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STANDARD OPERATING PROCEDURE PACKET

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STANDARD OPERATING PROCEDURE

SUBJECT: Anaesthesia and Reversal of Mice with Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) and Atipamezole Hydrochloride (Antisedan) for X-Rays – V1

SOP Number: SOP0024	**To be reviewed:**
Author(s): Signed: Date:	
Editor: Signed: Date:	
Risk Approver: Signed: Date:	
Date of Implementation:	

INTRODUCTION:

The purpose of this procedure is to place mice under general anaesthesia, allowing SOP0054 - Dual Energy X-ray Absorptiometry and X-ray Imaging to be performed on wild-type and genetically modified mice, and reversal.

ABBREVIATIONS:

DCF = Data Capture Form
IVC = Individually Ventilated Cage
K/X= Ketamine Hydrochloride/Xylazine Hydrochloride
LAA = Laboratory Animal Allergens
NACWO = Named Animal Care and Welfare Officer
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL= Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RSF = Research Support Facility
SLT = Senior Leadership Team
SMP = Sick Mouse Procedure
SOP = Standard Operating Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved 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'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
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-1475
Assessment Title: DEXA X-ray recovery anaesthesia
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - 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.
- 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 Licence (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 Licence (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. Weight scale
2. 70% Ethanol **- Hazardous substance: highly flammable**
3. Hydrex Pink hand spray **- Hazardous substance: highly flammable**
4. Hydrex Hard Surface spray - **Hazardous substance: highly flammable**
5. Tissues
6. One clean cage and 2 nestlets per cage of mice tested
7. Heat mats
8. 4x 1mL BD Plastipak syringes
9. BD Microlance 3, ½"G needles; one for each mouse
10. Yellow sharps container
11. 100mg/kg Ketamine Hydrochloride,10mg/kg Xylazine Hydrochloride (K/X) solution (Anaesthetic) - **non-hazardous in working form**
12. 1mg/kg Atipamezole hydrochloride (Antisedan) solution (Reversal) - **non-hazardous in working form**
13. Absorptiometry & Imaging Record Sheet
14. Anaesthesia recovery log
15. Anaesthesia cage labels
16. Tecniplast heated Individually Ventilated Cage (IVC) recovery rack
17. Tecniplast IVC rack
18. Transport rack
19. Diet (as defined by pipeline)
20. Water bottles (as required)
21. Countdown timer with alarm *(Supplier name; VWR International Ltd. Supplier product code; 609-0131)*
22. 110mg/Kg Ketamine; 11mg/Kg Xylazine dose calculation chart
23. 'Experimental mice removed for Procedure' labels

Associated SOPs/Documentation:

- **SOP0101** – Taking and Returning Cages for Procedures
- **SOP0031** – Recovery of Mice from Anaesthesia
- **SOP0032** – Preparation of Ketamine-Xylazine + Antisedan
- **SOP0045** – Weigh Mice
- **Ketamine_Xylazine_Antisedan calculation template**
- **Anaesthesia Recovery Log Sheet_main**
- **Absorptiometry & Imaging Record Sheet**
- **Anaesthesia cage labels**
- **'Experimental mice removed for Procedure' labels**

Staff: This test can be completed by one phenotyper.

NOTE:

For non-terminal anaesthesia, where stuffers are present, mice will remain in the home cage; add 'Experimental mice removed for Procedure' label and store on the Tecniplast Individually Ventilated Cage (IVC) rack. Once all anaesthetised mice are fully recovered, stuffers can then be relocated to the clean cage with cage mates.

The amount of K/X administered during the x-ray tests varied depending on the pipeline, though the amount of Antisedan remained the same. The amount each mouse received can be found in the DCF metadata.

Expected recovery times for K/X:

- First signs of recovery can be expected 5-10 minutes following injection of Atipamezole hydrochloride (Antisedan) - mice will display "nodding" or bobbing of heads as well as tail flicking and muscle tensing.

- 20-25 minutes post Atipamezole hydrochloride (Antisedan) injection, mice will begin to move around the cage and display more awareness. In some cases, some hyperventilation and tachycardia can be expected in this window, but this should not be long-lasting. Monitor respiratory movements.

- Mice should have fully recovered approximately 90-150 minutes following the injection of Atipamezole hydrochloride (Antisedan).

If any unexpected behaviour are observed during the anaesthesia process, seek advice from a Named Animal Care and Welfare Officer (NACWO) and inform the primary phenotyper for the test or Senior Leadership Team (SLT).

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. Prepare heat mats for use.

2. Take the anaesthetic and reversal solutions out from the fridge and allow them to warm to room temperature.

3. Collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (Refer to SOP0101 – Taking and Returning Cages for Procedures).

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

5. Complete an anaesthesia cage label per cage of mice to be anaesthetised.

6. Complete the top section of the anaesthesia recovery log with all the required information.

7. Whilst wearing the correct PPE, weigh mice (Refer to SOP0045 - Weigh mice) and record the body weight of each mouse on the Absorptiometry & Imaging Record Sheet.

8. Calculate the dose of Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) for each mouse at either 100mg/kg Ketamine; 10mg/kg Xylazine or using the 110mg/Kg Ketamine; 11mg/Kg Xylazine calculation chart (see Appendix 1) (pipeline dependent) and record on the Absorptiometry & Imaging Record Sheet.

9. Calculate the reversal agent dose of Atipamezole hydrochloride (Antisedan) required for each mouse, based on their true body weight using a dose of 0.1ml (100µl) per 10g and record on the Absorptiometry & Imaging Record Sheet.

10. Clearly label 2 syringes 'K/X' for the Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) anesthetic and 2 syringes with 'R' for the reversal agent Atipamezole hydrochloride (Antisedan).

11. Place a clean cage with an anaesthesia cage label on the heat-mat. If not already present, place 2 shredded nestlets within the cage.

12. Identify mouse and anaesthetise:
    - 12.1. Place a needle on a 'K/X' syringe.
    - 12.2. Load the syringe with the correct dose of Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) required for the mouse.
    - 12.3. Administer the dose via intraperitoneal injection on the mouse's right hand side.
    - 12.4. **Discard the used needle in the yellow sharps bin.**
    - 12.5. Place this mouse in a clean cage and allow it to undergo anaesthesia. The expected window of induction for anaesthesia with Ketamine Hydrochloride/ Xylazine Hydrochloride (K/X) is 5 to 10 minutes.
    - 12.6. Record the time of injection on the anaesthesia cage label.

13. Repeat step 12 for the second mouse, if processing in pairs.
    - 13.1.1. If mice are from the same home cage, they can be placed in the same clean cage to undergo anaesthesia.
    - 13.1.2. If from a different cage, each mouse requires a separate clean cage with anaesthesia cage label.
    - 13.1.3. If stuffers are present, they remain un-anaesthetised in the original home cage, with 'Experimental mice removed for Procedure' label added, re-joining test mice when the cage is fully recovered.

14. Once the first mouse has stopped showing signs of movement, check the level of anaesthesia:
    - 14.1. Righting reflex is absent.
    - 14.2. Tail and whiskers not twitching.

    If the mouse is not completely anaesthetised, leave it for another 5 minutes before proceeding.

15. If full anaesthesia has not been achieved up to 10-15 minutes after the injection, administer a top-up:
    - 15.1. Inject up to either 20% (if initially given 110mg/Kg Ketamine; 11mg/Kg Xylazine) or 30% (if initially given 100mg/kg Ketamine; 10mg/kg Xylazine) of the mouse's body weight (a minimum of 0.1ml), on the same side as the original injection for anesthesia.

15.2. Record the amount of the top-up administered in the comments section of the Absorptiometry & Imaging Record Sheet, the anaesthesia cage label, and in the 'anaesthetics comment' section on the mouse's DCF on the database.

16. When an entire cage has been anaesthetised, record the time on the anaesthesia cage label.

17. When test procedures are complete, but not less than 15 minutes after anaesthesia, Atipamezole hydrochloride (Antisedan) should be administered to reverse anaesthetised state:
    17.1. Place a needle on a syringe labelled "R".
    17.2. Load the syringe with the correct dose of Atipamezole hydrochloride (Antisedan) required for the mouse, not including top-up dose.
    17.3. Administer the dose via intraperitoneal injection to the mouse's left hand side (opposite side used to administer the K/X).
    17.4. **Discard the used needle in the yellow sharps bin.**
    17.5. Return mice to their cage for recovery.
        17.5.1. Place the mice gently on their front, ensuring the mouth/nose is not obstructed.
        17.5.2. Ensure that the mouse has been placed in an area clear of the nozzle of the water bottle. (Refer to SOP003 - Recovery of Mice from Anaesthesia)
    17.6. Record the time at which the reversal agent was administered to the first and the last mouse of the cage on the anaesthesia cage label.
    17.7. Ensure that all mice have access to food and water. If necessary add food pellets in the cage food hopper and a clean water bottle.

