	TLC Workplace Technical & Competency Training Document			
	Title	Blood Sampling in the Mouse		
	Reference	TLC-01-01	Version	1
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	Revision History	First Issue		

Objective

This document will describe the general procedure for blood collection from common routes in the conscious mouse. All equipment used must be calibrated, single-use, sterile and within use-by-date as appropriate.

Whilst outside of the home cage, animals should never be left unattended.

Read and fully understand the requirements of the protocol to ensure the correct procedure and equipment are used.

If the sample is difficult to collect, request assistance after two attempts. If after three attempts the sample is still unobtainable, stop the procedure and record in the documentation.

Consult either NACWO, NTCO or NVS for advice and guidance outside of this document.

Prior to being signed off as competent, the trainee must successfully perform the technique on a minimum of twenty occasions. Both the assessor and trainee must agree that competency has been achieved.

Terminology

A(SP)A – Animal (Scientific Procedures) Act 1986 Amendment Regulations 2012
PPL - Procedural Project Licence
PIL - Procedural Individual Licence
PEL – Establishment Licence Holder
NACWO – Named Animal Care & Welfare Officer
NVS – Named Veterinary Surgeon
NTCO – Named Training and Competency Officer
COSHH – Control of Substances Hazardous to Health Regulations 2002
Caudal/Lateral tail vein – the vessel of choice
Blood Tube – may be a blood tube, blood pot, blood spotting cards, capillary tubes, slides.
Protocol – refers to written instructions, lab notebook, study protocol detailing the precise requirements of the sample/s
TBV – Total Blood Volume

Regulated Procedures

Regulated procedures are carried out under the authority of A(SP)A. It is the responsibility of the individual carrying out the procedure to ensure their PIL and the PPL are adhered to, and the procedure room is listed on the PEL.

Read any associated risk assessments and COSHH assessments that apply to this procedure.

Personal Protective Equipment (PPE) and General Safety

Gloves	Masks	Safety Glasses	Absorbent Apron
Waste Bin	Sharps Bin	Safety Shoes	

Never re-sheath needles and immediately discard used needles into a sharps bin.

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Apparatus

Equipment required:

- Weighing scales
- Restraint tube
- Thermostatically controlled animal warming cabinet or water bath
- Labelled blood tubes
- Absorbent swabs
- Animal Record/cage label/paperwork/apparatus bleed sheets
- Absorbent material e.g. bench cote or similar

Optional, dependent on method:

- Needle – 25g or 27g
- Butterfly –25g
- Syringes
- Centrifuge
- Pipettes
- Sterile Scalpel
- Clippers
- Blood roller
- Petroleum jelly

Procedure

Prepare the procedural area and equipment in advance. Ensure area is clean and tidy and there is no evidence of contamination that may stress the animal.

Check animal identification prior to starting the procedure. Ensure animal ID, labels, blood tubes and paperwork correlate.

As a minimum requirement the blood tube must be labelled with study identifier, animal ID, date and if appropriate, an accurate record of the sample time point. Record any other observations, such as if the animal is slow to bleed on the proforma.

Care should be taken to avoid haemolysis. The sample should be of good quality, decanted into the correct blood tube for the purpose, labelled and stored at the correct temperature. Never decant through the needle as this will cause cell damage/ haemolysis. If un-clotted blood is required, mix the sample with anticoagulant as quickly as possible and place on a blood roller.

The animal ID is checked prior to returning to the correct home cage and the area is cleaned.

Method – Lateral Tail Vein

Weigh the mouse to calculate TBV.

Lateral tail veins are easily visible in a mouse and are located either side of the tail. The lateral tail veins require dilation. This can be obtained by using a thermostatically controlled warming cabinet at no more than 39^{o}C (102^{o}F) for between 5 and 15 minutes. Record when the animal is placed in the warming cabinet. Do not leave in the cabinet for more than 15minutes without prior authority from the NVS. Do not use hot cloths to dilate the veins as this can scald the tail.

A right handed person would hold the tail in the left hand and use the right hand to perform the procedure. A left handed person would reverse these instructions.

