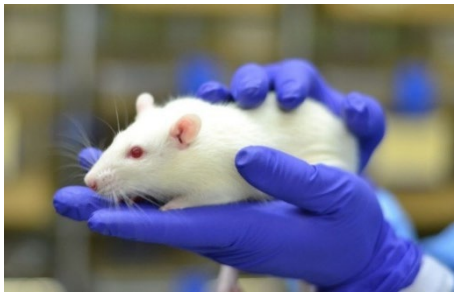
Welfare Guide to Blood Collection from Rodents

Overview:

This guide supports investigators and Animal Ethics Committees (AECs) in understanding the welfare and research impacts of commonly performed blood collection techniques and what can be done to provide support to the animals. It should be read alongside the relevant procedure-specific Standard Operating Procedures (SOPs), which include detailed guidance on safe blood volumes and recovery periods (not covered in this guide).



Reminder: all blood sampling methods, frequencies, and volumes must be detailed in approved animal ethics applications. Researchers must demonstrate consideration of welfare impacts and outline strategies to minimise pain and distress when designing experimental models.

1. Things to consider when choosing a blood sampling technique

All blood collection techniques carry some degree of risk to the animal. Pain and distress can be minimised by selecting the method that causes the least tissue damage and ensuring that sampling is performed by suitably trained personnel. Procedures should be completed as efficiently as possible in conscious animals to limit stress-induced physiological changes including a risk of excessive bleeding because of hypertension. When repeated sampling is required, using positive reinforcement (treats, pats and tickles) can help create a favourable association with the procedure.

The following items must be considered when selecting a blood collection procedure:

- The minimum blood volume required, the frequency of blood sampling and the total number of samples to be collected over the project i.e. the overall blood loss and required recovery time (see Appendix 1 and procedure-specific SOPs).
- The health status of the animals (e.g. collecting maximum allowable volumes only from healthy animals, consideration of age, pregnancy or strain-specific limitations; See Table 1)
- The welfare impacts of each procedure – blood collection procedures vary in their relative welfare impact and the refinements needed to minimise pain and distress (e.g. use of general or local anaesthesia)
- The influence of blood sampling technique on sample quality (e.g. higher haematocrit is typically observed in tail blood compared to retro-orbital blood; See Table 2)
- The availability of suitably trained personnel to perform particular techniques.
- The requirement for post-procedure supportive care (e.g. fluid replacement for large blood collections, nutritional support via hydrogel or mash; See section 4).

Always select the method with the **lowest cumulative welfare impact**. If a higher-impact method appears necessary, the experimental design should be reviewed to determine whether modifications could allow use of a less invasive technique.

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Table 1. Key animal health considerations when selecting an appropriate blood collection route

Factor	Key health considerations
Strain	• Strain-specific stress responses, vessel visibility, clotting and healing vary by strain e.g. BALB/c mice are highly stress-responsive, immunodeficient strains may have delayed haemostasis and dark-pigmented strains hinder vessel visualisation
Age	• Juveniles have fragile, narrow vessels • aged animals have reduced physiological reserve and higher risks from blood loss, temperature disturbance and anaesthesia
Sex	• Females have lower absolute blood volume and increased hormonal variability
Disease status	• Disease may reduce tolerance to blood loss or anaesthesia. E.g. Anaemia, tumours, and renal, hepatic or cardiovascular disease typically increase the risk of more invasive procedures
Obesity	• Poor landmark visibility, deeper vessels and reduced cardiopulmonary reserve. • Blood volume often overestimated based on body weight
Pregnancy	• Reduced circulating reserve, especially late gestation - Blood loss risks maternal hypotension and foetal compromise

Table 2. The choice of blood collection procedure may influence sample quality

Sampling considerations	Sample quality impact
Sample collection time	• Prolonged collection times associated with some techniques can affect sample haematocrit readings and blood chemistry values
Technique proficiency	• Inadequate skill increases stress and the risk of complications such as haemolysis, oversampling, tissue damage, and stress-related physiological changes.
Stress	• Stress due to handling and restraint can change some blood biochemistry parameters.
General anaesthetics	• General anaesthetics can cause respiratory depression and respiratory acidosis; • Anaesthetic substances can have effects on blood chemistry e.g. ketamine may cause elevated blood glucose and corticosterone levels.
Catheterisation	• Indwelling catheters may cause a local inflammatory response, bacteraemia and promote clotting. • Flushing catheters may lead to contamination of the sample with the flushing agent.
Contamination of samples	• Choice of technique can result in tissue fluid contamination of sample (e.g. retro-orbital bleeds) • Poor technique may lead to microbial or tissue fluid contamination of the sample

