does not require general anaesthesia but it is important for the animal to be properly restrained. Warming of the tail can increase obtainable volume with blood vessel's dilation.

3.3 Saphenous vein sampling (medial or lateral approach)

This method allows collection of around 5% of circulating blood volume from rats and mice. Anaesthestic is not required for this method, hence, highly appropriate for pharmacokinetic studies.

The lateral saphenous vein is located at the level of the tarsal joint. It can be easily visualized when the fur is shaved and wiped with alcohol. The animal can be immobilized using a restrainer for the operator to extends the hind leg. The vein is raised by gentle pressure above the joint and the vessel is punctured using 25–27 g for rats and mice. A microhematocrit can be used to collect a drop of blood at the puncture site for small volumes. After blood has been collected, apply pressure over the site to stop further bleeding. Removal of the scab will enable serial sampling. There are limited complications from blood collection with this method other than persistent (minor) bleeding.

3.4 Jugular vein sampling (limited to rat)

This method can yield medium to large blood volume and results in high quality sample. Anaesthesia is recommended to facility the procedure.

3.5 Retro-orbital sinus/plexus sampling

This method can be used in both rats and mice. Blood is collected by using a glass capillary tube to penetrate the retro-orbital sinus(mice)/plexus(rats).

References

NIH Office of Animal Care and Use: Guidelines for Survival Blood Collection in Mice and Rats. Revised 2022. https://oacu.oir.nih.gov/system/files/media/file/2022-12/b2-Survival_Blood_Collection_Mice_Rats.pdf