ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

BLOOD COLLECTION IN MICE AND RATS

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.

V1 Date of Approval:	11 July 2016	**Date of Review:**	11 July 2019

1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

- General anaesthesia of mice and rats
- Surgery and aseptic technique of mice and rats
- Analgesia
- Handling and restraining mice and rats
- Injections in mice and rats

2. SUMMARY

Blood may be collected to analyse biochemical, metabolic, toxicological, immunological or physiological changes. Some pathogens and drugs may also be detectable via blood samples.

3. BENEFITS & RISKS

3.1 Blood collection and analysis can assist in providing data for a study or determining the health status of an animal.

3.2 The method, frequency and volume of blood collected can all impact on the pain and distress imposed on an animal. Pain and distress may be caused by handling, restraint, venepuncture, blood loss, thrombosis, bruising and inflammation of the vein.

3.3 Heating devices used in some species prior to blood collection can potentially cause thermal damage to animals.

4. PROCEDURE/PROTOCOL

4.1 Training

4.1.1 Trainees must be assessed as competent in handling and restraint of the relevant species prior to commencing training in blood collection. Training specific to the procedures described in this Standard must be undertaken and the investigator must be deemed competent.

4.1.2 Practical trainers should provide the content, format and/or competency assessment sheets to the Animal Welfare Officer (AWO) prior to commencement of training. Trainers should have been observed delivering training to an appropriate standard as determined by the AWO.

4.2 Planning

4.2.1 The location selected for blood collection must comply with Table D4 of the NHMRC Guidelines (2008) where appropriate – refer to Appendix I.

4.2.2 The needle gauge selection should consider the potential for haemolysis and pain and distress. Larger gauges may reduce pain and distress in some situations due to quicker collection times. The following needle gauges must be used:

Vein (species)	**Gauge**
Submandibular (mice)	18-23G
Saphenous and tail (mice)	25-27G
Tail (rats)	23-26G
Saphenous (rats)	23-26G

4.2.3 The lancet gauge must be selected as outlined in 4.2.2, with the following lengths provided as a guide only. The length used will vary with animal size, age, site of use and volume of the required sample.

Lancet length	**Age of mice**
3mm	For 1 to 2 drops only (any age)
4mm	<2 months
5mm	2 to 6 months
5.5mm	>6 months

Lancet length	**Age of rat**
5.5mm or 6mm	< 3 months
6mm or 7mm	3 to 4 months
7mm or 8mm	> 5 months

4.2.4 Blood volume can be estimated as 70 mL/kg (NHMRC, 2008). Table D2 in the NHMRC Guidelines (2008) must be used to determine the recovery period after blood collection (Appendix II). Blood collection on any occasion must not exceed 15% of blood volume. Example calculations are shown here:

- 0.025 kg (25 g) mouse x 70 mL/kg = 1.75 mL total blood volume in mouse
  1.75 x 7% = 0.123 mL (123 µL) volume collection requires 1 week recovery
  1.75 x 10% = 0.175 ml (175 µL) volume collection requires 2 week recovery

- 0.25 kg (250 g) rat x 70 mL/kg = 17.5 mL total blood volume in rat
  17.5 x 7% = 1.23 mL volume collection requires 1 week recovery
  17.5 x 10% = 1.75 mL volume collection requires 2 weeks recovery

4.2.5 The pathology laboratory should be consulted to determine the appropriate tube for collection. As a general guide the following can be used:

Tube	**Test**
Clotted (plain)	Serology (antibody/antigen)
Lithium heparin	Biochemistry, trace elements, vitamin A & E
EDTA	Haematology, thiamine, PCR
Sodium Fluoride	Glucose

4.2.6 Where EDTA is indicated, it is beneficial to coat the inside and outside of the needle with EDTA prior to venepuncture.

4.2.7 Where haematology is performed a blood smear is advisable to assist with analysis.

4.3 Animal preparation

4.3.1 Most species will benefit from conditioning to regular handling prior to restraint for blood collection. Positive rewards such as treats or petting can assist in conditioning and at the time of blood collection.

4.3.2 Where the site of collection contains hair and restraint stress is unlikely to be exacerbated, the hair should be clipped or removed.

4.3.3 A solution of 80% alcohol (v/v) should be used on the site prior to collection. Chlorhexidine followed by water is an alternative.