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2. Welfare Implications of Commonly Used Blood Collection Techniques

Table 3. Rodent Blood Collection Techniques – Summary

Method	Typical Volume*	Anaesthesia	Repeat bleeds (subject to total blood volume collection approval)	Pros	Cons / Welfare Challenges
Tail tip 'prick'	Small: <0.05 mL (mice) <0.1 mL (rats)	Not required	Multiple and frequent - within 24 hours	Quick and reliable Minimal tissue trauma Minimal risk of post-procedure bleeding	Limited blood volumes obtainable Risk of damage to the tail by 'overmilking'
Tail vein 'nick'	Small: <0.05 mL (mice) <0.1 mL (rats)	Not required	Multiple and frequent - within 24 hours. Multiple samples can usually be taken by removing the clot.	Quick and reliable Minimal tissue trauma Minimal risk of post-procedure bleeding	Limited blood volumes obtainable Risk of damage to the tail by 'overmilking'
Tail tip (amputation)	Small: <0.05 mL (mice) <0.1 mL (rats)	Local or general anaesthesia required for animals >21 days old	Removal of the scab to collect blood can be performed multiple times. Note: Repeat 'trimming' of the tail must be limited to total removal of less than 1mm.	Quick and reliable;	**Not recognised by NHMRC as a suitable method for blood collection**; - a form of soft tissue mutilation; Results in permanent tail damage and risk of painful neuroma development; Mixed arterial/venous samples – resulting in different blood values compared to other methods. Variable sample quality (tissue fluid contamination); Risk of damage to the tail by 'overmilking' There should be clear limits on approval for not just the amount of tail removed (<1mm) but repeated cutting of the tail.
Saphenous vein	Small-medium: <0.2 mL (mice) ~0.3 mL (rats)	Not required but may be of benefit	May be performed as frequently as 4 times a day (alternating sides) but typically recommended no more than twice a week to allow for healing and reliably obtain a sample. Can use indwelling catheters for rats	**NHMRC preferred method of blood collection in rats and mice (along with facial bleeds in mice)** Reliable collection volumes; No general anaesthesia required Low potential for tissue damage	Relatively technically challenging; Takes longer and could cause more stress in mice compared to Facial bleeds May result in variable sample quality (due to clotting of larger samples) and quantity Risk of haematoma, tissue reactions, bruising and lameness.

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Lateral tail vein	Small-medium: <0.2 mL (mice) ~1 ml (rats)	Not required, but local anaesthetic cream should be considered	Depending on the volume, one or two samples per session (within 24 hours).	The vein is easily accessible in unpigmented animals; Less stressful than facial vein bleeds (according to cortisol studies); Low potential for tissue damage	Must be securely restrained (may not suit all strains of mice) Longer draw time compared with saphenous or facial bleeds Low visibility in black or pigmented mice Vasodilation (warming) may be necessary; animals must be monitored for heat stress
Facial Bleed (Submandibular) - Facial Vein or Superficial Temporal Vein (STV)	Medium–large: 0.15–0.2 mL (mice only)	Not required	Typically limited to twice weekly (once per face side)	**NHMRC preferred method of blood collection in mice** (along with Saphenous bleeds) Large collection volume possible; Rapid; Suitable for repeat bleeds (alternate sides) STV bleeds may cause the least damage	Adult mice only; Limited collection frequency; Risk of bleeding/haematoma; STV bleeds are technically more difficult particularly in dark mice, and result in smaller samples.
Retro-orbital / Retrobulbar Sinus	Large: up to 0.4 mL (recovery bleed in mice) Up to 2.0 mL (rats)	Required (unless otherwise AEC approved)	Twice weekly alternating between eyes Maximum total of 4 bleeds (2 per eye) Some sources recommend not repeating in the one eye within 2 weeks.	Rapid; Large samples possible;	**Generally controversial (ref. NHMRC Guideline)** Highest complication risk - eye and nerve injury; Typically, should only be performed by animal house technicians Greater risk of damage in rats so should only be performed with strong justification; Longer recovery between samples; Risk of contamination from tissue fluids;
Jugular vein	Large: up to 0.4 mL (mice) Up to 2.0 mL (rats)	Usually performed under anaesthesia	Can be cannulated	Low potential for tissue damage Suitable for repeat bleeds (using a catheter) High quality samples	Not typically used in mice at UQ but sometimes used in rats. No UQBR SOP or training.