18. When the last mouse of the first cage has been reversed, initiate recovery procedure (Refer to SOP0031 - Recovery of Mice from Anaesthesia).
        18.1.1. Start the countdown timer, pre-set to alarm every 30 minutes.
        18.1.2. From this point the timer should be worn at all times to facilitate recovery (Refer to SOP0031 – Recovery of Mice from Anaesthesia).

19. Complete the middle section of the anaesthesia recovery log with the relevant information and place the cages within the Tecniplast heated IVC recovery rack.

20. Repeat steps 12-19 for all the cages.

21. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container. Place used syringes and needles into yellow sharps bins. Bind used anaesthetic and reversal solutions using chemical binding agent and dispose in a yellow clinical waste bag.**

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Appendix 1: 110mg/kg Ketamine; 11mg/kg Xylazine dose calculation chart 15g-44g.

body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)
15	0.17	19.9	0.22	24.8	0.27
15.1		20		24.9	
15.2		20.1		25	0.28
15.3		20.2		25.1	
15.4		20.3		25.2	
15.5		20.4		25.3	
15.6		20.5	0.23	25.4	
15.7		20.6		25.5	
15.8		20.7		25.6	
15.9	0.18	20.8		25.7	
16		20.9		25.8	
16.1		21		25.9	0.29
16.2		21.1		26	
16.3		21.2		26.1	
16.4		21.3		26.2	
16.5		21.4	0.24	26.3	
16.6		21.5		26.4	
16.7		21.6		26.5	
16.8	0.19	21.7		26.6	
16.9		21.8		26.7	
17		21.9		26.8	0.30
17.1		22		26.9	
17.2		22.1		27	
17.3		22.2		27.1	
17.4		22.3	0.25	27.2	
17.5		22.4		27.3	
17.6		22.5		27.4	
17.7	0.20	22.6		27.5	
17.8		22.7		27.6	
17.9		22.8		27.7	0.31
18		22.9		27.8	
18.1		23		27.9	
18.2		23.1		28	
18.3		23.2	0.26	28.1	
18.4		23.3		28.2	
18.5		23.4		28.3	
18.6	0.21	23.5		28.4	
18.7		23.6		28.5	
18.8		23.7		28.6	0.32
18.9		23.8		28.7	
19		23.9		28.8	
19.1		24		28.9	
19.2		24.1	0.27	29	
19.3		24.2		29.1	
19.4		24.3		29.2	
19.5	0.22	24.4		29.3	
19.6		24.5		29.4	
19.7		24.6		29.5	
19.8		24.7		29.6	0.33

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body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)
29.7	0.33	34.6	0.38	39.5	0.44
29.8		34.7		39.6	
29.9		34.8		39.7	
30		34.9		39.8	
30.1		35	0.39	39.9	
30.2		35.1		40	
30.3		35.2		40.1	
30.4		35.3		40.2	
30.5	0.34	35.4		40.3	
30.6		35.5		40.4	
30.7		35.6		40.5	0.45
30.8		35.7		40.6	
30.9		35.8		40.7	
31		35.9	0.40	40.8	
31.1		36		40.9	
31.2		36.1		41	
31.3		36.2		41.1	
31.4	0.35	36.3		41.2	
31.5		36.4		41.3	
31.6		36.5		41.4	0.46
31.7		36.6		41.5	
31.8		36.7		41.6	
31.9		36.8	0.41	41.7	
32		36.9		41.8	
32.1		37		41.9	
32.2		37.1		42	
32.3	0.36	37.2		42.1	
32.4		37.3		42.2	
32.5		37.4		42.3	0.47
32.6		37.5		42.4	
32.7		37.6		42.5	
32.8		37.7	0.42	42.6	
32.9		37.8		42.7	
33		37.9		42.8	
33.1		38		42.9	
33.2	0.37	38.1		43	
33.3		38.2		43.1	
33.4		38.3		43.2	0.48
33.5		38.4		43.3	
33.6		38.5		43.4	
33.7		38.6	0.43	43.5	
33.8		38.7		43.6	
33.9		38.8		43.7	
34		38.9		43.8	
34.1	0.38	39		43.9	
34.2		39.1		44	
34.3		39.2		44.1	0.49
34.4		39.3		44.2	
34.5		39.4		44.3	

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STANDARD OPERATING PROCEDURE

SUBJECT: Anaesthesia of Mice with 2,2,2-tribromoethanol (Avertin) – V1

SOP Number: SOP0023	**To be reviewed:**	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Approver:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to place mice under general anaesthesia, allowing 'SOP0054 - Dual Energy X-ray Absorptiometry and X-ray Imaging' or 'SOP0046 – Retro-orbital bleed' to be performed on wild-type and genetically modified mice.

ABBREVIATIONS:

DCF= Data Capture Form
IVC = Individually Ventilated Cage
LAA = Laboratory Animal Allergens
NACWO = Named Animal Care and Welfare Officer
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL= Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RSF = Research Support Facility
SLT = Senior Leadership Team
SMP = Sick Mouse Procedure
SOP = Standard Operating Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved 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'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
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 Assessments (RA):

Name: WTSI-1475 & WTSI-1189
Assessment Title: DEXA X-ray recovery anaesthesia & RO Bleeds
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
  - Mask
- 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.
- 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 Licence (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 Licence (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. Weight scale
2. 70% Ethanol **- Hazardous substance: highly flammable**
3. Hydrex Pink hand spray **- Hazardous substance: highly flammable**
4. Hydrex Hard Surface spray - **Hazardous substance: highly flammable**
5. Tissues
6. One clean cage and 2 nestlets per cage of mice tested
7. Heat mats
8. Diet (as defined by pipeline)
9. Water bottles (as required)
10. 2x 1mL BD Plastipak syringes
11. BD Microlance 3, ½"G needles; one for each mouse
12. Yellow sharps container
13. 1.25% 2,2,2-tribromoethanol (Avertin) solution- **Non-hazardous in working form**
14. pH test strips (pH 4 to 7) *(Supplier name; Scientific Laboratory Supplies Ltd. Supplier product code; PAP2076)*
15. 2,2,2-tribromoethanol (Avertin) dose calculation sheet
16. Absorptiometry & Imaging Record Sheet
17. Anaesthesia recovery log (X-ray only)
18. Anaesthesia cage labels (X-ray only)
19. Tecniplast heated Individually Ventilated Cage (IVC) recovery rack (for X-ray procedure only)
20. Tecniplast IVC rack
21. Transport rack
22. Countdown timer with alarm. *(Supplier name; VWR International Ltd. Supplier product code; 609-0131)*. (X-ray only)
23. 'Experimental mice removed for Procedure' labels
24. Histology cards (Bleeds only)

Associated SOPs/Documentation:

- **SOP0101** – Taking and Returning Cages for Procedures
- **SOP0031** – Recovery of Mice from Anaesthesia
- **SOP0035** – Preparation of 2,2,2-tribromoethanol (Avertin)
- **SOP0045** – Weigh Mice
- **Anaesthesia Recovery Log Sheet_main**
- **Absorptiometry & Imaging Record Sheet**
- **Anaesthesia cage labels**
- **'Experimental mice removed for Procdure' labels**

Staff: This test can be completed by one phenotyper.

NOTE:

For non-terminal anaesthesia, where stuffers are present, mice will remain in the home cage; add 'Experimental mice removed for Procedure' label and store on the Tecniplast Individually Ventilated Cage (IVC) rack. Once all anaesthetised mice are fully recovered, stuffers can then be relocated to the clean cage with cage mates.

Expected recovery times for 2,2,2-tribromoethanol (**Avertin):**

- First signs of recovery can be expected 20-25 minutes following initial injection of 2,2,2-tribromoethanol (Avertin) - mice will right themselves, and respond to the foot pinch reflex, but continue to appear "asleep".

