WELLCOME SANGER INSTITUTE

STANDARD OPERATING PROCEDURE PACKET

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SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Necropsy and Tissue Collection – Mouse Pipelines

INTRODUCTION:

This document details the processes involved in the terminal tissue collection from control and KO adult mice (approx.16 weeks old).
Tissues are collected for both the Sanger Biobank and for collaborators dependent on the requirements of the pipeline and collaboration at the time of collection.

Task	Number of males collected per line	Number of females collected per line
Sanger Biobank (full necropsy)	2	2
3i ears	2	2
3i Spleen (CTL)	2	2
3i spleen (flow)	3	3
3i Right hind limb	3	3
3i mesenteric lymph nodes	3	3
OBCD Left hind limb and tail (bones)	0	6 (2 for pilot lines)
OBCD Left hind limb (joints)	7	0
Brain	3	0
Eyes	3	0
Skin Histopathology	0	1
Tail Epidermis Wholemount	0	2

ABBREVIATIONS:

3i = Immunity, infection and immunophenotyping (strategic award)
CTL = Cytotoxic T cells
DCF = Data capture form
HBSS = Hank's balanced salt solution
L-HL = Left hind limb
mLN = Mesenteric lymph node
NBF = Neutral buffered formalin
PBS = Phosphate buffered saline
OBCD = Origins of bone and cartilage disease (strategic award)
QC = Quality control
PFA = Paraformaldehyde
R-HL = Right hind limb
RO = Reverse osmosis
RSF = Research support facility
RT = Room temperature
SOP=Standard operating procedure

HEALTH & SAFETY:

This procedure is covered by the following risk assessment **WTSI_1204**

- Appropriate personal protective equipment (PPE) is to be worn at all times when handling the samples (RSF scrubs, gown, safety shoes, overshoes, gloves, safety glasses and appropriate mask).
- Entry procedure to the RSF should be followed including the wearing of scrubs.
- In addition to the above, when sources for LAA (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or down flow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- A functional down-flow table must be used when performing mouse necropsies and using fixatives.
- Safety glasses must be worn at all times when carrying out this procedure.
- Fire extinguishers are located in the clean corridor opposite the end of Yellow
- Lone working is not routinely done for this procedure.
- New workers are to be supervised until deemed competent to perform this assay.
- Individual risk assessments for lone workers, young persons and new or expectant mothers would be performed to define any exclusion for performing this assay.

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).

STAFF: This procedure requires one member of staff per assigned task.

RESOURCES

Equipment

1. Downflow table- Check reading is in green safety zone
2. Balance for organ weights
3. Camera + Lens and measuring scale
4. Dissection Tools
5. PPE – gloves, safety glasses, surgical gown, overshoes and masks.
6. Dissection boards and pins
7. Petri dishes
8. Absorbent tissues
9. Biopsy Cassettes
10. Spray bottle with 70% Ethanol Highly flammable. To be used on a downflow table wearing correct PPE including safety glasses
11. Disposable bags for cadavers

Reagents

1. PBS 1 x pH7.4 and plastic container
2. Igloos and wet ice
3. Orange tube rack and baskets

4. Trigene
5. Alcohol wipes
6. Liquid detergent
7. 70% ethanol Highly flammable. To be used on a downflow table wearing correct PPE including safety glasses. Refer to Appendix 1 for recipe.
8. 1x HBSS: Hank's balanced salt solution (Invitrogen Cat No 14170-138)
9. 10% Neutral buffered formalin (Leica 5 litre NBF Cat No.38BHP500NBF). Toxic, corrosive, carcinogenic, irritant causes damage to organs and skin and respiratory sensitizer. To be used on a downflow table wearing correct PPE including safety glasses.
10. Zinc chloride fixative for Eyes. Harmful, corrosive and causes damage to organs. To be used on a downflow table wearing correct PPE including safety glasses. Refer to Appendix 1 for recipe.
11. Davidson's Fluid (Fixative for Sanger Eye Collection). Highly flammable, toxic, corrosive, irritant and causes damage to organs. To be used on a downflow table wearing correct PPE including safety glasses. Refer to Appendix 1 for recipe.
12. 1x PBS pH 7.4 (-$MgCl_2$, -$CaCl_2$) (tail) – Dilution of 10x PBS solution

ASSOCIATED SOPS/DOCUMENTATION:

- SOP IC1 Entry procedure to RSF

Procedure

Appropriate personal protective equipment (PPE) is to be worn at all times (RSF scrubs, gown, safety shoes, overshoes, gloves, safety glasses and appropriate mask).

Work flow of terminal collections

During the terminal collections each mouse will be processed as following:

Preparation of collection tubes & dissection work space

1. All collection tubes are prepared in advance with labels and media required.

2. Set up the downflow table with the following tools:
    a. Dissection tools
    b. Pen
    c. Disposable bag for cadavers
    d. Absorbent tissue to dry hearts
    e. Petri dish on top of the scale
    f. Cork board
    g. Biopsy cassettes
    h. 70% ethanol spray bottle
    i. Plastic cup containing PBS 1x pH7.4

3. Switch on scales, once calibrated, place a petri dish on the weighing platform and press "tare".

4. Adjust the dissection table height and switch on overhead lighting as required.

METHOD - Necropsy collections

1. For each mouse, perform a check to ensure the information on the cage card match the mouse and the collection tubes:

    a. Identify the mouse by sex and ear mark
    b. Check ear and tail genotyping samples have been taken
    c. Check the collection tubes match the list on the mouse necropsy card
    d. Check the collection tubes have the correct mouse barcode.