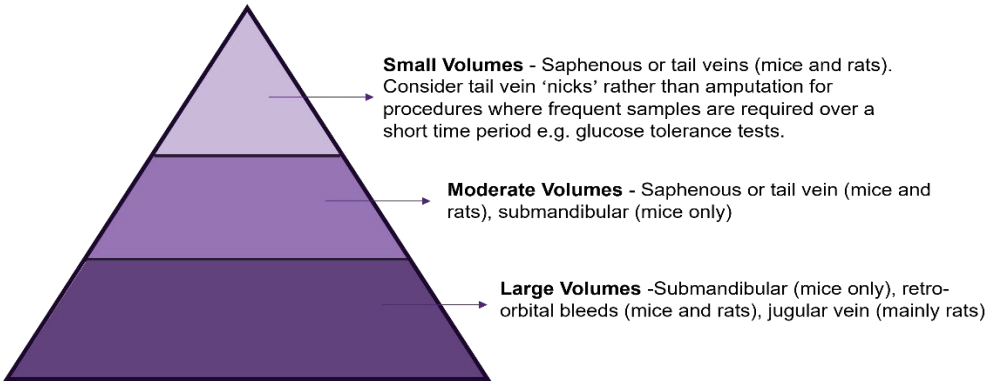
*Typical volume: The values presented are the maximum typically obtainable from a single sample using that method, NOT recommended maximum blood collection volumes. For guidance on selecting an appropriate sampling volume, refer to the relevant blood collection SOPs. See Appendix 1 for total blood volume collection guidelines.

Other blood collection techniques such as submental, sublingual, pedal and ventral tail artery bleeds are not typically used at UQ and there are no UQBR SOPs or training for these methods. For these reasons they have not been included in this table.

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3. Balancing sample needs and animal welfare

The choice of blood collection technique can be managed to achieve both the scientific outcome of the study and to have the lowest welfare impact on the animals. In the below example, required blood volume is considered against the relative welfare impact of the collection procedure. This example serves as a guide only; specific project considerations must be taken into account when deciding on which technique to use.



There are times when a 'higher impact' technique will result in better research outcomes in which case there should be additional monitoring and consideration of procedure refinements to support animal welfare (Section 4).

4. Refinements to reduce the welfare impact of blood sampling techniques on animals

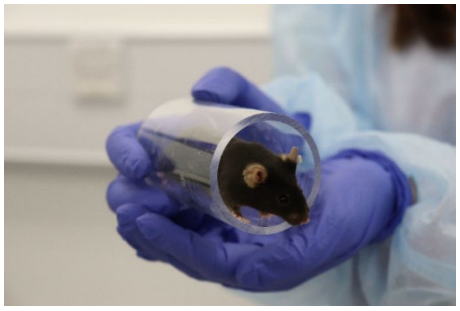
I. Training

Poor sampling technique compromises animal welfare, blood sample quality and research results. Training should begin with inanimate objects (e.g. oranges) to develop needle and syringe handling skills then culled animals (euthanised for other purposes) before working with live animals. UQBR provides training in standard blood collection techniques, and trained UQBR staff can perform blood collections on request.

II. Handling and Restraint

Handling should be as refined as possible, with tunnel or cupping methods preferred to reduce stress. Restraint must be firm but empathetic; poor restraint prolongs the procedure, increases risk and welfare impacts, and compromises sample quality.

Manual restraint can be better than restraint devices, particularly for warmed animals as confinement increases the risk of syncope and thermal stress. If restraint devices are used, they must be correctly sized and adjusted for the individual animal and cleaned between animals to prevent stress or cross-infection.