4.3.4 Investigators should refer to the Analgesia Standard to determine indications for local analgesia.

4.4 Repeated attempts

4.4.1 No more than three skin punctures may be made in the one attempt to collect blood for scientific purposes. For submandibular bleeds, only one attempt per cheek (a total of 2) is permitted.

4.4.2 The Animal Facility Manager (AFM) or AWO must be notified if more than one in every ten animals requires three attempts or more than three in every ten animals requires two attempts.

4.5 Collection

4.5.1 Blood may be collected by puncture of a blood vessel, from the heart under general anaesthesia as a terminal procedure, or via catheters.

4.5.2 Submandibular/facial vein collection in MICE (preferred route for mice)

4.5.2.1 Shaving of hair is not done with this technique due to the proximity of venepuncture to vital structures and the need to minimise restraint time.

4.5.2.2 The mouse is grasped by the base of the tail and placed on a rough surface such as the cage lid. A one or two handed technique can be used.

4.5.2.3 Gentle rear traction is applied to the tail.

4.5.2.4 The thumb and forefinger (of the other hand if using two hands) are used to grasp the scruff of the neck extending down the back. The scruff grip should include some flank skin and extend to the base of the ears which is more than with routine restraint. A more extensive scruff grip is required to ensure minimal mobility of the head.

4.5.2.5 There is a higher risk of airway/blood vessel pressure from the operator with this technique compared to routine restraint. Respiration and pink areas of skin should be closely monitored during and after the procedure. Trainees should be closely observed to ensure the scruff grip is not too loose or tight.

4.5.2.6 The puncture location is determined by extending an imaginary vertical line down from the lateral canthus of the eye to the point where it meets the mandible. A needle of 18-23G or a lancet may be used for puncture.

4.5.2.7 Once blood has been collected, the scruff should be slightly loosened and pressure should be applied to the puncture location for a few seconds.

4.5.2.8 It is important to minimise the time of restraint to prevent respiratory problems. Maximum restraint time should be 1 minute, or less if the mouse is showing signs of distress.

4.5.3 Warming and lateral tail vein collection in MICE and RATS

4.5.3.1 Heating assists in dilation of the veins and can be done via direct exposure to a lamp (maximum 10 minutes at 40°C), inside a heating box (maximum 10 minutes at 40°C), or by dipping the tail in warm water (maximum 1 minute at 45°C). It is important to assess the temperature at the location of the animal, particularly where there is potential for adjusting the distance between the heat source and animal (eg. heat lamps). Burns and hyperthermia are potential adverse impacts associated with warming at high temperatures or for extended periods.

4.5.3.2 An independent thermometer must be used to validate the temperature of the warming device.

4.5.3.3 Animals must be observed constantly during heating for signs of hyperthermia and pain including: respiration rate, salivation, altered activity/ambulation. If these signs are observed the animal should be immediately removed from the heat source and placed in a darkened box for additional observation. Oxygen should be provided by mask or into the box if available until respiration returns to normal. The animal should be allowed to fully recover (seen as normal activity, respiratory rate and effort) and blood collection should not be attempted until at least 2 hours after recovery. Caution should be exercised for any future warming attempts in affected individuals.

4.5.3.4 Restrainer size should be small enough to prevent the animal from turning around but large enough to allow normal respiration. The technique for insertion of the animal in the restrainer will depend on the type of restrainer.

4.5.3.5 The tail should be held in the distal third, extended, and rotated 90° to place the vein dorsally. Ensure the tail is not pulled as this may cause trauma injury.

4.5.3.6 The tail is disinfected and the needle is placed into the vein at a 15-20° angle starting approximately one third from the end of the tail. The bevel should face up and the needle is inserted only far enough so that the bevel has entered, or 2-4 mm. Blood can be collected with a capillary tube or syringe. Where inserting the needle is unsuccessful, a 23G needle or a scalpel blade can be used to nick the vein. However, due to the increased risk of trauma to the tail if this procedure is performed incorrectly, a scalpel blade should only be used by competent investigators.

4.5.3.7 Pressure should be applied to the tail to stop bleeding for at least 30 seconds.

4.5.4 Saphenous vein collection in RATS (preferred route for rats)

4.5.4.1 1-2 mm of local analgesia cream may be applied 30-60 minutes prior to the procedure.