2. Check reflexes before dissection starts and spray the mouse with 70% ethanol to dampen the fur.

3. Cut both sides of the rib cage to expose the heart and lungs.

4. Confirm death by dissecting the heart out, remove any fat and blot dry on tissue.
    a. To avoid missing tissues, if the mouse requires a *Full Necropsy* and *Spleen for collaborator,* collect the spleen then take the heart. **This is the only scenario where the heart is not the first organ to be removed.**

5. Place the heart on the balance to be weighed if required. This value should be recorded on the Phlebotomy DCF on the mouse database by pressing “Print” icon on the scale.

6. Mark the procedure as complete and save the Phlebotomy DCF.

7. Record the heart weight on the necropsy mouse card.

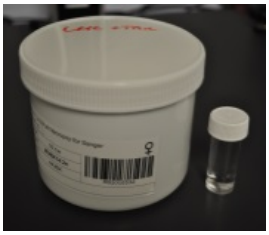
8. Place the heart in formalin pot (Full Necropsy) or discard it in the disposable cadaver bag.

9. Collect all expected tissues as described below for each task

10. Dissection board should be washed and tools should be cleaned with ethanol between mice.

Task - Full Necropsy for Sanger (2 males and 2 females)

All tissues must be placed in the labelled formalin pot (except eyes - Davidson’s tube)



1. **Left ear pinna -** Harvest left ear pinna, cutting right from the base.

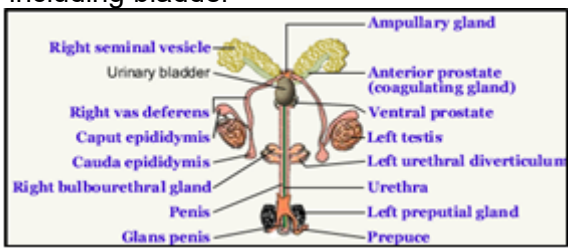
2. Open the peritoneal cavity by a single midline incision through the abdominal wall from sternum to pubis. *Be careful not to cut the bowel loops below.*

3. Cut across below the ribs and pin down as required on the board to ease access.

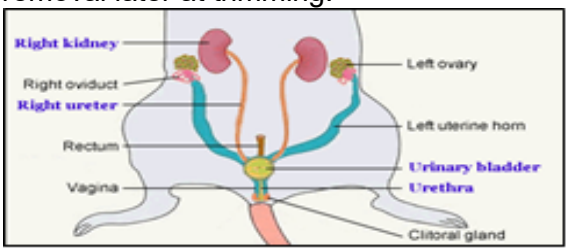
4. **Skin and mammary glands -** Detach skin and peritoneal cavity and collect left inguinal mammary glands and skin. Inspect for enlarged superficial lymph nodes and inguinal mammary glands with overlying skin.

5. **Reproductive organs –**

5.1 **Male-** Remove the testes, epididymis and seminal vesicles in one block including bladder



5.2 **Female -** Remove the uterus with bladder and ovaries for females. Cut well below the uterus at the level of the cervix to remove the uterus intact with ovaries. Leave urinary bladder attached to uterus, for removal later at trimming.

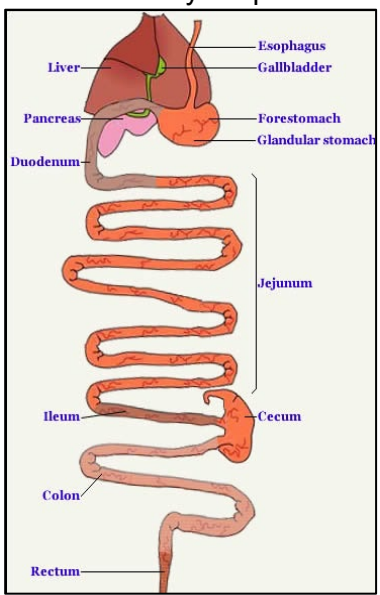


6. **GIT –**

6.1 Identify the abdominal organs in situ first before cutting any further. Identify the stomach and cut the oesophagus just before it enters the stomach and the rectum as low as possible. Gently pull the entire GIT out including the spleen.

