SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Retro-orbital bleed

INTRODUCTION:

The purpose of this procedure is to perform anaesthesia and retro-orbital bleed as a terminal procedure in wild type and genetically altered mice.

ABBREVIATIONS:

DCF = Data Capture Form
EDTA = Ethylenediaminetetraacetic acid
HFD = High Fat Diet
IVC = Individually Ventilated Cage
K/X = Ketamine Xylazine solution
LAA = Laboratory animal allergens
PAF = Project authorisation form
PIL = Procedure Individual Licence
PPL = Procedure Project Licence
PCR = Polymerase Chain Reaction
RSF = Research Support Facility
SOP = Standard Operating Procedure
QC = Quality Control

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved Quality Control (QC) fail comments steps to be used during procedures.
If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

In-Life Procedures:

Problem / Issue	QC fail reason
At any point during the procedure the mouse is deemed sick and processed through Sick Mouse Procedure (SMP)	Fail whole DCF as 'Sick mouse' – for all tests that day
Mouse incorrectly scheduled at wrong week	Fail whole DCF as 'Scheduling Issue'
Insufficient anaesthesia level affects the whole test DCF	Fail whole DCF as 'Anaesthesia Issue'
Insufficient anaesthesia level affects specific parameter(s)	Fail parameter(s) as 'Anaesthesia issue'
A welfare issue makes it impossible to collect specific parameters	Fail parameter(s) as 'Welfare issue'
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'

A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Glucose meter unable to record low blood values	Fail parameter(s) as 'Meter reading LO'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure' – old core temp reason
Parameter on the current DCF is not required for that specific test/pipeline	Fail parameter(s) as 'Not required'

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-3289
Assessment Title: Retro-orbital bleed

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Disposable gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or downflow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- Locate eye wash stations, fire extinguishers, standard spill kits and solvent/formaldehyde spill kit.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project License (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual License (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

Equipment:

1. Balance
2. BD Plastipak 1ml syringes (*Supplier: ThermoFisher; Product code 12359289*)
3. BD Microlance 3 27G ½" needles (*Supplier: SLS; Product code BD300635*)
4. Capillaries Minicaps 10µL (*Supplier: VWR; Product code: 612-2448*)
5. Yellow sharps container
6. Paper towels
7. Tubes for blood collection according to pipeline/DCF + racks
   7.1. EDTA (Ethylenediaminetetraacetic acid) powder coated tubes (*Supplier name;* SLS (Scientific Laboratory Supplies)*, Supplier product code; 078035*).
   7.2. Lithium Heparin powder coated tubes (*Supplier name;* SLS (Scientific Laboratory Supplies)*, Supplier product code;* 078042)
8. Igloo filled with ice
9. Polymerase Chain Reaction (PCR) 96 well plates + cover slips
10. PCR plate coolers
11. Scissors, forceps and ear punch device
12. Heat mats
13. Falcon tube warmer
14. Hydrex Pink hand spray **- Hazardous substance: highly flammable**
15. Hydrex Hard Surface spray - **Hazardous substance: highly flammable**
16. 70% Ethanol **Hazardous substance: highly flammable**
17. 1% Trigene solution
18. 100mg/kg Ketamine Hydrochloride,10mg/kg Xylazine Hydrochloride (K/X) solution (Anaesthetic) - **non-hazardous in working form**
    See Appendix 1 for preparation instructions
19. 1.25% 2,2,2-tribromoethanol (Avertin) solution (as required)- **Non-hazardous in working form**
20. Tecniplast Individually Ventilated Cage (IVC) rack
21. Transport rack

Associated SOPs/Documentation:

- **SOP0023** - Anaesthesia of mice with 2,2,2,-tribromoethanol (Avertin)

Staff: This test normally requires 3 phenotypers (1 anaesthetist and 2 phlebotomists) plus required histologists if necropsy collections are needed.

NOTE:

Retro-orbital blood collection is a terminal (non-recovery) procedure. After blood removal has commenced the mouse must not be allowed to regain consciousness and following the bleed it must be culled by a schedule 1 method.

Anaesthesia times are dependent on diet, the times indicated relate to mice fed a normal chow. For mice fed High Fat Diet (HFD) increase anaesthesia time by 5 minutes.

This procedure involves moving mice between downflow tables out of containment – **a face mask which has been fit tested must be worn**.

PROCEDURE:

Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).

1. **Set up**
   1.1. Remove the anaesthetic from the lockable box inside the necropsy fridge and place into the Falcon tube warmer to bring the solution close to body temperature.