- 35–50 minutes post injection, mice will show some movement or attempts to move, yet continue to appear sedated or mildly sedated. Where mice have not moved, closely observe respiratory movements and heartbeat.

- Mice should have fully recovered approximately 150–200 minutes following the initial injection of 2,2,2-tribromoethanol (Avertin). Throughout recovery, breathing movements and heartbeat should be closely observed.

If any unexpected behaviour in an animal is observed during the anaesthesia process, seek advice from a Named Animal Care and Welfare Officer (NACWO) and inform the primary phenotyper for the test and Senior Leadership Team (SLT).

PROCEDURE:

Before performing the procedure, verify that this is the correct procedure at this point in the pipeline by consulting the cage card(s) and confirming that the procedure has not already been performed on the mouse.

1. **Anaesthesia for X-ray/DEXA/ABR only.**

    1.1. Prepare heat mats for use.

    1.2. Remove 1.25% 2,2,2-tribromoethanol (Avertin) from 4°C refrigerator and allow to warm to room temperature.

    1.3. Test the pH of the 2,2,2-tribromoethanol (Avertin) solution using a pH test strip:
        1.3.1. A pH of 5 or above is deemed acceptable for use.
        1.3.2. If the pH is below 5, assume that 2,2,2-tribromoethanol (Avertin) has degraded and dispose of the solution.

    1.4. Collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (Refer to SOP0101 – Taking and Returning Cages for Procedures).

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

    1.6. Complete an anaesthesia cage label per cage of mice to be anaesthetised.

1.7. Complete the top part of the anaesthesia recovery log with all the required information.

1.8. Wearing the correct PPE, weigh mice (refer to SOP0045 - Weigh mice) and record the body weight of each mouse on the Absorptiometry & Imaging Record Sheet.

1.9. Calculate the dose of 2,2,2-tribromoethanol (Avertin) for each mouse, using the 2,2,2-tribromoethanol (Avertin) dose calculation sheet (see Appendix 1) and record on the Absorptiometry & Imaging Record Sheet. Reversal agent is not used with 2,2,2-tribromoethanol (Avertin).

1.10. Place a clean cage with an anaesthesia cage label on the heat-mat.
- 1.10.1. If not already present place 2 shredded nestlets within the cage.

1.11. Identify mouse and anaesthetize:
- 1.11.1. Place a needle on the 2,2,2-tribromoethanol 'Avertin' labelled syringe.
- 1.11.2. Load the syringe with the correct dose of 2,2,2-tribromoethanol (Avertin) required for the mouse.
- 1.11.3. Administer the dose by intraperitoneal injection to the mouse's right hand side.
- 1.11.4. **Discard the used needle in the yellow sharps bin.**
- 1.11.5. Place this mouse in a clean cage and allow it to undergo anaesthesia. The expected window of induction for anaesthesia with 1.25% 2,2,2-tribromoethanol (Avertin) is 2 to 5 minutes.
- 1.11.6. Record the time of injection on the anaesthesia cage label.

1.12. Repeat step 1.11 for the second mouse, if processing in pairs.
- 1.12.1. If mice are from the same home cage, they can be placed in the same clean cage to undergo anaesthesia.
- 1.12.2. If from a different cage, each mouse requires a separate clean cage with anaesthesia cage label.
- 1.12.3. If stuffers are present, they remain un-anaesthetised in the original home cage, with 'Experimental mice removed for Procedure' label added, re-joining test mice when the cage is fully recovered.

1.13. Once the first mouse injected has stopped showing signs of movement, check the level of anaesthesia:
- 1.13.1. Righting reflex is absent.
- 1.13.2. Tail and whiskers not twitching.
- 1.13.3. If the mouse is not completely anaesthetised, leave it for another 5 minutes before proceeding. No top-up should be given to mice injected with 2,2,2-tribromoethanol (Avertin) as the lethal dose is too close to the working dose.

1.14. When an entire cage has been anaesthetised:
- 1.14.1. Record the time on the anaesthesia cage label.
- 1.14.2. Start the countdown timer, pre-set to alarm every 30 minutes.
- 1.14.3. From this point the timer should be worn at all times to facilitate recovery.

1.15. When test procedures are complete return mice to their cage for recovery (refer to SOP0031 – Recovery of Mice from Anaesthesia).
- 1.15.1. Place the mice gently on their front, ensuring the mouth/nose is not obstructed.

1.15.2. Ensure that the mouse has been placed in an area clear of the nozzle of the water bottle.

1.15.3. Ensure that all mice have access to food and water. If necessary add food pellets in the cage food hopper and a clean water bottle.

1.16. Complete the middle section of the anaesthesia recovery log with the relevant information and place the cages within the Tecniplast heated IVC recovery rack.

1.17. Initiate recovery procedure (Refer to SOP0031 - Recovery of Mice from Anaesthesia).

1.18. Repeat steps from 1.11 to 1.18 for all the cages.

1.19. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container. Place used syringes and needles into yellow sharps bins. Bind used anaesthetic and reversal solutions using chemical binding agent and dispose in a yellow clinical waste bag.**

2. Anaesthesia for Bleeds only.

2.1. Prepare heat mat(s) for use.

2.2. Remove 1.25% 2,2,2-tribromoethanol (Avertin) from 4°C refrigerator and bring to room temperature.

2.3. Test the pH of the 2,2,2-tribromoethanol (Avertin) solution using a pH test strip.

2.3.1. A pH of 5 or above is deemed acceptable for use.

2.3.2. If the pH is below 5, assume that 2,2,2-tribromoethanol (Avertin) has degraded and dispose.

2.4. Collect scheduled mice from the animal room, transport them to the procedure room (Refer to SOP0101 – Taking and Returning Cages for Procedures).

2.5. Wearing the correct PPE, identify mouse and weigh (see SOP0045 - Weigh mice) and record the body weight on their respective necropsy card.

2.6. Calculate the dose of 2,2,2-tribromoethanol (Avertin) using the 2,2,2-tribromoethanol (Avertin) dose calculation sheet (see Appendix 1).

2.7. Identify mouse and anaethetise:

2.7.1. Place a needle on the 2,2,2-tribromoethanol ‘Avertin’ labelled syringe.

2.7.2. Load the syringe with the correct dose of 2,2,2-tribromoethanol (Avertin) required for the mouse.

2.7.3. Administer the dose by intraperitoneal injection to the mouse’s right hand side.

2.7.4. **Discard the used needle in the yellow sharps bin.**

2.7.5. Place this mouse in a clean cage and allow it to undergo anaesthesia. The expected window of induction for anaesthesia with 1.25% 2,2,2-tribromoethanol (Avertin) is 2 to 5 minutes.

2.7.6. Record the time of injection on the histology card.

2.8. Repeat step 2.5-2.7 for all the mice in the cage.

2.9. Once the first mouse injected has stopped showing signs of movement, check the level of anaesthesia:
  2.9.1. Righting reflex is absent.
  2.9.2. Tail and whiskers not twitching.
  2.9.3. If the mouse is not completely anaesthetised, leave it for another 5 minutes before proceeding. No top-up should be given to mice injected with 2,2,2-tribromoethanol (Avertin) as the lethal dose is too close to the working dose.