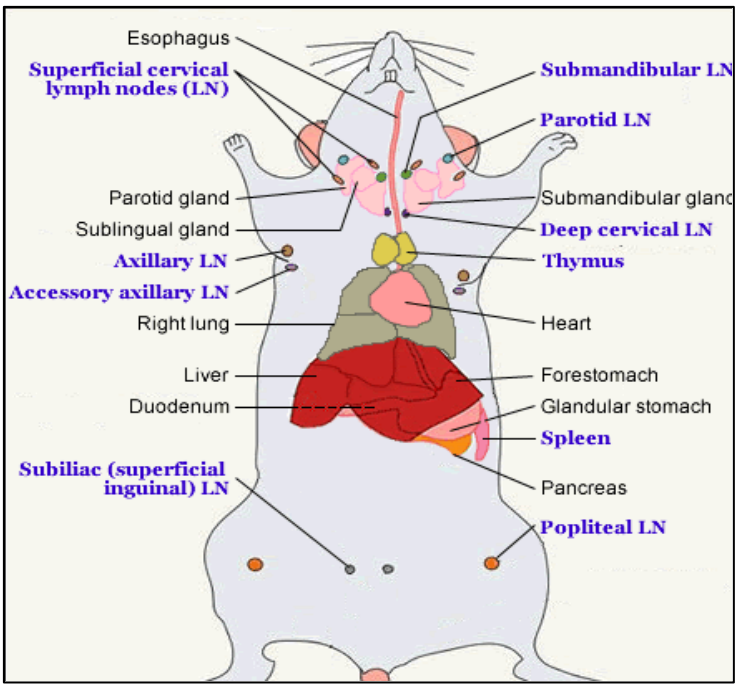
6.2 Examine the duodenum, jejunum, ileum, caecum, colon and rectum. Retain all samples intact for trimming later.

6.3 Examine Peyers patches and leave mesenteric lymph nodes attached to aid identification at trimming.



Mouse GI tract

7. **Liver -** Remove all the liver lobes. Inspect all lobes of the liver for abnormalities. Take one slice through the left and right median lobe to include gall bladder.



Thoracic organs of a mouse

8. **Kidneys and adrenals –**

   8.1 Inspect kidneys, adrenals and ureters for any abnormalities.

   8.2 Remove both kidneys and detach the adrenal glands.

   8.3 Biopsy cassettes must be used for the adrenal glands to aid identification at trimming and to prevent loss of the tissue.

   8.4 Cut the right kidney to aid identification at trimming.

9. **Lungs and thymus -** Remove the lungs and thymus together by grasping the trachea at top of the rib cage and pulling forward and down.

10. **Salivary glands, trachea and oesophagus –**

    10.1 Take both salivary glands

    10.2 Collect the thyroids in place with the trachea and oesophagus.

    10.3 Place thyroids, trachea and oesophagus into biopsy cassette with the adrenal glands.

11. **Spine -** Take a segment of the vertebral column (lumbar part) after removing the rest of the organs.

12. Turn the animal over onto its ventral surface.

13. **Brown fat -** Remove the brown fat, from between the shoulder blades.

14. **Eyes –**

    14.1 Check the eyes and orbital contents (especially the Harderian gland)

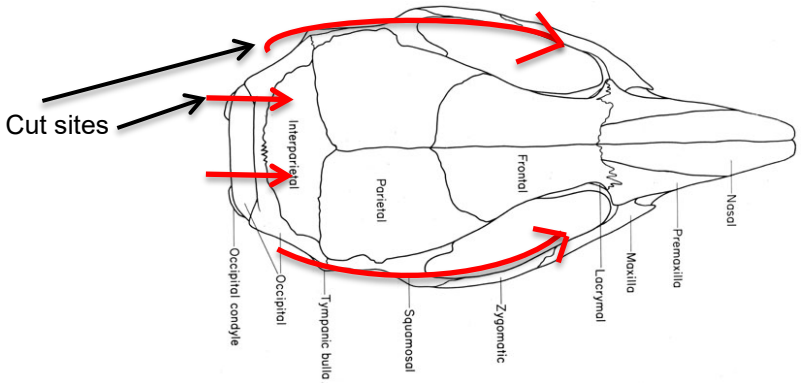
    14.2 Harvest the eyes by gripping the eyelids with forceps and elevating the attached globes.

    14.3 Place the eyes in Davidson's fluid for Sanger Biobank.

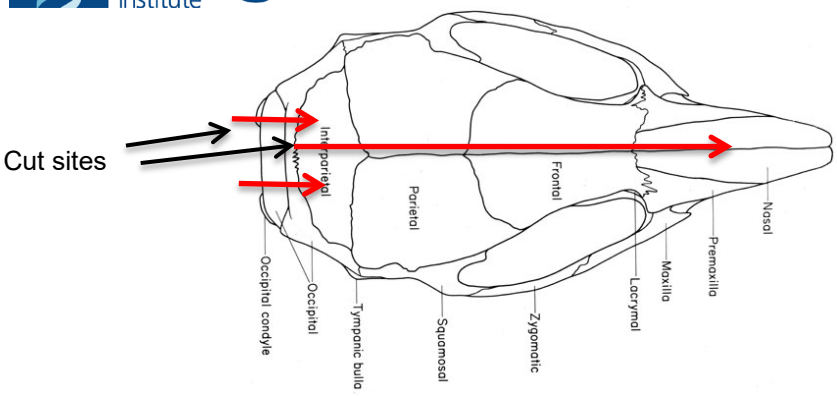
    14.4 Place the eye collection tube inside formalin pot.

15. **Brain – Two methods can be used for brain removal**

    15.1 Make a vertical incision through the left and right side of the bone protecting the cerebellum. Then lift bone up to expose cerebellum. Use small black handled scissors to very gently cut both sides of the cranium, and then peel the cranium gently away from the brain. Then remove the brain using a fine spatula. Place brain and skull in the formalin pot.



