This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Experimentation Ethics Committee (AEEC). It is designed to provide guidance regarding current best practice to institutional animal users and carers on the care and use of animals for scientific purposes. It has been prepared in consultation with the Australian code for the care and use of animals for scientific purposes 8th edition 2013.

Document 024_Blood Collection from Mice Standards_V1.0

Background

Collection of blood from mice is a necessary and frequent procedure performed at the ANU.

The methods used must be suitable for the research outcomes and must ensure minimal possible impact to the animal.

Collection of blood from mice is particularly challenging given their small size and veins can be difficult to access.

Training and competency are key to maintaining high standards of animal welfare when collecting blood samples. Competency assessments must be undertaken as per "AEEC 015_Animal handling and Procedure Training Standards."

The code requires that specific care is taken when performing blood sampling

3.3.7 When performing injections, blood sampling and non-surgical procedures, procedures used must:

> *i) minimise the risk of an animal developing complications (e.g. tissue damage, infection, haematoma, bleeding)*
>
> *ii) be performed under aseptic conditions if there is a potential risk of infection*
>
> *iii) if the procedure involves transplantation of cells or tissues, include management of the effects of tissue rejection and immunosuppression.*

Definitions

The Code: Australian code for the care and use of animals for scientific purposes 8th Edition 2013

Competency: the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments.

General Information and Considerations

Retro-Orbital Eye Bleeding

Training Requirements

Must only be completed by competent individuals and must follow the APF Training Guidelines for this procedure.

This procedure can be highly controversial and therefore the individuals chosen to be trained in this procedure must show a high regard for animal welfare, excellent restraint ability and the skill to adapt their technique where necessary.

Equipment

- Capillary Tube (Heparanised or non-heparanised as required for the experiment)
- Collection container (containing heparin, plain, EDTA or another buffer as required for the experiment)
- NOTE: All equipment should be sterile to minimise the chances of eye infection and complications after the procedure.

Volume

This procedure must only be used for large volumes unless otherwise approved in the protocol (100-200uL). Smaller volumes should be able to be collected from other routes including tail vein with a needle.

Frequency

Retro-orbital blood collection must not be performed more than once per week, preferably once per fortnight. Volumes and frequency are outlined in the maximum bleed amount table below.

Anaesthesia/Restraint Requirements

Anaesthesia is generally recommended however, highly skilled technicians may have approval to perform the procedure without anaesthesia.

The restraint is a manual restraint and requires special training and a high level of competency and confidence. Prior to being allowed to perform this on experimental animals, the operator must be signed off as competent by an approved trainer who is deemed competent by the ANU Veterinarians or other considerably experienced individual.

Special Considerations

If the right eye is damaged but blood is still able to be collected (i.e. the eye is still able to bulge out) it is preferable to use the damaged eye to minimise the risk of reducing the mouse's eyesight further. (NB This may be the opposite for a left handed technician).

Left eye bleeds must only be undertaken by staff that are highly competent in the procedure as it requires a different approach. If the staff member is at all unsure the bleed should not occur. (NB this may be the opposite for a left handed technician).

No more than two attempts are to be made.

As per the AEEC Approved procedure 015_Animal Handling and Procedure Training Standards, assessment of retro-orbital bleed technique must be completed every year.

A maximum of six (6) bleeds can be performed on a single animal over their lifetime.

Tail Vein (needle advanced into the vein)

Training requirements

Individuals must be trained by competent persons in collecting the required volume of blood.

Equipment

Use of needle to penetrate vein is the only acceptable method. Nicking the side of the tail vein with a scalpel blade is not suitable.

Volume

Samples may be 5uL-100uL in volume from the tail vein. Highly skilled individuals may be able to collect 200uL using a needle inserted into the vein.

Frequency

There is a preference for tail cannulation if multiple samples (i.e. 4 or more) required over a 24 hour period unless otherwise approved in the ethics protocol.

Anaesthesia/Restraint Requirements

This procedure does not require anaesthesia but individuals may choose to use anaesthesia for larger volumes and tail cannulation procedure. Mice do need to be restrained in a restraint device.

For larger volumes there may be benefit from warming the tail however this must be done cautiously. Localised warming with a warm (not hot) compress or warming the cage under a lamp may be suitable however animals must not be warmed for more than 5 minutes and must be continuously monitored for any signs of heat stress.

Special Considerations

Any bleeding after 1 minute post collection should be addressed immediately with pressure applied and careful handling. If the mouse continues to bleed veterinary advice should be sought by calling the veterinarian on call on 6125 1130.