2.10. Repeat steps from 2.5-2.9 for all the cages.

2.11. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container. Place used syringes and needles into yellow sharps bins. Bind used anaesthetic and reversal solutions using chemical binding agent and dispose in a yellow clinical waste bag.**

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Appendix 1: 1.25% 2,2,2-tribromoethanol (Avertin) Dose Calculation Sheet

Weight Range (g)	Avertin Dose (mL)	Weight Range (g)	Avertin Dose (mL)
14.8 - 15.3	0.30	32.8 - 33.3	0.66
15.4 - 15.7	0.31	33.4 - 33.7	0.67
15.8 - 16.3	0.32	33.8 - 34.3	0.68
16.4 - 16.7	0.33	34.4 - 34.7	0.69
16.8 - 17.3	0.34	34.8 - 35.3	0.70
17.4 - 17.7	0.35	35.4 - 35.7	0.71
17.8 - 18.3	0.36	35.8 - 36.3	0.72
18.4 - 18.7	0.37	36.4 - 36.7	0.73
18.8 - 19.3	0.38	36.8 - 37.3	0.74
19.4 - 19.7	0.39	37.4 - 37.7	0.75
19.8 - 20.3	0.40	37.8 - 38.3	0.76
20.4 - 20.7	0.41	38.4 - 38.7	0.77
20.8 - 21.3	0.42	38.8 - 39.3	0.78
21.4 - 21.7	0.43	39.4 - 39.7	0.79
21.8 - 22.3	0.44	39.8 - 40.3	0.80
22.4 - 22.7	0.45	40.4 - 40.7	0.81
22.8 - 23.3	0.46	40.8 - 41.3	0.82
23.4 - 23.7	0.47	41.4 - 41.7	0.83
23.8 - 24.3	0.48	41.8 - 42.3	0.84
24.4 - 24.7	0.49	42.4 - 42.7	0.85
24.8 - 25.3	0.50	42.8 - 43.3	0.86
25.4 - 25.7	0.51	43.4 - 43.7	0.87
25.8 - 26.3	0.52	43.8 - 44.3	0.88
26.4- 26.7	0.53	44.3 - 44.7	0.89
26.8 - 27.3	0.54	44.8 - 45.3	0.90
27.4 - 27.7	0.55	45.4 - 45.7	0.91
27.8 - 28.3	0.56	45.8 - 46.3	0.92
28.4 - 28.7	0.57	46.4 - 46.7	0.93
28.8 - 29.3	0.58	46.8 - 47.3	0.94
29.4 - 29.7	0.59	47.4 - 47.7	0.95
29.8 - 30.3	0.60	47.8 - 48.3	0.96
30.4 - 30.7	0.61	48.4 - 48.7	0.97
30.8 - 31.3	0.62	48.8 - 49.3	0.98
31.4 - 31.7	0.63	49.4 - 49.7	0.99
31.8 - 32.3	0.64	49.8 - 50.3	1.00
32.4 - 32.7	0.65	50.4 - 50.7	1.01

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Recovery of Mice from Anaesthesia – V1

SOP Number: SOP0031	To be reviewed:	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Approver:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to provide guidance on making appropriate observations and ensuring good welfare during recovery of mice following procedures that require non-terminal general anaesthesia.

ABBREVIATIONS:

ACH = Air Changes per Hour
DCF = Data Capture Form
IVC = Individually Ventilated Cage
LAA = Laboratory Animal Allergens
K/X = Ketamine/Xylazine
NACWO = Named Animal Care and Welfare Officer
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL= Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RSF = Research Support Facility
SOP = Standard Operating Procedure
SLT = Senior Leadership Team
SMP = Sick Mouse Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved 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'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
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-1475
Assessment Title: DEXA X-ray recovery anaesthesia
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - 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.
- 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 Licence (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 Licence (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. 70% Ethanol **- Hazardous substance: highly flammable**
2. Hydrex Pink hand spray **- Hazardous substance: highly flammable**
3. Hydrex Hard Surface spray - **Hazardous substance: highly flammable**
4. Tissues
5. Transport rack
6. Diet (as defined by pipeline)
7. Tecniplast heated Individually Ventilated cage (IVC) Recovery Rack
8. Heat-mats
9. Nestlets (*when required*)
10. Long-spouted water bottles (*when required*)
11. Small Petri dishes (*when required*)
12. Anaesthesia Recovery log
13. Anaesthesia cage labels.
14. Countdown timer with alarm. *(Supplier name; VWR International Ltd. Supplier product code; 609-0131)*
15. Testo thermohygrometer (model 608-H1)
16. Temperature probe (model PCE-310)
17. Post Procedure Check labels

Associated SOPs/Documentation:

- **SOP0101** - Taking and Returning Cages for Procedures
- **SOP0023** - Anaesthesia of Mice with 2,2,2-tribromoethanol (Avertin)
- **SOP0024** - Anaesthesia of Mice with Ketamine hydrochloride/ Xylazine Hydrochloride (Ketamine / Xylazine) + Atipamezole hydrochloride (Antisedan) (Reversal) for X-Rays
- **Anaesthesia Recovery Log Sheet_main**
- **Absorptiometry & Imaging Record Sheet**
- **Anaesthesia cage labels**

Staff: This procedure can be completed by one phenotyper.

NOTE:

When a mouse has recovered from anaesthesia, it will no longer be lying on its side and will display normal exploratory behaviour or will avoid capture. Recovered mice may also display grooming, feeding and other socially interactive behaviours.

If any unexpected behaviour in an animal is observed during the recovery process, seek advice from a Named Animal Care and Welfare Officer (NACWO) and inform the primary phenotyper for the test or the Senior Leadership Team (SLT).

Expected recovery times for Ketamine hydrochloride / Xylazine Hydrochloride (Ketamine /Xylazine (K/X)) + Atipamezole hydrochloride (Antisedan)

- First signs of recovery can be expected 5-10 minutes following injection of atipamezole hydrochloride (Antisedan) - mice will display "nodding" or bobbing of heads as well as tail flicking and muscle tensing.

- 20-25 minutes post atipamezole hydrochloride (Antisedan) injection, mice will begin to move around the cage and display more awareness. In some mice, some hyperventilation and tachycardia can be expected in this window, but this should not be long-lasting. Monitor respiratory movements.

- Mice should have fully recovered approximately 90-150 minutes following the injection of atipamezole hydrochloride (Antisedan).

Expected recovery times for 2,2,2-tribromoethanol (Avertin):

- First signs of recovery can be expected 20-25 minutes following initial injection of 2,2,2-tribromoethanol (Avertin) - mice will right themselves, and respond to the foot pinch reflex, but continue to appear “asleep”.

- 35–50 minutes post injection, mice will show some movement or attempts to move, yet continue to appear sedated or mildly sedated. Where mice have not moved, closely observe respiratory movements and heartbeat.

- Mice should have fully recovered approximately 150–200 minutes following the initial injection of 2,2,2-tribromoethanol (Avertin). Throughout recovery, breathing movements and heartbeat should be closely observed.

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. Ensure that the Tecniplast heated IVC Recovery Rack is switched on and the control panel parameters are checked.
   1.1. The correct parameters (indicated in figure below):
      1.1.1. Negative pressure
      1.1.2. Temperature value of the heated compartment is between 29°C-31°C
      1.1.3. Temperature value of the exhaust vent is between 24°C-26°C.
      1.1.4. 55-65 Air Changes per Hour (ACH)

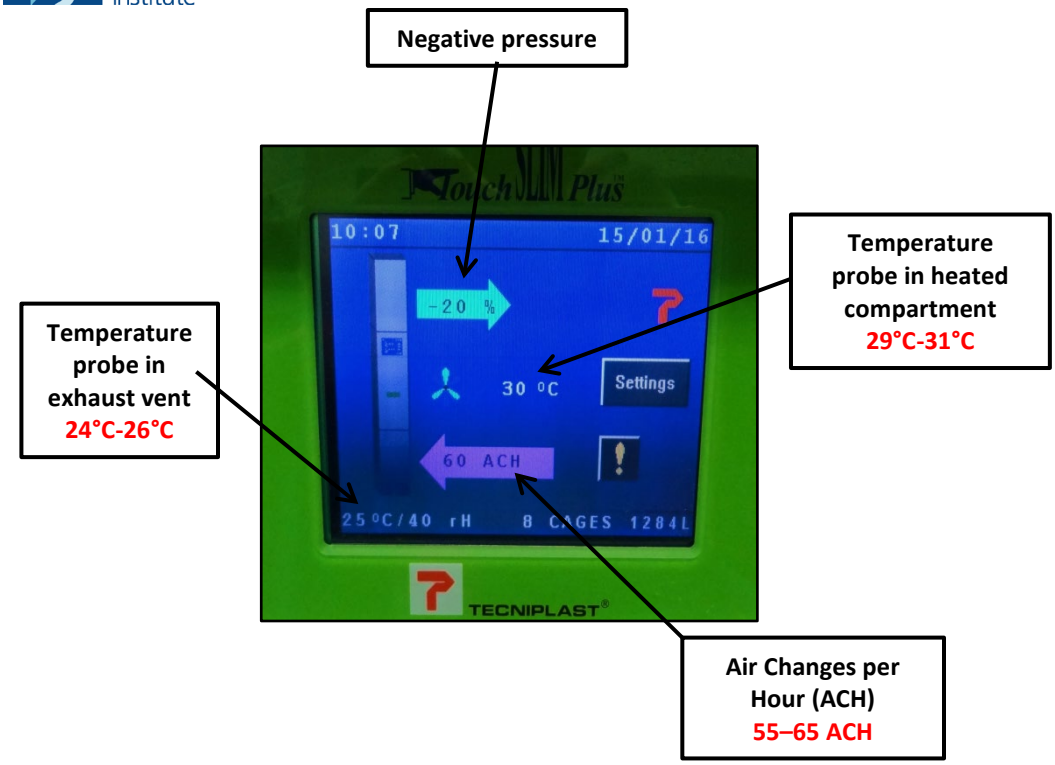

Figure: IVC Recovery Rack Control Panel

1.2. If either of the temperature readings are outside of the range, action should be taken:
   1.2.1. Check that the exhaust vent on the left side of the unit is clear.
   1.2.2. Monitor the temperature recorded on the Testo Thermohygrometer.
   1.2.3. Measure the temperature in at least 3 cages with the temperature probe.
   1.2.4. Record the details in the lab book.
   1.2.5. Inform the primary phenotyper for the test or SLT.