   1.2. Turn on all heat mats.

   1.3. Collect scheduled mice from the animal room, transport them to the procedure room

   1.4. Collect PCR 96 well plates and place in PCR coolers (from -20^{0}C freezer).

   1.5. Place 'Phenotyping in progress' sign on the outside of the door.

   1.6. Select the first cage of mice and place on the large heat mat next to the weight scales **on a downflow table.**

   1.7. Remove water bottle, discard food from the hopper, and place all mice on the cage grid.

   1.8. Weigh the first mouse required (as identified by sex and earmark) and record the weight on the necropsy card. Check the mouse for fight wounds, if present record this on the necropsy card for that mouse stating if 'open' or 'healed'.

2. **Anaesthesia with Ketamine Xylazine (K/X) solution**

   2.1. Identify mouse and determine the amount of anaesthetic required using a dose of 0.1ml per 10g body weight and administer via intraperitoneal injection to the mouse's right side.

   2.2. Place mouse back in its home cage. Record the time of injection on the necropsy card.

   2.3. Repeat for each pipeline mouse in the cage. Change the needle after every mouse, **disposing of the old needle into a sharps bin. Needles are never to be re-sheathed.**

   2.4. If stuffers (non-test mice used to maintain cage density) are present, Schedule 1 cull by cervical dislocation and confirm by femoral cut.
   2.4.1. Record stuffer as culled on the database.

   2.5. Replace the cage lid with necropsy cards attached, do not replace water bottle, grid or original cage card.

   2.6. Monitor the mice until immobile and then place in the centre of the cage in injection order, ensuring their breathing is not obstructed. Transfer to the bleeders within 5 minutes of first injection.

2.7. Determine the number of mice which can be anaesthetised based on the requirements. Ideally do not exceed 4 mice waiting to be bled at any one time, ensuring that the anaesthesia timeframe is maintained. Mice should be suitably anaesthetised for approximately 20 minutes after anaesthesia is reached.

2.8. If after 5 minutes of being injected a mouse is still able to move around the cage, a top-up of anaesthetic can be given, up to 50% of the original dose depending on level of movement, to the mouse's left side. Mice which are only showing signs of 'tail-flicking' or 'whiskers-twitching' can be left for up to a further 3 minutes before a top-up is administered. Record any top ups with the time and additional volume given on the necropsy card.

2.9. If there is a delay and anaesthetised mice recover, possibly due to fire alarm, administer top up or re-anaesthetise to complete the collection session.

2.10. If the collection period has passed, allow any mice to recover and re-schedule the session.

Note: Anaesthesia with Avertin

If mice have been deemed unsuitable to be anaesthetised with K/X solution they must be anaesthetised with Avertin. Refer to SOP0023 - Anaesthesia of mice with 2,2,2,-tribromoethanol (Avertin).

3. **Retro-orbital bleed procedure**

3.1. Identify mouse to be bled by sex and earmark and confirm anaesthesia.

3.1.1. Mice anaesthetised with Ketamine /Xylazine do not lose the pedal ("toe pinch") reflex, therefore this cannot be used to assess level of anaesthesia. Confirmation of anaesthesia is assessed by the corneal ("eye closure") reflex or by whisker movement. If these are still present allow a further few minutes, if they do not subside a top-up can be given, as described in step 2.8, if not already administered.

3.2. Collect relevant tubes and/or pots as required for the tasks listed on the necropsy card. These should be placed in a rack and then in a basket. This can also be done after the bleed if there is not enough time before.

3.3. Start the Phlebotomy Data Capture Form (DCF) for the mouse and:

3.3.1. Set PIL performing the bleed.

3.3.2. Enter the body weight.

3.3.3. If a top up has been administered, add the 'Anaesthesia comment' "[volume] additional anaesthetic given".

3.3.4. If fight wounds were noted, select 'Yes' for the visible wound parameter and add a comment to detail the location and the severity of this wound (only for open wounds and scabs).
Check the individual mouse page for any with fight wounds and ensure a health record is present. If not, add an appropriate health record with the current date as the end date.

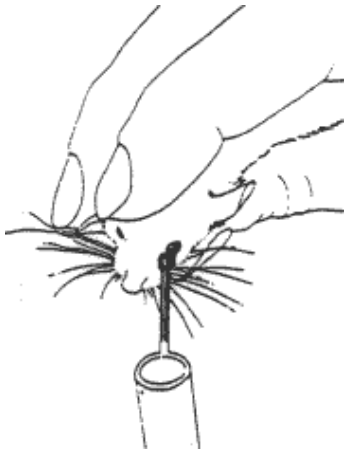
3.3.5. Ensure correct anaesthetic is selected from drop down list

3.4. Hold the mouse in a loose scruff against a flat surface and tighten the skin over the sides of the face. This retracts the eyelids and protracts the eyes.