No more than three attempts are to be made per animal.

Tail Nick (lateral small incision of the tail vein)

Training Requirements

Individuals must be trained by competent persons in collecting the required volume of blood.

Equipment

The tail vein can be ‘nicked’ with a small sharp instrument to create a small localised bleed.

Use of a needle (25-57G) or small lancet to nick the lateral tail vein is the only acceptable method. **Nicking of the tail with a scalpel blade is not acceptable.**

Volume

A tail nick is used for small volumes of blood up to 10-20uL.

Anaesthesia/Restraint Requirements

This procedure does not require anaesthesia. The mouse must be restrained in a restraint device.

Special Considerations

Repeated small volume collections may be achieved by removing the clot or scab over the previous sample collection point if possible, rather than performing numerous tail nicks.

No more than three attempts are to be made in a single session and animals must be provided with an opportunity to recover and the vessel to heal before further attempts are made.

Tail Tip

Training Requirements

Individuals must be trained by competent persons in collecting the required volume of blood.

Equipment

A sharp, sterile scalpel blade or sharp and sterilised scissors must be used.

Volume

Repeated small volume collections (5-10uL) should be achieved by removing the clot or scab over the previous sample collection point rather than removing additional length of tail.

Frequency

A maximum of 1mm of tail must be removed from a mouse. If a mouse has had a previous tail tip for bleeding or genotyping purposes it must not have further tail removed and can only have a scab knocked off. The scab may be knocked off repeatedly in a small window of time or over a number of days. Once the removal of the scab is no longer sufficient to create the blood collection required an alternative method would need to be used.

Special Considerations

This is not a preferred method for volumes over 5uL, due to the risk of exposing the nerve endings and bone of the tail. Special justification must be included in the approved animal ethics protocol to utilise this technique.

Lateral Saphenous

Although rarely used at the ANU, this is considered an acceptable method of blood collection if performed by competent individuals.

Training Requirement

As this procedure isn't often used at the ANU the individual must be able to demonstrate competency in the procedure as assessed by the ANU veterinarian or a suitable delegate.

Equipment

A needle must be used to penetrate the vein. A capillary tube or syringe with needle is used to collect the blood.

Volume

This procedure is suitable for up to 200uL blood.

Frequency

This procedure should not be performed more than once per week. Volumes and frequency are outlined in the maximum bleed amount table below.

Anaesthesia/Restraint Requirements

This procedure requires restraining the mouse in a suitable restraint device. The vein is punctured with a needle only. This procedure can be used for aseptic blood collection as the skin over the vein can be prepared by shaving the hair and cleaning the skin with chlorhexidine prior to blood collection. You may require two individuals to perform this technique.

Special Considerations

Further bleeding may occur after the required volume is collected. Pressure must be applied at the end of the bleed to ensure adequate haemostasis before removing the animal from the restraint and returning them to the cage.

Submandibular Cheek Bleeding

Although rarely used at the ANU, this is considered an acceptable method of blood collection if performed by competent individuals.

Training Requirement

As this procedure isn't often used at the ANU the individual must be able to demonstrate competency in the procedure as assessed by the ANU veterinarian or a suitable delegate.

Equipment

A sterile needle or lancet must be used to penetrate the vein. A capillary tube or syringe with needle is used to collect the blood.

Volume

This procedure is suitable for up to 200uL blood (noting the frequency restrictions in the Minimum Requirements below).

Frequency

This procedure should not be performed more than once per week at a maximum (noting the frequency restrictions in the Minimum Requirements below).

Anaesthesia/Restraint Requirements

Anaesthesia may or may not be necessary. A good restraint of the mouse is a requirement for this procedure.

Special Considerations

This procedure may result in some contamination of the sample and should not be used where sterile collection is required.

Further bleeding may occur after the required volume is collected. Pressure must be applied at the end of the bleed to ensure adequate haemostasis after releasing the animal and before returning them to the cage.

Terminal Cardiac Bleed

Training Requirement

Individuals must be competent in rodent anaesthesia to be able to undertake this method.

Equipment

Anaesthesia equipment as per the approved animal ethics protocol. A syringe and needle (23-27G) is used to withdraw the blood direct from the heart.

Volume

This is the only method that can be used to withdraw more than 200uL from a mouse as it is a terminal procedure. A skilled individual can collect up to 1ml.

Frequency

This is a terminal procedure and the animal must not be allowed to recover.

