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Blood collection in mice: Submandibular vein (cheek pouch) method

SCOPE

The collection of blood from mice using the submandibular vein method minimises mouse distress and is a relatively easy collection technique to learn and perform on conscious mice. The blood contacts the skin of the mouse thus the sample is not aseptic and can also be haemolysed following contact of the blood with the atmosphere.
Blood volume collected can be partially controlled by lancet size used to puncture the site.
Repeat sampling is possible using alternate sides of the face.
Sample may be a mixture of venous and arterial blood.
This technique is more difficult in mice that are black, have been irradiated or are old.
No more than 6 samples in total to be collected from one mouse using alternate sides of face.

AUTHORISATION TO UNDERTAKE PROCEDURE

To perform this technique animal technician or investigator must be competent as determined in BRC training program for submandibular vein blood collection in mice.
Investigators must be approved by AEC to perform this technique.

SPECIAL REQUIREMENTS/SAFETY

- Correct restraint of the mouse
- Disposal of sharps into approved container
- Wear protective personal equipment

MATERIALS/EQUIPMENT

- Blood lancet of suitable size:

Mouse weight	Lancet Size
<20g	4.0mm
20-40g	5.0mm
>40g	5.5mm

- Blood sample tube (heparinised or non-heparinised depending on sample required)

- Gauze swab or sterile tissue to stop any bleeding after collection completed

PROCEDURE

Location of superficial temporal vein
The superficial temporal vein runs diagonally across the face from the orbit to the base of the ear. In white or light coloured mice a small whisker spot provides a good mark. This is harder to locate in black mice.

Mouse restraint and blood collection
The mouse is restrained with the lateral surface of the head facing the operator. Holding the lancet in the other hand a stab is made into the cheek, approximately halfway between the ear and the mandible with enough pressure to produce a small incision. Drops of blood will exude from the stick point and are collected into a suitable sample container.

Recovery
To stop the bleeding light pressure is applied to cheek using a sterile gauze pad or tissue for approximately 5 seconds. After the mouse is released it is immediately mobile and alert and will usually groom the cheek.

Blood volume and collection frequency
The acceptable volume is dependent on the circulating blood volume and in a mouse this is 0.05 -0.07mL/g. Approx. 10% can be removed safely at one collection every 2-4 weeks without fluid replacement as this allows time for replacement blood cells to be made.
Any adverse reaction at the blood collection site precludes any further attempt to use the site for blood collection.

Blood sample volume recommendations:

Mouse weight	Total blood volume	Blood sample size (10%)
20g	1.10 – 1.40mL	0.11 - 0.14mL
25g	1.37 – 1.75mL	0.14 – 0.18mL
30g	1.50 – 2.10mL	0.15 – 0.20mL

A maximum of six blood collections is permitted over duration of experiments in AEC approved projects with a minimum of one week between bleeds. This is provided the recommended volume is not exceeded and the blood collection site is alternated between cheeks. If more frequent blood collection is required AEC approval must be sought.

MONITORING REQUIREMENTS

Mice observed for 10 mins following blood collection for normal behaviour and haemostasis

EXPECTED RISKS

Blood can flow quickly after puncture, however after mouse restraint is released the bleeding quickly stops. Too deep a puncture can lead to blood into the oral cavity and risk of haemorrhage. Haematoma on side of face can be an occasional occurrence.

REFERENCES

Australian code for the care and use of animals for scientific purposes (8th Edition 2013)

www.medipoint.com video of technique

'Sublingual v submandibular bleeding in mice' Lab Animals (2010) 14, 352-358, Heimann M, Roth, DR, Ledieu D, Pfister R, Classen W.

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