Animals should be habituated to blood collection procedures in advance, including mock sessions with restraint devices and where appropriate, food rewards – check first that these will not create an unwanted variable in the model. Training can be achieved in a few short sessions and improves animal welfare, handler experience, and sample quality. Well-habituated animals may be sampled with minimal or no restraint. For further information, refer to the NC3Rs website.

III. Fluid replacement

For medium to major bleeds (≥10% blood volume in a single sample or >15% over two weeks), replacement fluids should be given to reduce physiological impact e.g. if taking around 0.18mL from a 25g mouse or 1.6 mL from a 250g rat.

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Fluids are given via subcutaneous injection in the scruff of the neck. Sterile saline or lactated Ringer's solution should be warmed to body temperature (~38 °C) and administered immediately after blood collection. The recommended volume is one to two times the blood removed. To minimise pain and skin distension, large volumes should be divided across multiple sites, with a maximum of ~5 mL/kg per site (≈0.125mL per site for a 25g mouse).

IV. Mash

Giving mash both prior to and after bleeds helps to support hydration and recovery.

V. Warmth

Consider using warming pads, heat lamps, or warmed bedding before and after moderate to major bleeds. Animals must be monitored to avoid overheating (those nearing the humane endpoint of a study will be particularly sensitive to both hypothermia and hyperthermia) and note that warming methods are contra-indicated for pregnant animals.

VI. Analgesia

Topical anaesthetic creams applied 30–60 minutes before venipuncture have been shown to significantly reduce discomfort in dogs, cats, and rabbits. The evidence is less clear with rodents however in the interest of animal welfare it is something that should be considered and used if practical. Many institutions now use anaesthetic creams routinely before tail vein bleeds for example.

VII. Needle size

Needle size should be selected with care. Evidence suggests that a larger-bore needle relative to the vein may not cause more stress than a smaller one, as it allows faster sampling and shorter handling time. If you are unsure, consult with someone who has further experience in that blood collection method.

VIII. Haemostasis

It is important to ensure effective haemostasis with any blood collection technique to minimise blood loss and bruising. Apply gentle pressure to the sampling site with a swab or tissue until bleeding stops and monitor the animal closely after their release so that you can respond immediately if bleeding restarts.

IX. Proactive Monitoring

Frequent or long-term sampling increases the risk of developing anaemia and so regular monitoring is important. Signs of chronic anaemia include pale mucous membranes, reduced activity, exercise intolerance, increased respiratory rate, and loss of muscle mass. For models where there is a risk of anaemia developing, signs of this should be included in the scoresheet associated with that experimental work. If unsure of what signs should be monitored, contact the UQBR Vets (br.vetservices@uq.edu.au) or animal ethic veterinary officer (ae.welfare@uq.edu.au).

X. Catheterisation for repeat blood sampling

This guide does not cover catheterisation (IV cannulation) but the NC3Rs website has a practical section on the potential improved welfare outcomes with cannulation.

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Appendix 1

Calculation of blood collection volume

Blood collection volume is generally calculated as a percentage of total blood volume. This is the clinically relevant value. The average blood volume of a mouse is 7% of body weight where 1mL of blood is assumed to be equivalent to 1g of body weight.

Example calculation to obtain 10% of the total blood volume of a 20gram mouse
total blood volume is 7% of 20g
0.07 x 20 = 1.4mLs
10% of total blood volume is 0.10 x 1.4 = 0.14 mL i.e. 140µL

Recommended blood collection volumes based on a mouse's live body weight (NHMRC 2008)

Mouse Weight	**TOTAL BLOOD VOLUME (TBV)** *[equates to 5-7% of body weight]*	**Minor bleed** *(<7.5% of TBV)*	**Moderate bleed** *(7.5-10% of TBV)*	**Major Bleed** *(10-15% of TBV)*
Minimum Recovery period required between bleeds:		1 week recovery	2 weeks recovery	3 weeks recovery
18g	1.2mL	<90uL	90-120uL	120-180uL
22g	1.5mL	<115uL	115-150uL	150-225uL
26g	1.8mL	<140uL	140-180uL	180-270uL

Table and blood calculation information from UQBR Blood Collection SOPs.

Note on minimum recovery periods: These are based on the volume of blood lost to the animal (as compared to recovery of the sampling site). All blood lost during the procedure counts toward the total blood volume collected from the mouse and must be considered when determining whether the next AEC-approved collection timeframe can proceed.