Needle and Syringe Method

- Place mouse into restraining device
- Insert the needle, bevel uppermost, no more than 1/3 the length into the vein
- Wait for blood to appear in hub of needle before starting to withdraw
- Withdraw slowly and only collect the correct amount of blood
- Apply swab over point of insertion and withdraw needle
- Apply gentle pressure over the puncture until blood flow ceases. To assist cessation, elevate the tail
- Remove needle from syringe and decant blood into chosen blood tube
- Discard needle into sharps bin
- Wipe tail clean if necessary, and return animal to home cage
- Ensure sample quality i.e. no visible clots
- Update protocol information, proforma and labels
- Continue with repeated bleeds, next sample or sanitise the procedure area and store all equipment
- Follow the protocol for blood tube storage
- Check mouse after 5 minutes to ensure puncture site has not started to bleed again

Cut-off Butterfly Method

- Place mouse into restraining device
- Hold the cut-off butterfly so as not to obscure the vein
- Insert the cut-off butterfly, bevel uppermost, no more than 1/3 into the vein
- Allow blood to drip into blood tube
- Hold the tail lower than the animals body to increase blood flow
- If the blood flow is slow or ceases remove the cut-off butterfly and the blood will usually flow. It can be dripped directly into the blood pot
- Apply swab over point of insertion and withdraw needle
- Discard cut-off into sharps bin
- Apply gentle pressure over the puncture until blood flow ceases. To assist cessation, elevate the tail
- Wipe tail clean if necessary, and return animal to home cage
- Ensure sample quality i.e. no visible clots
- Update protocol information, proforma and labels
- Continue with repeated bleeds, next sample or sanitise the procedure area and store all equipment
- Follow the protocol for blood tube storage
- Check mouse after 5 minutes to ensure puncture site has not started to bleed again

Tail Nick Method

- Place mouse into restraining device
- With the point of the scalpel make a shallow puncture in the vein
- Use a capillary tube to collect blood
- Transfer to chosen blood tube
- Apply swab and gentle pressure over the puncture until blood flow ceases. To assist cessation, elevate the tail
- Discard scalpel into sharps bin
- Wipe tail clean if necessary, and return animal to home cage
- Ensure sample quality i.e. no visible clots
- Update Protocol information, proforma and labels
- Continue with repeated bleeds, next sample or sanitise the procedure area and store all equipment
- Follow the protocol for blood tube storage
- Check mouse after 5 minutes to ensure puncture site has not started to bleed again

Method – Lateral Saphenous Vein

Weigh the mouse to calculate TBV.

Shave a hind leg to expose the saphenous vein, located along the anterior aspect of the limb. Place the mouse in a thermostatically controlled warming cabinet set to 37°C (98.6°F) for about 5 minutes to aid peripheral dilation. Record when the animal is placed in the warming cabinet.

Blood can be collected by either allowing it to drip into a collection tube or modifying a pipette, blood collection tube and syringe. Attach a blood collection tube to a 2ml syringe with an adapted attachment made by using a sharp implement to cut a pipette with a tip to size. Ensure that the pipette tip is securely fitted to the syringe and forms a good seal into the blood collection tube.

- Place mouse into restraining device or have an assistant to restrain the animal
- Apply a small amount of petroleum jelly over the area of the vein to prevent blood from running onto the skin and surrounding fur
- Extend the limb by gently pinching in front of it. This will expose and raise the vein
- Use a needle to puncture the vein at a 90° angle without piercing the other side of the vein
- Hold a blood tube to the puncture site and allow blood to drip into it, or use the adapted blood tube to collect the blood pooling at the puncture site by gently drawing back on the syringe
- Apply swab and gentle pressure over the puncture until blood flow ceases
- Discard needle into sharps bin
- Wipe limb clean and return animal to home cage
- Ensure sample quality i.e. no visible clots
- Update protocol information, proforma and labels
- Continue with repeated bleeds, next sample or sanitise the procedure area and store all equipment
- Follow the protocol for blood tube storage
- Check mouse after 5 minutes to ensure puncture site has not started to bleed again

Repeated Bleeds

These may be required by the protocol. Ensure the blood collected per animal does not exceed the legal requirements. You may take up to 10% TBV in 24 hours or 15% TBV in 28 days. For example 70ml blood/Kg body weight means a mouse weighing 20g has a TBV of 0.14ml. For example a 10% blood loss in a mouse equates to 2-3 drops of blood. The volumes above are a general guide; refer to appropriate PPL for more precise information.

If larger samples are required, additional animals will need to be used and /or the sampling points adjusted accordingly.

The tail nick method allows for removal of the scab to obtain more blood within a given time.

Potential Adverse Effects

Animal

Heat exhaustion in the warming cabinet (often seen as excessive salivation in the first instance)
Injury and pain to the tail or limb due to poor technique
Onset of injury to sampling sites
Excessive blood loss
Poor handling and restraint technique, incorrect restraint tube size
Haematoma at sampling site

Human

Bite or scratch
Needle stick injury