2. Whilst wearing the correct PPE, place the mice gently on their front in the recovery cage, ensuring the mouth/nose is not obstructed. Ensure that the mouse has been placed in an area clear of the nozzle of the water bottle.

3. Ensure that all mice have access to diet and water. If necessary add diet to the cage food hopper and a clean water bottle.

4. Place the cage within the Tecniplast heated IVC Recovery Rack for recovery ensuring the current cage card and anaesthesia cage label are displayed.

5. Begin the first observation 30 minutes after the LAST mouse in the FIRST cage received its final injection; anaesthetic agent or reversal agent, depending on method (see SOP0023 – Anaesthesia of Mice with 2,2,2-tribromoethanol (Avertin) and SOP0024 – Anaesthesia of Mice with Ketamine-Xylazine + Antisedan for X-rays).

6. Indicate the recovery status of the mice as:
   6.1.1. N= Not recovered (no movement – *comment as "asleep/ anaesthetised/ sedated"*).
   6.1.2. P = Partially recovered (displaying righting reflex or laboured movement – comment as "partial").

6.1.3. Y = Yes (displaying normal behaviour – *comment as "recovered/ awake"*).

7. Record observations on the post-anaesthetic care checklist **at least once every 30 minutes** until full recovery is achieved.
   7.1. Ensure a countdown timer with alarm is used and worn at all times to make certain that time between checks does not exceed 30 minutes.

8. Throughout recovery make thorough checks by bringing the cage out of the Tecniplast heated IVC recovery rack. Where cages are placed outside the Tecniplast heated IVC recovery unit for extended periods, place the cage on the heat mat. During observations:
   8.1. Breathing movements should be closely observed.
   8.2. Ensure that mice do not remain immobilised directly under the nozzle of the water bottle.
   8.3. Check that mice are in a comfortable position if they have returned to a partially sedated state.
   8.4. Ensure no mice are atop each other and that snouts are not buried under bedding or any other obstruction.

9. If any abnormal behaviour is observed (poor recovery, bleeding, extended periods of hyperventilation, vocalisation etc.), consult a NACWO immediately. If a NACWO determines that the recovery is in a severe state and instructs that the mouse be culled, inform the primary phenotyper for the test or SLT.

10. If any mice are not ambulant 3 hours post final injection:
    10.1. Consult a NACWO and allow a further 1 hour observing for significant signs of improvement.
    10.2. If any mice are recovering slower than others, but are still showing exploratory activity and are able to rear within 4 hours of final injection, initiate the slow recovery procedure:
      10.2.1. Provide the cage with extra nestlets (shredded), floor food or mash placed in a small Petri dish.
      10.2.2. Replace the regular water bottle with a long spouted water bottle and place a "long spout" label at the bottom of the water bottle.
      10.2.3. Place a 'SLOW RECOVERY FROM ANAESTHESIA' label on the cage, marked with the date and animal details.
      10.2.4. Make a note of any slow recovery mice on the recovery sheet.
    10.3. If mice are not fully ambulant after 4 hours initiate the SMP immediately.

11. When an entire cage has been recorded as fully recovered for 2 consecutive checks:
    11.1. Remove the cage from the Tecniplast heated IVC recovery rack.
    11.2. If stuffers were originally present in the cage, allow these to re-join the test mice in the new home cage.
    11.3. Place a 'POST PROCEDURE CHECK REQUIRED' label on the cage.
    11.4. Record the time at which the cage recovered on the anaesthesia cage label.
    11.5. Place the cage on the Tecniplast IVC rack.

12. When all mice from all cages have recovered:
    12.1. Remove and discard the anaesthesia cage labels.
    12.2. Make a record on the post anaesthetic care checklist and place it in the Anaesthesia Monitoring Log folder.

12.3. If required, top-up the food hoppers with diet and provide new water bottles.

12.4. If cages have been provided with mash due to health concerns, ensure the mash pot is also topped up, or prepare a fresh mash pot using a new small Petri dish.

13. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

14. **All cages must display the updated cage card. Place a 'POST PROCEDURE CHECK REQUIRED' label on all cages and register them to the correct rack whilst returning them to their destination/home rack in the animal room. (Refer to SOP0101 – Taking and Returning Cages for Procedures).**

15. Only once all animals have been returned should the countdown timer be stopped and removed.

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Anaesthesia, Reversal and Recovery of Mice using Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) and Atipamezole Hydrochloride (Antisedan) – V1

SOP Number: SOP0182	**To be reviewed:**	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Assessor:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to induce and subsequently reverse anaesthesia and provide guidance on making appropriate observations and ensuring good welfare during recovery of mice following procedures that require non-terminal general anaesthesia.

ABBREVIATIONS:

ACH = Air Changes per Hour
DCF= Data Capture Form
IVC = Individually Ventilated Cage
K/X= Ketamine Hydrochloride/Xylazine Hydrochloride
LAA = Laboratory Animal Allergens
NACWO = Named Animal Care and Welfare Officer
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL= Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RSF = Research Support Facility
SLT = Senior Leadership Team
SMP = Sick Mouse Procedure
SOP = Standard Operating Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved 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'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
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-1944
Assessment Title: DEXA X-ray recovery anaesthesia
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - 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.
- 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 Licence (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 Licence (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. Weight scale
2. 70% Ethanol **- Hazardous substance: highly flammable**
3. Hydrex Pink hand spray **- Hazardous substance: highly flammable**
4. Hydrex Hard Surface spray - **Hazardous substance: highly flammable**
5. Tissues
6. One clean cage and 2 nestlets per cage of mice tested
7. Heat mats
8. 4x 1mL BD Plastipak syringes
9. BD Microlance 3, ½"G needles; one for each mouse
10. Yellow sharps container
11. 100mg/kg Ketamine Hydrochloride,10mg/kg Xylazine Hydrochloride (K/X) solution (Anaesthetic) - **non-hazardous in working form**
12. 1mg/kg Atipamezole hydrochloride (Antisedan) solution (Reversal) - **non-hazardous in working form**
13. Appropriate Phenotyping worksheet
14. Anaesthesia recovery log
15. Anaesthesia cage cards
16. Tecniplast heated Individually Ventilated Cage (IVC) recovery rack
17. Tecniplast IVC rack
18. Transport rack
19. Diet (as defined by pipeline)
20. Water bottles (as required)
21. Nestlets (as required)
22. Long-spouted water bottles (as required)
23. Small Petri dishes (as required)
24. Countdown timer with alarm *(Supplier name; VWR International Ltd. Supplier product code; 609-0131)*
25. 110mg/Kg Ketamine; 11mg/Kg Xylazine dose calculation chart
26. Testo thermohygrometer (model 608-H1)
27. Temperature probe (model PCE-310)
28. Post Procedure Check labels
29. 'Experimental mice removed' labels
30. Falcon tube warmers

Associated SOPs/Documentation:

- **SOP0101** – Taking and Returning Cages for Procedures
- **SOP0032** - Preparation of Ketamine-Xylazine + Antisedan

- **SOP0045** – Weigh Mice
- **Ketamine_Xylazine_Antisedan calculation template**
- **Anaesthesia Recovery Log Sheet_main**
- **Anaesthesia cage cards 'Experimental mice removed' labels**

Staff: This test can be completed by one phenotyper.

NOTE:

For non-terminal anaesthesia, where stuffers are present, mice will remain in the home cage; add 'Experimental mice removed' label and store on the Tecniplast Individually Ventilated Cage (IVC) rack. Once all anaesthetised mice are fully recovered, stuffers can then be relocated to the clean cage with cage mates.