    15.2 Using small black handled scissors make a vertical incision through the left and right side of the bone protecting the cerebellum (occipital bone) and remove. Gently cut along the midline of the brain towards the nose and peel away the cranium. The brain can be removed using a fine spatula.



16. **Right leg -** Take the right leg for the knee joint, leave muscles intact and remove skin.

17. **Collaborator tissues –** if required collect any collaborator tissues as described in the relevant section below.

18. **Carcass** - Place the carcass in the formalin pot

19. Close the lid and write the time of start of the fixation on the formalin pot lid.

20. Fixed Full necropsy tissue kept at room temperature.

21. Post collection fixed full necropsy tissues should be transported, using a metal carrier box, to the lab for processing.

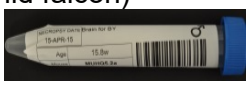
Note:

1. If there is any missing or damaged tissue this must be reported to a manager with the appropriate collection status used against this task on the Mouse necropsy page.

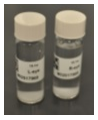
2. If any abnormalities are found:
   - 2.1 Record them in the General comments and tissue abnormalities section of the on the Mouse necropsy page
   - 2.2 Take macroscopic pictures using the camera and macro lens

Task - Brain (3 males)

Collection instructions	Collect brain as described in full necropsy description above
Brain weight	Place brain on the petri dish and weigh it. Enter the weight in the 'Brain weight' provided space on the Phlebotomy DCF. Write the value on the Necropsy mouse card. **Weigh the brain before placing it in 10% NBF**

Collection tube	With care drop fix the brain in 10% NBF 15ml tube (blue lid falcon) 
Storage	Keep at RT
Transport	**Metal carrier box transported to lab**
Post-collection	Stored in cold room until shipment
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

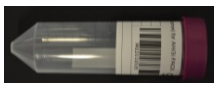
Task - Eyes (3 males)

Collection instructions	Collect eyes as described in full necropsy description above
Collection tubes	Zinc chloride fixative in labelled glass tubes 
Storage	Keep at RT
Transport	**Metal carrier box transported to lab**
Post-collection	Stored at room temperature in lockable cabinet.
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task - Spleen and mesenteric lymph nodes for 3i flow (3 males and 3 females) and Spleen for 3i CTL (2 males and 2 females)

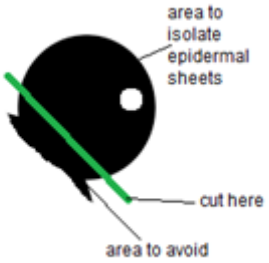
Collection instructions	Open peritoneal cavity and collect spleen and mesenteric lymph nodes. Remove all the extra fat.
Collection tubes	labelled 1.7 ml eppendorfs (blue, red and green) with fresh HBSS 
Storage	Keep on ice
Transport	Igloo transported to lab
Post-collection	Same day shipment to collaborator on ice
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task – Right hind limb for 3i (3 males and 3 females)

Collection instructions	Turn the animal over onto its ventral surface. Remove the right leg cutting between the spine and hemi-pelvis. Remove the skin and extra fat. Quickly rinse with tap water and wash the leg thoroughly on a plastic cup with PBS 1x pH7.4 to remove any fur.
Collection tubes	labelled 50 ml falcons (purple lid) with fresh HBSS 
Storage	Keep on ice
Transport	Igloo transported to lab

Post-collection	Same day shipment to collaborator on ice
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task - Ears for 3i (2 males and 2 females)

Collection instructions	Turn the animal over onto its dorsal surface. Cut both ears straight, grabbing the loose pinnae only. Avoid any fat or connective tissue. 
Collection tubes	PBS labelled 1.7 ml eppendorfs (clear) 
Storage	Keep at RT
Transport	**Small rack transported to lab**
Post-collection	Processed on the same day in lab
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task – Left hind limb and tail (OBCD bone) (6 females)

Collection instructions	Turn the animal over onto its ventral surface. Remove the left leg cutting between the spine and hemi-pelvis. Remove the skin and extra fat from the limb. Isolate the tail and remove the skin.
Collection tubes	Ethanol 30 ml tubes (white lid) 
Storage	Keep at RT
Transport	**Metal carrier box transported to lab**
Post-collection	Stored in cold room until shipment
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task – OBCD Joint/LHL (7 males)

Collection instructions	Turn the animal over onto its ventral surface. Remove the left leg cutting between the spine and hemi-pelvis. Remove the skin and extra fat from the limb. Isolate the tail and remove the skin.
Collection tubes	10% NBF 30ml tube (green lid) 
Storage	Keep at RT
Transport	**Metal carrier box transported to lab**
Post-collection	Stored in cold room until shipment
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task – Skin histopathology (1 female)

Collection instructions	Turn the animal over onto its dorsal surface. Remove a piece of dorsal skin from the lumbar region
Collection tubes	60 ml pot with formalin 
Storage	Keep at RT
Transport	**Metal carrier box transported to lab**
Post-collection	Processed in lab and stored as paraffin blocks at RT until shipment
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task – Tail epidermis wholemount (2 females)