4.5.4.2 Restraint can be done with the hands, a restraint tube, or a towel ensuring a hind limb is exposed.

4.5.4.3 The selected limb is extended by pushing just above the knee joint. The same hand is usually used to place pressure on the proximocranial portion of the limb to dilate the saphenous vein.

4.5.4.4 Hair is removed and antiseptic applied to the lateral side of the limb between the ankle and knee.

4.5.4.5 A 23-26G needle is used at 15-20° with the bevel facing up and is advanced until the bevel has entered or 2-4 mm.

4.5.4.6 Pressure should be applied to the venepuncture site to stop bleeding for at least 30 seconds.

4.6 Post-collection sample care

4.6.1 Rapid temperature changes, moisture and rough handling should be avoided.

4.6.2 Where a syringe is used for collection the needle should be removed before expressing blood into the tube to prevent haemolysis.

4.6.3 Samples should be left to cool to room temperature before refrigeration.

4.6.4 Plain tubes should be left to clot whilst anticoagulant tubes (eg. EDTA) should be gently tilted from end to end.

4.6.5 Haematology samples should be delivered to the testing laboratory within 24 hours and biochemistry within 48 hours.

5. MONITORING & INTERVENTION

5.1 Monitoring must be done for the first 5 minutes immediately after blood collection and include: assessment for further bleeding, activity, skin colour (for blue cyanosis) and respiration rate. If abnormal signs occur during restraint or collection the animal should be immediately released.

5.2 Monitoring of animals that may be prone to anaemia from blood loss can include: haematology (packed cell volume, haemoglobin, red cell count, reticulocyte count), blood pressure, mucous membrane colour, weight loss (chronic), activity levels and respiration rate. Animals in this category would include those where more blood than anticipated was lost or for repeat sampling within 10% of the NHMRC maximum amounts and frequencies (eg. 6.3-7% blood volume collected weekly, 9-10% blood volume collected every 2 weeks).

Monitoring of these animals must be a minimum of twice weekly including the day after blood collection. Twice daily monitoring should be performed for those with expected clinical signs of anaemia.

5.3 Animals with moderate signs should be given parenteral fluids and monitored twice daily. For rodents this can be 3 to 4 mL of warm sterile fluids (0.95% NaCl or Lactated Ringer's) per 100 g body weight by subcutaneous injection. Animals with severe signs should be euthanased.

6. ADDITIONAL INFORMATION

6.1 The APN Histopathology service provides Complete Blood Count/Differentiated Blood Count/Reticulocyte Count for rodents as part of a comprehensive whole animal histopathology service: http://www.apn-histopathology.unimelb.edu.au/

6.2 The University of Melbourne Veterinary Hospital provides blood services for many species: http://www.u-vet.com.au/services/clinical-pathology-lab

6.3 NHMRC, 2008. Guidelines to promote the wellbeing of animals used for scientific purposes. (Table D2 & D4 referenced in Standard): https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/ea18.pdf

7. ENFORCEABLE REQUIREMENTS

7.1 Performance of the procedure by competent investigators or trainees under the direct supervision of competent investigators.

7.2 Table D2 (Appendix II) of the NHMRC Guidelines utilised to determine maximum volumes and frequency.

7.3 Hair removal and 80% alcohol (v/v) or chlorhexidine applied (except for submandibular collection).

7.4 Contact the AFM or AWO if three unsuccessful attempts on an individual animal.

7.5 Pressure applied to site after collection.

7.6 Monitor the animals as above for a minimum of 5 minutes post collection.

7.7 Twice weekly monitoring of animals where repeat samples are collected and volume/frequency are within 10% of NHMRC Guidelines.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.

9. UNEXPECTED ADVERSE INCIDENTS

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University's AWO and relevant AFM must be notified of the event. The AWO will advise researchers of the appropriate response.

10. GLOSSARY

Scientific Term	Lay Description
Canthus	Corner of the eyelids.
Haemolysis	Destruction of red blood cells. Can be caused by blood collection with a needle that is too small.
Mandible	Lower jaw bone.
Proximocranial	Towards the body on the side directed towards the head.
Saphenous vein	A vein that runs close to the surface of the skin, on the outside of the lower limb just above the hock (ankle).
Venepuncture	Puncture of a vein performed for blood collection or intravenous administration.