When a mouse has recovered from anaesthesia, it will no longer be lying on its side and will display normal exploratory behaviour or will avoid capture. Recovered mice may also display grooming, feeding and other socially interactive behaviours.

Expected recovery times for K/X:

- First signs of recovery can be expected 5-10 minutes following injection of Atipamezole hydrochloride (Antisedan) - mice will display "nodding" or bobbing of heads as well as tail flicking and muscle tensing.

- 20-25 minutes post Atipamezole hydrochloride (Antisedan) injection, mice will begin to move around the cage and display more awareness. In some cases, some hyperventilation and tachycardia can be expected in this window, but this should not be long-lasting. Monitor respiratory movements.

- Mice should have fully recovered approximately 90-150 minutes following the injection of Atipamezole hydrochloride (Antisedan).

If any unexpected behaviour in an animal is observed during the recovery process, seek advice from a Named Animal Care and Welfare Officer (NACWO) and inform the primary phenotyper for the test or the Senior Leadership Team (SLT).

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. Prepare heat mats and falcon tube warmers for use.

2. Remove the anaesthetic and reversal solutions from the fridge and place in the falcon tube warmers to allow them to warm up.

3. Ensure that the Tecniplast heated IVC Recovery Rack is switched on and the control panel parameters are checked.
   3.1. The correct parameters (indicated in figure below):
      3.1.1. Negative pressure.
      3.1.2. Temperature value of the heated compartment is between 29°C-31°C.
      3.1.3. Temperature value of the exhaust vent is between 24°C-26°C.
      3.1.4. 55-65 Air Changes per Hour (ACH).

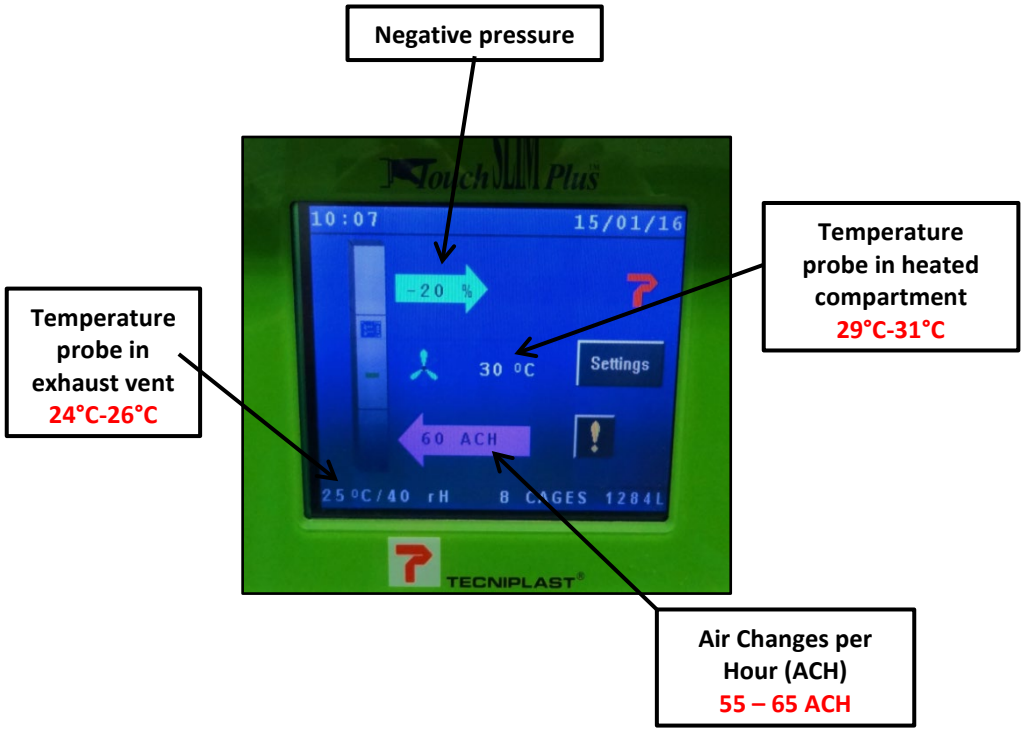

Figure: IVC Recovery Rack Control Panel

3.2. If either of the temperature readings are outside of the range, action should be taken:

3.2.1. Check that the exhaust vent on the left side of the unit is clear.

3.2.2. Monitor the temperature recorded on the Testo Thermohygrometer.

3.2.3. Measure the temperature in at least 3 cages with the temperature probe.

3.2.4. Record the details in the lab book.

3.2.5. Inform the primary phenotyper for the test or SLT.

4. Collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (Refer to SOP0101 – Taking and Returning Cages for Procedures)

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

6. Print an anaesthesia cage card for each cage of mice to be anaesthetised.

7. Complete the top section of the anaesthesia recovery log with all the required information.

8. Whilst wearing the correct PPE, weigh mice (Refer to SOP0045 - Weigh mice) and record the body weight of each mouse on the relevant phenotyping worksheet.

9. Calculate the dose of Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) for each mouse using the appropriate Ketamine/Xylazine calculated volume (for example see Appendix 1 for 110mg/Kg Ketamine; 11mg/Kg Xylazine dose calculation chart) and record on the relevant phenotyping worksheet.

10. Calculate the reversal agent dose of Atipamezole hydrochloride (Antisedan) required for each mouse, based on their true body weight using a dose of 0.1ml (100µl) per 10g and record on the relevant phenotyping worksheet. The volume of anaesthetic might not equal to the volume of reversal agent for an individual mouse.

11. Clearly label 2 syringes 'K/X' for the Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) anesthetic and 2 syringes with 'R' for the reversal agent Atipamezole hydrochloride (Antisedan).

12. Place a clean cage with an anaesthesia cage card on the heat-mat. If not already present, place 2 shredded nestlets within the cage, add food pellets in the cage food hopper and a clean water bottle.

13. Identify mouse and anaesthetise:
    - 13.1. Place a needle on a 'K/X' syringe.
    - 13.2. Load the syringe with the correct dose of Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) required for the mouse.
    - 13.3. Administer the dose via intraperitoneal injection on the mouse's right hand side.
    - 13.4. **Discard the used needle in the yellow sharps bin.**
    - 13.5. Place this mouse in the clean cage and allow it to undergo anaesthesia. The expected window of induction for anaesthesia with Ketamine Hydrochloride/Xylazine Hydrochloride (K/X) is 5 to 10 minutes.
    - 13.6. Record the time of injection on the anaesthesia cage card.

14. Repeat step 13 for the second mouse, if processing in pairs.
    - 14.1.1. If mice are from the same home cage, they can be placed in the same clean cage to undergo anaesthesia.
    - 14.1.2. If from a different cage, each mouse requires a separate clean cage with anaesthesia cage card.
    - 14.1.3. If stuffers are present, they remain un-anaesthetised in the original home cage, with 'Experimental mice removed' label added, re-joining test mice when the cage is fully recovered.

15. Once the first mouse has stopped showing signs of movement, check the level of anaesthesia.
    - 15.1. If full anaesthesia has been achieved:
      - 15.1.1. Righting reflex is absent.
      - 15.1.2. Tail and whiskers are not twitching.
    - 15.2. If the mouse is not completely anaesthetised, leave it for another 5 minutes before proceeding.

16. If full anaesthesia has not been achieved up to 10-15 minutes after the injection, administer a top-up:
    - 16.1. Inject up to 20% of the mouse's body weight (a minimum of 0.1ml), on the same side as the original injection for anaesthesia.
    - 16.2. Record the amount of the top-up administered in the comments section of the worksheet, the anaesthesia cage card, and in the 'anaesthesia comment' section on the mouse's DCF on the database.

17. When an entire cage has been anaesthetised, record the time on the anaesthesia cage card.

18. When test procedures are complete, but not less than 15 minutes after anaesthesia, Atipamezole hydrochloride (Antisedan) should be administered to reverse the anaesthetised state of the mouse:
    18.1. Place a needle on a syringe labelled “R”.
    18.2. Load the syringe with the correct dose of Atipamezole hydrochloride (Antisedan) required for the mouse, not including top-up dose.
    18.3. Administer the dose via intraperitoneal injection to the mouse’s left hand side (opposite side used to administer the K/X).
    18.4. **Discard the used needle in the yellow sharps bin.**
    18.5. Return mice to their cage for recovery.
        18.5.1. Place the mice gently on their front, ensuring the mouth/nose is not obstructed.
        18.5.2. Ensure that the mouse has been placed in an area clear of the nozzle of the water bottle.
    18.6. Record the time at which the reversal agent was administered to the first and the last mouse of the cage on the anaesthesia cage card.
    18.7. Ensure that all mice have access to food and water. If not already done so, add food pellets in the cage food hopper and a clean water bottle.