Collection instructions	Turn the animal over onto its ventral surface. Cut off the tail just below the base of the spine.
Collection tubes	1x PBS pH 7.4 (-$MgCl_2$, -$CaCl_2$) 15ml tube (blue lid falcon)

	
Storage	Keep on ice
Transport	Igloo transported to lab
Post-collection	Processed on the same day in lab
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

Task - QC Samples

Collection instructions	Collect brain, liver and kidneys as described in full necropsy
Collection tubes	60 ml pot with formalin 
Storage	Keep at RT
Transport	**Metal carrier box transported to lab**
Post-collection	Processed in lab
Missing / Damaged tissues	Report & add appropriate collection status to task on mouse necropsy page

METHOD - After each necropsy task collection

1. After finishing the basic dissection, check all required organs are collected including the carcass for a full necropsy.

2. Fill out the database with a short record of the necropsy and of any abnormalities found.
    a. Comments may be added to the general comments box as required.
    b. Abnormalities can be added using the drop down list called 'Abnormal tissues'. Use an MP term (http://www.informatics.jax.org/vocab/mp_ontology)

3. Change status from expected to collected for the tasks assigned for the mouse, check the necropsy mouse card to confirm all tasks have been collected.

4. Record the time for the start of the fixation and technician name.

5. If database is not working, record all times and keep a record of all weights and abnormalities on the mouse collection card.

Method- At the end of all necropsy sessions

1. At the end of a collection check the following:-
    1.1 Necropsy reports have been filled in and assigned tasks collected (located on database).
    1.2 Heart & brain weights have all be entered (located on database)
    1.3 No empty tubes are left.

2. Run a missing tissue report to check if any tissues were not marked as “Collected” on the database and investigate these.

MISSING TISSUE

If any tissues are found to be missing from the collection, according to the histology report it must be replaced. Tissue missing from a full necropsy will be replaced by another full necropsy.

CLEANING & MAINTENANCE

- **The residual animal carcass, any tissues soiled with formalin or body fluids, should be disposed of in a cadaver bag and placed in the cadaver freezer.**
- **All dissection instruments should be scrubbed clean of blood in the sink with antibacterial liquid soap, then rinsed and dried. Check that all instruments are accounted for.**
- **Clean the downflow table inside and out with liquid detergent.**
- **All baskets and racks should be cleaned with Trigene or alcohol wipes after each collection.**
- **Clean all surfaces and equipment.**
- **Transfer all other waste to an offensive waste bag or clearly labelled container.**

Appendix 1 – reagent preparation

1. Davidson’s fixative – **Contains Acetic acid - Irritant, flammable substance that causes damage to organs. Must be handled on a down-flow table and wearing correct PPE. 1L bottles stored inside fixatives safety cabinet. This solution is made up in a fumehood using the following reagents:**

   Preparation: **Wearing correct PPE and using a fumehood**

   - 1L glass bottle
   - 300 ml of 95% ethanol
   - Gently add 200 ml of formalin (Leica Cat 38BHP500NBF) avoiding splashing
   - Add 100 ml of glacial acetic acid (Sanger Stores Cat BCAC0013) – **an irritant, highly flammable substance, damaging to organs.**
   - Add 300 ml of water.
   - Mix gently by agitating the bottle.
   - Seal the lid with parafilm,
   - Label the bottle with flammable and irritant hazard labels.
   - Store in fixatives safety cabinet

2. 70% ethanol – Prepared fresh during Set Up. **Highly flammable substance made up with 100% ethanol and RO water Must be handled on a down-flow table and wearing correct PPE.**

   Preparation: **wearing correct PPE**

   - Using a 1L measuring cylinder to measure 700ml of 100% ethanol
   - Top up to 1L with RO water

3. 2% zinc fixative - **Harmful, corrosive and causes damage to organs. 1L bottles stored in fixatives safety cabinet. Must be handled on a down-flow table and wearing correct PPE. This solution is made up in a fumehood using the following reagents:**

   Preparation: **Wearing correct PPE and using a fumehood**

   - 1L glass bottle
   - Prepare 500 ml of 4% PFA solution (Electron Microscopy Sciences Cat No 15714-S) - **toxic, corrosive, carcinogenic, irritant, respiratory and skin sensitizer** in PBS pH7.4 (Sanger stores) in a 1 lt bottle (62.5ml 32% PFA made up to 500ml PBS pH 7.4)
   - Add 20g Zinc chloride (Fluka Cat No 96470) - **harmful, corrosive substance and causes damage to organs**. Final concentration 2%
   - Add 200 ml of isopropyl alcohol - **highly flammable, irritant, and damaging to organs**. Final concentration 20%
   - Add RO water to make a total volume of 1000 ml
   - Mix all the above using a stirrer/agitator
   - Seal the lid with parafilm
   - Label the bottle as harmful and corrosive
   - Store in flammables safety cabinet

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Trimming Tissue Samples

INTRODUCTION

Instruction on how to trim and orient tissues.