11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- Department of Agriculture and Food, Government of Western Australia. Selecting the right blood test tube and techniques for an accurate diagnosis.
- Harkness, J.E., Turner, P.V., and VandeWoude, S. (2010). Harkness and Wagner's Biology and Medicine of Rabbits and Rodents. pp113-114.
- Medipoint International. Available: http://www.medipoint.com/
- National Centre for the Replacement, Refinement & Reduction of Animals in Research. Blood Sampling. Available: https://www.nc3rs.org.uk/our-resources/blood-sampling
- National Health and Medical Research Council (NHMRC). 2008. Guidelines to promote the wellbeing of animals used for scientific purposes.
- Parasuraman, S., Raveendran, R., and Kesavan, R. (2010). Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother,* 1(2): 87-93.

Appendix I. Wellbeing issues to consider for blood collection in mice and rats (taken from Table D4 – NHMRC, 2008)

Species	Routes of blood collection	Preferred route	Suitable for repeat bleeds	Volume of blood able to be collected	Wellbeing			Reference
					Potential for tissue damage	Anaesthesia required	Comment	
Mouse	Anterior facial vein or submandibular puncture	Yes	Yes (consider alternating sides)	+	Low	No	Needle used to puncture vein or a lancet may be used.	Gole et al (2005)
	Lateral tail vein		Yes	+ or ++	Low	No	Vasodilation may be necessary to promote bleeding by applying warmth to the tail. Animals must be monitored for heat stress.	Diehl (2001)
	Saphenous vein		Yes (consider alternating sides)	+ or ++	Low	No, although anaesthesia is recommended	Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed.	Diehl (2001); Hem et al (1998); Flecknell et al (1990); Lumley et al (1990)
	Cardiac puncture		No	+++	Moderate	Yes, general	Can lead to pericardial bleeding and cardiac tamponade. Should only be used for terminal bleeds.	Diehl (2001)
Rat	Jugular vein		Yes	+++	Low	Yes, general	Suitable for serial sampling using a catheter	Diehl (2001); Flecknell et al (1990)
	Lateral tail vein		Yes	++(+)	Low	No	Vasodilation may be necessary to promote bleeding by applying warmth to the tail. Animals must be monitored for heat stress.	Diehl (2001)
	Saphenous vein	Yes	Yes (consider alternating sides)	++(+)	Low	No	Monitor the site for tissue reaction. The site should be changed if site becomes red, swollen or inflamed. Consider the use of indwelling catheters for repeated sampling.	Diehl (2001); Hem et al (1998); Flecknell et al (1990); Lumley et al (1990)
	Cardiac puncture		No	+++	Moderate	Yes, general	Can lead to pericardial bleeding and cardiac tamponade. Should only be used for terminal bleeds.	Diehl (2001)

Note: +, volume in the order of 0.1 mL; ++, volume in the order of 0.1–1.0 mL; +++, volume in the order of more than 1.0 mL

References:

- Diehl KH (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of Applied Toxicology* 21:15–23.
- Flecknell PA, Liles JH and Williamson HA (1990). The use of lignocaine–prilocaine local anaesthetic cream for pain-free venepuncture in laboratory animals. *Laboratory Animals* 24:142–146.
- Gole WT, Gollobon P and Rodriguez LL (2005). A rapid, simple and humane method for submandibular bleeding of mice using a lancet. *Laboratory Animals* 43:39–43.
- Hem A, Smith J and Solberg P (1998). Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink. *Laboratory Animals* 32:364–368.
- Lumley JSP, Green CJ, Lear P and Angell-James JE (1990). Essentials of Experimental Surgery, Butterworths, London.

Appendix II. Maximum volumes and recovery periods for blood collection (Table D2 – NHMRC, 2008)

Period of collection	% of blood volume collected	Approximate recovery period in weeks
Single bleed	Up to 7% (minor bleed)	1
	10% (moderate bleed)	2
	15% (severe bleed)	3
Over a 24–hour period	Up to 7%	1-2
	10%	2-3
	15%	4-6

Note: These recommendations make no allowance for pregnancy, lactation, illness, or the effects of genetic manipulation. Blood volume can be estimated as 70 mL/kg.