19. When the last mouse of the first cage has been reversed, initiate the recovery procedure.
    19.1.1. Complete the middle section of the anaesthesia recovery log with the relevant information
    19.1.2. Place the cage within the Tecniplast heated IVC recovery rack for recovery ensuring the current cage card and anaesthesia cage card are displayed.
    19.1.3. Start the countdown timer, pre-set to alarm every 30 minutes.
    19.1.4. From this point the timer should be worn at all times to facilitate recovery and ensure that time between checks does not exceed 30 minutes.

20. Repeat steps 12–19.1.2 for all the cages.

21. Begin the first observation 30 minutes after the LAST mouse in the FIRST cage received its injection of reversal agent.

22. Indicate on the recovery log sheet the recovery status of the mice as:
    22.1.1. N = Not recovered (no movement – *comment as “asleep/ anaesthetised/ sedated”*).
    22.1.2. P = Partially recovered (displaying righting reflex or laboured movement – comment as “partial”).
    22.1.3. Y = Yes (displaying normal behaviour – *comment as “recovered/ awake”*).

23. Record observations on the recovery log sheet **at least once every 30 minutes** until full recovery is achieved.

24. Throughout recovery make thorough checks by bringing the cage out of the Tecniplast heated IVC recovery rack. Where cages are placed outside the Tecniplast heated IVC recovery unit for extended periods, place the cage on the heat mat. During observations:
    24.1. Breathing movements should be closely observed.
    24.2. Ensure that mice do not remain immobilised directly under the nozzle of the water bottle.
    24.3. Check that mice are in a comfortable position if they have returned to a partially sedated state.

24.4. Ensure no mice are atop each other and that snouts are not buried under bedding or any other obstruction.

25. If any abnormal behaviour is observed (poor recovery, bleeding, extended periods of hyperventilation, vocalisation etc.), consult a NACWO immediately. If a NACWO determines that the recovery is in a severe state and instructs that the mouse be culled, inform the primary phenotyper for the test or SLT.

26. If any mice are not ambulant 3 hours post final injection:
    26.1. Consult a NACWO and allow a further 1 hour observing for significant signs of improvement.
    26.2. If any mice are recovering slower than others, but are still showing exploratory activity and are able to rear within 4 hours of final injection, initiate the slow recovery procedure:
        26.2.1. Provide the cage with extra nestlets (shredded), floor food or mash placed in a small Petri dish.
        26.2.2. Replace the regular water bottle with a long spouted water bottle and place a “long spout” label at the bottom of the water bottle.
        26.2.3. Place a ‘SLOW RECOVERY FROM ANAESTHESIA’ label on the cage, marked with the date and animal details.
        26.2.4. Make a note of any slow recovery mice on the recovery log sheet.
    26.3. If mice are not fully ambulant after 4 hours initiate the SMP immediately.

27. When an entire cage has been recorded as fully recovered for 2 consecutive checks:
    27.1. Remove the cage from the Tecniplast heated IVC recovery rack.
    27.2. If stuffers were originally present in the cage, allow these to re-join the test mice in the new home cage.
    27.3. Place a ‘POST PROCEDURE CHECK REQUIRED’ label on the cage.
    27.4. Record the time at which the cage recovered on the anaesthesia cage card.
    27.5. Place the cage on the Tecniplast IVC rack.

28. When all mice from all cages have recovered:
    28.1. Remove and discard the anaesthesia cage cards.
    28.2. Make a record on the recovery log sheet and place it in the Anaesthesia Monitoring Log folder.
    28.3. If required, top-up the food hoppers with diet and provide new water bottles.
    28.4. If cages have been provided with mash due to health concerns, ensure the mash pot is also topped up, or prepare a fresh mash pot using a new small Petri dish.

29. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container. Place used syringes and needles into yellow sharps bins. Bind used anaesthetic and reversal solutions using chemical binding agent and dispose in a yellow clinical waste bag.**

30. **All cages must display the updated cage card. Place a ‘POST PROCEDURE CHECK REQUIRED’ label on all cages and register them to the correct rack whilst returning them to their destination/home rack in the animal room. (Refer to SOP0101 – Taking and Returning Cages for Procedures).**

31. Only once all animals have been returned should the countdown timer be stopped and removed.

Appendix 1: 110mg/kg Ketamine; 11mg/kg Xylazine dose calculation chart 15g-44g.

body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)
15	0.17	19.9	0.22	24.8	0.27
15.1		20		24.9	
15.2		20.1		25	0.28
15.3		20.2		25.1	
15.4		20.3		25.2	
15.5		20.4		25.3	
15.6		20.5	0.23	25.4	
15.7		20.6		25.5	
15.8		20.7		25.6	
15.9	0.18	20.8		25.7	
16		20.9		25.8	
16.1		21		25.9	0.29
16.2		21.1		26	
16.3		21.2		26.1	
16.4		21.3		26.2	
16.5		21.4	0.24	26.3	
16.6		21.5		26.4	
16.7		21.6		26.5	
16.8	0.19	21.7		26.6	
16.9		21.8		26.7	
17		21.9		26.8	0.30
17.1		22		26.9	
17.2		22.1		27	
17.3		22.2		27.1	
17.4		22.3	0.25	27.2	
17.5		22.4		27.3	
17.6		22.5		27.4	
17.7	0.20	22.6		27.5	
17.8		22.7		27.6	
17.9		22.8		27.7	0.31
18		22.9		27.8	
18.1		23		27.9	
18.2		23.1		28	
18.3		23.2	0.26	28.1	
18.4		23.3		28.2	
18.5		23.4		28.3	
18.6	0.21	23.5		28.4	
18.7		23.6		28.5	
18.8		23.7		28.6	0.32
18.9		23.8		28.7	
19		23.9		28.8	
19.1		24		28.9	
19.2		24.1	0.27	29	
19.3		24.2		29.1	
19.4		24.3		29.2	
19.5	0.22	24.4		29.3	
19.6		24.5		29.4	
19.7		24.6		29.5	
19.8		24.7		29.6	0.33

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body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)	body weight (g)	K/X dose (mL)
29.7	0.33	34.6	0.38	39.5	0.44
29.8		34.7		39.6	
29.9		34.8		39.7	
30		34.9		39.8	
30.1		35	0.39	39.9	
30.2		35.1		40	
30.3		35.2		40.1	
30.4		35.3		40.2	
30.5	0.34	35.4		40.3	
30.6		35.5		40.4	
30.7		35.6		40.5	0.45
30.8		35.7		40.6	
30.9		35.8		40.7	
31		35.9	0.40	40.8	
31.1		36		40.9	
31.2		36.1		41	
31.3		36.2		41.1	
31.4	0.35	36.3		41.2	
31.5		36.4		41.3	
31.6		36.5		41.4	0.46
31.7		36.6		41.5	
31.8		36.7		41.6	
31.9		36.8	0.41	41.7	
32		36.9		41.8	
32.1		37		41.9	
32.2		37.1		42	
32.3	0.36	37.2		42.1	
32.4		37.3		42.2	
32.5		37.4		42.3	0.47
32.6		37.5		42.4	
32.7		37.6		42.5	
32.8		37.7	0.42	42.6	
32.9		37.8		42.7	
33		37.9		42.8	
33.1		38		42.9	
33.2	0.37	38.1		43	
33.3		38.2		43.1	
33.4		38.3		43.2	0.48
33.5		38.4		43.3	
33.6		38.5		43.4	
33.7		38.6	0.43	43.5	
33.8		38.7		43.6	
33.9		38.8		43.7	
34		38.9		43.8	
34.1	0.38	39		43.9	
34.2		39.1		44	
34.3		39.2		44.1	0.49
34.4		39.3		44.2	
34.5		39.4		44.3	

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Preparation of Ketamine Hydrochloride + Xylazine Hydrochloride (Ketamine/Xylazine) solution and reversal Atipamezole Hydrochloride (Antisedan) – V1

SOP Number: SOP0032	**To be reviewed:**
Author(s): Signed: Date: **Editor:** Signed: Date:	
Date of Implementation:	

INTRODUCTION:

The purpose of this procedure is to formulate the required volumes and concentrations of the anaesthetic Hydrochloride (Ketamine/Xylazine (K/X)) and reversal Atipamezole Hydrochloride (Antisedan).