HEALTH & SAFETY

- **RA003** – Hazardous Substance ; Section RA003.3.
- **RA004** – Physical Hazards; Section RA004.4.2
- **RA005** – Biological Hazards : Section RA005.1,RA005.6

RESPONSIBILITIES

All staff responsible for performing this procedure should ensure that this SOP has been read understood and is followed.
The procedure is to be performed inside a specified histopathology room on a functioning downflow table.
The procedure is to be carried out by a member of staff or scientist who has been properly trained in the procedure.

RESOURCES

PPE – gloves, goggles, and lab coat
Properly functioning downflow table
Cutting up board
Dissection tools
Blue Absorbent tissues
Blue Biopsy Pads
Pre-printed Histology cassettes
10% Formalin
50% Ethanol

Staff : one member of staff required to carry out this task.

METHOD

Before entering the room put on the appropriate PPE – gloves, goggles and lab coat.

1. Use the cassettes with mouse ID and relevant tissue code (normally printed in advance).

2. Check that the fixation is less than 24 hours for soft tissue, up to 8 hours for Brain and up to 24 hours for bones. This is recorded on the database.

3. Use a cutting board and a scalpel to trim the organs, if necessary. Trim excessive fat off all tissues, especially the uterus, ovaries, mammary glands, testis, epididimus, mesenteric lymph nodes and brown fat.

4. Several organs can be processed together in the same cassette – see table below.

5. Wrap ALL urinary bladders and small tissues in blue biopsy tissue. Trigeminal ganglions must be placed between 2 biopsy pads.

6. After the organs are trimmed the cassette is sealed with a lid. Soft tissues are placed in neutral buffered Formalin (NBF) and bones are placed in 50% ethanol. Ready to load onto the tissue processor.

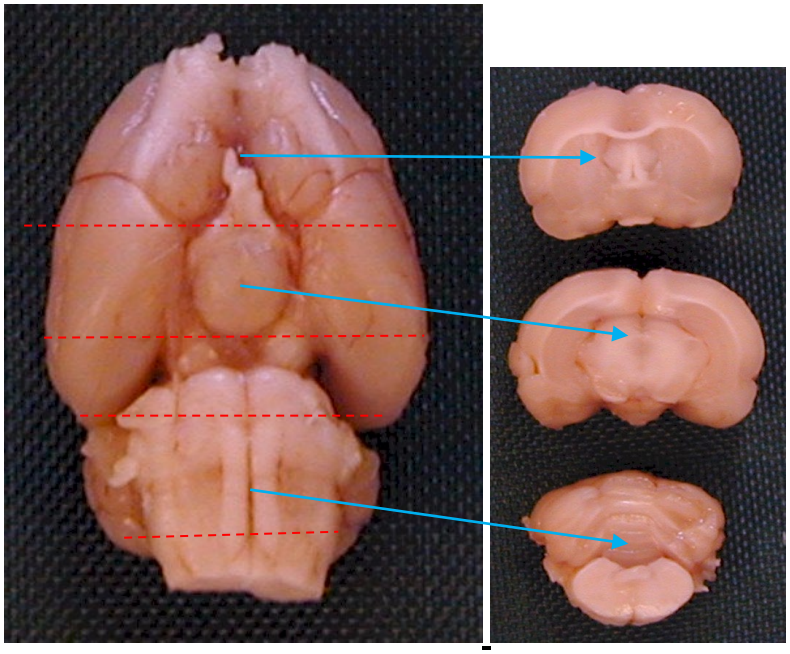
7. Bones in labelled cassettes are left in labelled pot, filled with formalin. These are then placed into EDTA the following morning.

8. Put all the cassettes in the tissue processor. Select the right program and start the processing.

Block Number	**Tissue**	**Orientation** LS: Longitudinal section ; TS: Tranverse sections
1	Lung	Left lobe only, LS along bronchi.
1	Thyroids,Trachea + Oesophagus	Trim T.S to include Thyroids, oesophagus + Trachea. Cut off excessive trachea, exposing thyroids. Embed trachea side down.
2	Heart	Cut one third off L.S, exposing both auricles, ventricles and aorta. Only process this part, discard off cut.
2	Thymus	Process whole
2	Brown Fat	Process one lobe.
3	Mesenteric L/N	Process whole
3	Adrenals x2	Process whole (LS)
4	Liver/gall bladder	Left and Right Median lobes cut to include Gall bladder
4	Spleen	One TS sample
4	Kidneys	Left kidney only (LS)
4	Urinary Bladder	TS
5	Uterus + ovaries	TS sample through bifurcation. LS sample through uterine horn including ovary
5	Testes + Epididymides x2	Process both LS
5	Prostate	Whole
5	Seminal Vesicles	TS x 2 (including coagulating glands)
6	Sternum	L.S (embed on edge) Trim to expose bone marrow.