3.5. Insert the capillary tube into the space between the globe and the lower eyelid at approximately a 45 degree angle. A slight thrust may be needed to puncture the tissue and enter the plexus or sinus. **Taking care not to break the capillary.**

3.6. Gently direct the capillary in a ventrolateral direction while rotating the capillary tube (rolling it between the thumb and forefinger of the dominant hand).

3.7. As the blood begins to flow into the capillary tube orientate the mouse so that the head is the lowest point and the end of the capillary is directed towards the collection tube, as close as is possible. (See diagram below).



3.8. Fill the tubes in the following order as required by pipeline:

3.8.1. **RED - EDTA** tube (haematology & flow assay): 100 µl (5-8 drops), unless otherwise requested. Mix and keep at room temperature.

3.8.2. **ORANGE - Lithium Heparin** tube (clinical chemistry): a minimum of 750 µl is required. Mix by 3 full inversions, and keep at room temperature.

3.9. When the blood flow slows, rotate the capillary to remove any clots and to maintain flow. If difficulty arises, change to a new capillary either in the same eye or the other eye if available. **Dispose of used capillaries in yellow sharps container.**

3.10. If the mouse starts to wake at any time during the bleed it should be culled immediately by a schedule 1 method. A note should be made of this on the necropsy card and in the comments on the DCF.

3.11. Cull the mouse by cervical dislocation.

3.11.1. Note for mice due to have brain collection for collaborator: Cervical dislocation should be performed carefully on these mice as the skull needs to be intact to maintain correct morphology for analysis.

3.12. Take an ear clip and a tail sample for genotyping and place in the well specified on the necropsy card.

3.13. Enter the blood volumes collected into the DCF and click ‘Done’

3.14. If there was an issue which affected the volume collected, enter the volume which was achieved, even if zero, and if appropriate, apply the relevant QC fail reason from the table above.

3.15. If not done before the bleed, collect relevant tubes and/or pots as required for the tasks listed on the necropsy card. These should be placed in a rack and then in a basket along with the mouse and necropsy card. Pass the basket to the next available histologist.

3.16. Repeat steps 3.1-3.15 for all mice.

3.17. If members of staff are present in the lab, contact them when samples from half the mice have been collected to arrange sample collection.

3.18. After all scheduled mice have been bled, check all ear and tail samples have been collected, cover plates with a cover slip and register them on the database.

3.19. Check all Phlebotomy DCFs have brain weights where required and have been completed.

3.20. If required, contact the lab to collect the final blood samples, tissue tubes and genotyping plates. Please inform at this point if any of the expected samples have not been collected (e.g. due to a welfare issue/anaesthesia not achieved). Genotyping samples should be taken to the relevant lab and stored in the labelled freezers there.

3.21. **Place used syringe and needle(s) into yellow sharps container for disposal.**

3.22. Clean all equipment, surfaces and the floor, **checking for loose capillaries. Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

3.23. **Place the empty dirty cages and water bottles on the trolley in the necropsy room**.

Appendix 1 – Preparation of Ketamine-Xylazine

Controlled drugs are supplied via the Named Veterinary Surgeon (NVS) and order and stock supply is monitored by the institute-nominated Designated Individual.

To access the drugs cabinet, the key will need to be issued by a designated key holder. All volumes must be carefully logged in the appropriate record books for each agent.

Excess diluted volumes should be absorbed into Uni-Safe gel and disposed of by incineration routes. Any undiluted expired/excess volumes should be returned to the NVS via the Designated Person.

1. Calculate the required volumes of each drug plus water based on the following ratio for a final dosage of 100mg/kg Ketamine/ 10mg/kg Xylazine when given at 0.1ml per 10g body weight.

Example for 10 animals up to 50g in weight, scale up as required:

Volume of dH2O	8.5 ml
Volume of Ketamine	1.0 ml
Volume of Xylazine	0.5 ml
Final volume of solution	**10 ml**

2. Pipette the required amount of dH_2O into a labelled falcon tube. Discard the stripette.

3. Withdraw the required volume of Ketamine Hydrochloride (Ketaset® / Ketalar®/ Narketan®) using a syringe/needle and add this to the dH_2O in the falcon tube. Dispose of the needle and syringe into the yellow sharps container. Record the volume taken in the Ketamine Hydrochloride log book.

4. Using a fresh needle/syringe, withdraw the required volume of Xylazine Hydrochloride (Rompun®) and add this to the diluted Ketamine Hydrochloride in the falcon tube. Dispose of the needle and syringe into the yellow sharps container. Record the volume taken in the Xylazine Hydrochloride log book.

5. Replace the lid to seal the tube and invert several times to ensure it has mixed well. Store in the 4°C refrigerator.