ABBREVIATIONS:

K/X = Ketamine/Xylazine
NVS = Named Veterinary Surgeon
PIL = Procedure Individual License
PPL = Procedure Project License
SOP = Standard Operating Procedure

HEALTH & SAFETY:

RA003 – Hazardous Substances; Sections RA003.11, RA003.12, RA003.12 & RA003.14
RA004 – Physical Hazards; Sections RA004.4.3

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) has 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).

RESOURCES:

Equipment

1. Fisherbrand® Electronic Pipette Filler
2. Fisherbrand®10ml/50ml Disposable pipettes
3. BD Plastipak™ 1ml syringes/ 2.5ml syringes
4. BD Microlance™3 27G ½” needles
5. Plastic 50ml falcon tubes
6. Yellow sharps container

7. Yellow pipette container
8. Permanent marker pen
9. Uni-Safe powder *(Supplier name; VWR International Ltd. Supplier product code;116-0000)*
10. Ketamine hydrochloride 100mg/ml (e.g. Ketaset® / Ketalar®/ Narketan®)
11. Xylazine hydrochloride 20mg/ml (Rompun®)
12. Atipamezole hydrochloride 5mg/ml (Antisedan®)
13. Filter-Sterilized Water for Embryo Transfer (dH_2O) *(Supplier name; Sigma-Aldrich Co. Ltd. Supplier product code; W1503-100ML)*
14. Controlled drugs log books;
    14.1. Atipamezole hydrochloride
    14.2. Ketamine Hydrochloride/Xylazine Hydrochloride
15. Printed out version of Ketamine_Xylazine_Antisedan calculation template

Staff: This task can be completed by one phenotyper.

NOTE:

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.

PROCEDURE:

1. Enter the estimated number of mice to be anaesthetised into the Ketamine_Xylazine_Antisedan calculation template, to determine the correct volumes required of each drug.

2. **ANAESTHETIC: Ketamine Hydrochloride/Xylazine Hydrochloride**

    2.1. Label a falcon tube with;
        2.1.1. “100 mg/kg Ketamine” or “110mg/kg Ketamine” (pipeline & assay dependent)
        2.1.2. “10 mg/kg Xylazine” or “11 mg/kg Xylazine” (pipeline & assay dependent)
        2.1.3. Date of preparation
        2.1.4. Date of expiry (one week from the date of preparation)
        2.1.5. Your initials
        2.1.6. Write “K/X” + the test it is made for on the cap of the falcon tube.

    2.2. Pipette the required amount of dH_2O, as indicated on the calculation sheet, into the falcon tube. Discard the pipette.

    2.3. Withdraw the required volume of Ketamine Hydrochloride (Ketaset® / Ketalar®/ Narketan®), as indicated on the calculation sheet, 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.

2.4. Using a fresh needle/syringe withdraw the required volume of Xylazine Hydrochloride (Rompun®), as indicated on the calculation sheet, and add this to the diluted Ketamine Hydrochloride (Ketaset® / Ketalar®) 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.

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

3. **REVERSAL: Atipamezole Hydrochloride (Antisedan)**

3.1. Label a falcon tube with;
- 3.1.1. "1 mg/kg Antisedan"
- 3.1.2. Date of preparation
- 3.1.3. Date of expiry (one week from the date of preparation)
- 3.1.4. Your initials
- 3.1.5. Write "Antisedan" + the test it is made for on the cap of the falcon tube.

3.2. Pipette required amount of dH_2O, as indicated on the calculation sheet, into the falcon tube. Discard the pipette.

3.3. Withdraw the required volume of Atipamezole Hydrochloride (Antisedan®), as indicated on the calculation sheet, 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 Atipamezole Hydrochloride log book.

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

4. Clean all equipment and surfaces. Dispose of the pipette in the yellow pipette container, and transfer all other waste to a yellow offensive waste bag or clearly labelled waste container.

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Preparation of 2,2,2-tribromoethanol (Avertin) – V1

SOP Number: SOP0035	**To be reviewed:**
Author(s): Signed:	Date:
Editor: Signed:	Date:
Risk Assessor : Signed:	Date:
Date of Implementation:	

INTRODUCTION:

The purpose of this procedure is to correctly and safely create predesignated volumes and concentrations of the anaesthetic Avertin.

ABBREVIATIONS:

LAA = Laboratory Animal Allergens
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
RA = Risk Assessment
RSF = Research Support Facility
SOP = Standard Operating Procedure

HEALTH & SAFETY:

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

Name: WTSI-1520
Assessment Title: Preparation of working solution of anaesthtetics Ketamine/Xylazine, Avertin, or reversal Antisedan from stock solution.
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
  - Safety spectacles
- 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.
- 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 Licence (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 Licence (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. Fisherbrand® Electronic Pipette Filler
2. Fisherbrand®10ml/50ml Disposable pipettes
3. Plastic 50ml falcon tube (*from Sanger stores; product code PLTU0002*)
4. Yellow sharps disposal bin
5. Permanent marker pen
6. Beaker
7. Precision scale (*Mettler Toledo model AB104S*)
8. Whatman paper *(Supplier name; Sigma Aldrich Co.Ltd. Supplier product code; WHA10313209)*
9. Spatula
10. Stirrer/heater and magnetic flee
11. Sterile Filter Unit Millex 0.22 *(Supplier name; Scientific Laboratory Supplies Ltd.. Supplier product code; SLGS033SS)*
12. BD Plastipak™ 10mL Syringe (*from Sanger stores; product code PLSY0004*)
13. 2,2,2 Tribromoethanol (97%) *(Supplier name; Sigma Aldrich Co.Ltd. Supplier product code; T48402-25G)* **Hazardous substance, must be handled inside the chamber of the safety cabinet, whilst wearing gloves and safety spectacles.**
14. 2-methyl-2-butanol (Tertiary amyl alcohol) *(Supplier name; Sigma Aldrich Co.Ltd. Supplier product code; 152463-250ML)* **Hazardous substance, must be handled inside the chamber of the safety cabinet, whilst wearing gloves.**
15. Embryo transfer water
16. Biological safety cabinet (Esco model LA2-3A1)
17. pH testing strip (pH 4 to 7) *(Supplier name; Scientific Laboratory Supplies Ltd. Supplier product code; PAP2076)*
18. Aluminium foil

Associated SOPs/Documentation:

- SOP0023 – Anaesthesia of mice with Avertin

Staff: This task can be completed by one phenotyper.

PROCEDURE:

1. Put on disposable gloves and **safety spectacles**.

2. Prepare the biological safety cabinet for use.

3. Switch on the stirrer/warmer and allow to heat to 40 ^{O}C.

4. Weigh 0.625 grams 2,2,2-tribromoethanol onto a folded square of chromatography paper then transfer into a clean 250ml beaker.

5. **In the biological cabinet**, measure 1.25mL 2-methyl-2-butanol and dispense over the 2,2,2 tribromoethanol in the beaker.

6. Add the magnetic stirrer into the beaker and place on the stirrer/warmer, setting the rpm to provide vigorous stirring (approx. 30rpm).

7. When dissolved, add 24mL of embryo transfer water, stirring continuously for approximately 20-30 minutes. Keep the beaker covered with aluminium foil during this time.

8. Add another 24.5mL of embryo transfer water when solution is no longer cloudy, up to a final volume of 50ml.

9. Test a drop of the solution with pH testing strip.
   9.1. If the pH of the solution is less than 5, it should be presumed to have degraded and must be disposed of according to the current practice.

10. Prepare label with:
    10.1. '1.25% Avertin'
    10.2. The date it was prepared
    10.3. Expiry Date (1 month from preparation date)
    10.4. The initials of the person who prepared it

11. Filter the solution through a cellulose acetate filter attached to a syringe.

12. Wrap the outside of the tube with a layer of foil and clearly label outside the foil wrap.

13. Store in the 4°C refrigerator.

14. Rinse and dry equipment thoroughly.

15. Bag up used equipment in autoclave bags, seal and place into the basket near the autoclave.

16. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**