7	Pancreas	Process whole
7	Skeletal Muscle	TS
7	Salivary Glands	Process whole x 2
8	Stomach	Process 2 LS samples through glandular + non glandular area.
8	Ileum, Colon, Duodenum Rectum,Jejunum with Peyer's patch	TS through each piece
9	Pinna	LS Trim to 1 cm.
9	Skin & Mammary	LS Skin + Mammary. Trim to 1cm and remove all fat.(Take care not to remove mammary tissue)
10	Eyes x 2	Process whole- sagittal
11	Spinal Cord	TS Lumbar region x 2 trim off excessive muscle
12	Brain	Transverse Section x 3 (See Diagram in appendix)
13	Femur + joint	LS through knee joint. Trim off excessive muscle.
13	Tibia + fibula + muscle	TS
14	Trigeminal ganglion x2	whole
15	Extra tissues	To be determined

Appendix – brain sections



- Make the first cut immediately posterior to the Optic Chiasma.
- Make the second cut at the posterior edge of the Mammillary Body.
- Make the third cut through the Pons as shown.
    - Embed all samples on POSTERIOR surface.
- Posterior surfaces shown.
- Trim off anterior portion to fit cassette.

Missing Tissue

If any tissue is found to be missing from necropsy at trimming please enter on the database ASAP. Tissue missing from a full histology collection will be replaced by another full histology collection.

CLEANING & MAINTENANCE

Any tissues soiled with formalin should be disposed of in a clinical waste bag.
All dissection instruments should be scrubbed clean of blood in the sink, soaked in antibacterial solution (Hibiscrub or equivalent) rinsed and dried.
Wipe down the downflow table.

Transfer all other waste to a yellow clinical waste bag or clearly labelled container. This is then transferred to a designated area, for waste disposal.

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Decalcification of bones for histology

INTRODUCTION

EDTA is used to remove the calcium from bones collected during necropsy. This method is used to facilitate generation of wax sections of bones and vertebrae.

HEALTH & SAFETY

- **RA003** – Hazardous Substances V1 ; Section RA003.3
- **RA004** – Physical Hazards V1 ; Section RA004.4.2

RESPONSIBILITIES

All staff performing this procedure are responsible for ensuring that this SOP has been read, understood and where applicable is followed in accordance with the relevant PPL. All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure.

RESOURCES

- PPE- lab coat, gloves and safety goggles
- 10% Neutral buffered formalin Cat No. 00840E (supplier Leica Biosystems Peterborough).
- EDTA 10% buffered solution, pH 7.4 (Dissolve 100 g of EDTA in 280 ml of 1N NaOH and 600 ml of deionised water and adjust with water to obtain a volume of 1 litre). Made by the Media Kitchen.
- Rocker

STAFF:

One person required to carry out this task.

Associated SOPs:

SOP0012 - Trimming Tissue Samples
SOP0004 - Use of the VIP Tissue Processor
SOP0169 - Embedding and archiving tissue samples

METHOD

Knee joint, vertebrae and sternum

- Dispose of the neutral buffered formalin within the pot containing the organs in the appropriate waste container for collection by the waste team.
- Fill each pot with 10 % buffered EDTA.
- Place the pots on a rocking platform under gentle agitation for 72 hours at room temperature.
- Then change the EDTA solution and place the pots on the rocking platform under gentle agitation for a further 72 hours. The tissues can stay in EDTA for 6 to 7 days.
- Trim the tissue. (refer to SOP0012 Trimming Tissue Samples).
- Place in a cassette and process the samples on appropriate program in the Tissue- Tek VIP 5 Tissue Processor (refer to SOP0004 - Use of the VIP Tissue Processor)
- Embed in paraffin wax (refer to SOP0169 - Embedding and archiving tissue samples)

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Use of the Tissue-Tek VIP 5 Tissue Processor

INTRODUCTION

To process tissues through selected reagents, into paraffin blocks to preserve cell morphology.

HEALTH & SAFETY

- **When carrying out this procedure a lab coat, safety goggles and gloves (Marigold Industrial Blue Nitrile) must been worn.**
- **The solution changes must be carried out in a fumehood.**
- **RA003** – Hazardous Substances ; Sections RA003.1, RA003.3
- **RA004** – Physical Hazards ; Sections RA004.1.1, RA004.1.2, RA004.5.4, RA004.10

RESPONSIBILITIES

All staff performing this procedure are responsible for ensuring that this SOP has been read, understood and where applicable is followed in accordance with the relevant PPL. All staff should be trained and competent to perform the procedure.

RESOURCES

PPE: Labcoat, gloves, safety goggles.
Chemicals: Xylene, Ethanol, IMS, Formalin.
Wax: Gurr Paramat pastillated wax congealing point approximately 58c.
Water: RO water for flushing processor
Filters: Charcoal filters
Drip tray for processing baskets loaded with cassettes.
Processing baskets and metal lids.

Staff: One member of staff is required to carry this out.

METHOD

Operation of tissue processor

Processing tissues

- Open lid of processing chamber and place baskets containing samples inside. Replace basket lid.
- Close chamber lid with latch and slide locking bar across to lock.
- Press enter using access code
- Press “tissue processing” and select program, using arrow keys.
- Check correct end time and date are selected.
  Bottle check will take place, before delayed start will appear at the bottom of the screen.

  Monitor solution usage by keeping track of runs done on VIP.

After processing

- The VIP indicates process complete and drain.
- Press button to drain.
- Remove your cassettes (use a drip tray to transfer cassettes to embedding station).
- Shut lid using latch and sliding locking bar.