Anaesthesia/Restraint requirements

The animal must be deeply anaesthetised to a surgical plane of anaesthesia prior to beginning this procedure. The preferred method is using isoflurane as inhalational anaesthesia as it is fast acting and easy to manage.

Special Considerations

There are a number of methods that can be undertaken. Open cardiac bleeds require the opening of the chest cavity. If this is the preferred method then care must be taken to perform the bleed quickly to ensure that the animal does not suffer from an inability to breathe in the anaesthetic due to lung collapse.

At the end of the procedure the animal must be humanely killed whilst still under anaesthesia by a secondary method (removal of the heart or cervical dislocation are acceptable secondary methods).

Cardiac puncture should not be used if the peritoneum needs to be lavaged to harvest cells as this technique can cause blood contamination of the peritoneum.

Monitoring, Intervention and Reporting

Mice must be monitored for at least 5 minutes after any survival blood collection or until it has fully recovered.

Any mouse that is unwell, small or dehydrated must not be used for blood collection. The investigator must be contacted and the animal must be supported to recovery before blood collection can be considered. The ANU veterinarian should be consulted if there is any concerns over mouse wellbeing.

Any animal that has an injury after blood collection must be attended to immediately. This may include the provision of pain relief, supportive fluid therapy, treatment and veterinary care. The animal's immediate welfare needs must be met and the veterinarian called if there is ongoing concern for the animal's wellbeing.

Any procedure that has been undertaken on an animal must be adequately notated in the animal room. There must be a notation on the animal cage card and further detail available on either the animal management database (e.g. Musterer) or a lab book/record book available in the room (for satellite areas). The records must be able to be understood by anyone inspecting the animals and must include;

- Name of the individual performing the procedure
- Date of the procedure

- Details of the procedure

Minimum Requirements

- The method of blood collection should be described in the approved animal ethics protocol.
- The method of blood collection used should be most suitable for the volume required, the training of the individuals involved and the purpose of the blood use.
- Prior to completing the blood collection the technician or investigator must confirm that they have the correct method, collection vehicles and buffer requirements for the intended experiment.
- The method of blood collection must be the least painful and least stressful for the proposed use.
- All staff and investigators must be adequately trained to achieve competency in the required techniques. The procedure must be undertaken by competent persons or under the direct supervision of a competent person.
- The use of Retro-orbital eye bleeding is restricted to highly trained individuals and training in this technique may not be suitable for all persons and will be determined as per AEEC Approved procedure 015_Animal Handling and Procedure Training Standards.
- The volume of blood collected must be the minimum required to achieve the required aim. This should be clearly stipulated in your animal ethics protocol and you must provide specific justification for any bleed that is between 10-15% of the circulating blood volume.
- For volumes greater than 150uL, small mice must be weighed to ensure the maximum percentage of circulating blood volume of 15% is not exceeded (see Jackson Labs reference).
- Animals must be monitored for a minimum of 5 minutes after blood collection.
  A record of the procedure undertaken must be made in the animal room.
- The following maximum volumes and frequency of bleeds is allowed for any technique;

Maximum Bleed Amounts	% of blood volume collected	Maximum Frequency	Example volume for 20g mouse
Single bleed	Up to 7%	Every 1 week	0.1ml = 100uL
	10%	Every 2 weeks	0.14ml = 140uL
	15%	Every 2-3 weeks	0.20ml = 200uL
Over a 24 hr period	Up to 7%	Every 1 week	0.098ml = 100uL
	10%	Every 2-3 weeks	0.14ml = 140uL
	15%	Every 4-6 weeks	0.20ml = 200uL

References and Resources

NHMRC. Australian code for the care and use of animals for scientific purposes 8th Edition 2013 (Section 4.4.3) https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes [accessed 3rd December 2020]

Parasuraman, S., Raveendran, R., Kesavan, R. (2010) Blood sample collection in small laboratory animals. *Journal of Pharmacology & Pharmacotherapeutics*, **1(2)**, 87-93 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3043327/ [accessed 3rd December 2020]

ANU. Procedure for Managing & Reporting Unexpected Adverse Events https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms [accessed 3rd December 2020]

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Jackson Laboratories. How much blood can I take from a mouse without endangering its health? https://www.jax.org/news-and-insights/2005/october/how-much-blood-can-i-take-from-a-mouse-without-endangering-its-health [accessed 3rd December 2020]

NC3Rs. Blood sampling https://www.nc3rs.org.uk/3rs-resources/blood-sampling [Accessed 8th September 2020]

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