Flush Cycle

- It is necessary to flush the processor before starting another processing programme.
- Place baskets/cassette carriers and lids in chamber to clean.
- Clean off excess wax from seal and make sure seal is pushed in place in VIP lid.
- Select Cleaning cycle 1 or 2 (they are the same)
- When the flush cycle is complete, empty chamber and dry using tissues.
- Remove screw and circular grid at bottom of chamber and mop/wipe out, then replace.

Routine Maintenance and Waste disposal

This part must be done in a fumehood.

- Change formalin, 50% ethanol, 70% ethanol and ethanol 90% every five runs.
- Rotate and change one 100% ethanol, one xylene and one wax every five runs.
- Change flush reagents: xylene, IMS and RO water every five runs.
- Machine will alarm when flush reagents require changing.
- After changing flush solutions, reset usage to zero.
- Once changed go to solution control, setup and clear count.
- Then go to clear line, for each of the solutions.
- Remove excess wax from the o ring / seal in the lid of the VIP and make sure firmly pushed in place.
- Remove all excess wax from the VIP cabinet.
- Transfer all waste to a yellow clinical waste bag or clearly labelled waste container. This is then transferred to a designated area.

Appendix

Processing schedules used.

Sakura Tissue Tek VIP 5 Processor - Program for Brains, Eyes and Bones.				
Solution	**Time**	**Temperature**	**PV**	**Mix**
50% Ethanol	45 minutes	Ambient	On	Slow
70% Ethanol	45 minutes	Ambient	On	Slow
90% Ethanol	45 minutes	Ambient	On	Slow
100% Ethanol	60 minutes	Ambient	On	Slow
100% Ethanol	60 minutes	Ambient	On	Slow
100% Ethanol	60 minutes	Ambient	On	Slow
Xylene	60 minutes	Ambient	On	Slow
Xylene	60 minutes	Ambient	On	Slow
Xylene	60 minutes	Ambient	On	Slow
Wax	75 minutes	60°c	On	Slow
Wax	75 minutes	60°c	On	Slow
Wax	75 minutes	60°c	On	Slow
Wax	75 minutes	60°c	On	Slow

Sakura Tissue Tek VIP 5 Processor - Program for soft tissues.				
Solution	**Time**	**Temperature**	**PV**	**Mix**
10% NBF	Depending on delay	35°c	On	Slow
50% Ethanol	30 minutes	35°c	On	Slow
70% Ethanol	30 minutes	35°c	On	Slow
90% Ethanol	30 minutes	35°c	On	Slow
100% Ethanol	40 minutes	35°c	On	Slow
100% Ethanol	40 minutes	35°c	On	Slow
100% Ethanol	40 minutes	35°c	On	Slow
Xylene	20 minutes	35°c	On	Slow
Xylene	20 minutes	35°c	On	Slow
Xylene	20 minutes	35°c	On	Slow
Wax	25 minutes	60°c	On	Slow
Wax	25 minutes	60°c	On	Slow
Wax	25 minutes	60°c	On	Slow
Wax	25 minutes	60°c	On	Slow

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Embedding and Archiving Tissue Samples

INTRODUCTION

This SOP will describe how to carry out tissue embedding using the Thermo Scientific Histo Star Embedding Station.

This SOP will also describe how to carry out archiving/storage of tissue samples.

HEALTH & SAFETY

- **RA003 –** Hazardous Substances; Sections RA003.3
- **RA004** – Physical Hazards; Sections RA004.1.1, RA004.4.2 RA004.5.4.
- **RA005** – Biological Hazard; Sections RA005.2, RA005.6

RESPONSIBILITIES

All staff performing this procedure are responsible for ensuring that this SOP has been read, understood and where applicable is followed in accordance with the relevant PPL. All staff should be trained and competent to perform the procedure.

RESOURCES

- Thermo Scientific Histo Star Embedding Station, in 2 parts:

  1 -The embedding module consists of:
  a. User interface
  b. Hot and cold spots
  c. Wax tank with adjustable wax dispenser
  d. Tissue tank
  e. Metal mould storage space

  2 – Cold module

- Gurr Paramat pastillated wax
- Tissue Tek metal moulds/range of sizes
- Forceps
- Solid scalpel/blunt knife
- Plastic scraper

Staff: One member of staff required for this procedure.

METHOD

Switching on and preparing the embedding station

The Wax tank and Tissue tank both contain molten wax (+65°C) and are permanently switched on, all other components have been pre-set to switch on at set times on set days. Outside of these times the embedding station can be switched on by pressing the **ON** button on the touch screen user interface.

Allow time for the Cold module to reach operating temperature before use (+5°C)

If required, additional lighting may be switched on by following the menu sequence on the user interface (Menu > Options > Light) and selecting the required light settings. Refer to the manufacturer's user guide to carry out any other functions.

Remove processed tissues from the Tissue Processor and place in the Tissue tank of the embedding module, ensuring that all tissue cassettes are fully immersed in molten wax.

Forceps are stored in a heated forceps block, forceps tips should be kept clean and clear of tissue and wax.

Embedding tissues

Dispense molten wax in to an appropriate sized metal mould and place on one of the hot spots regions of the embedding station.
Remove a tissue cassette from the Tissue tank, place on a hotspot region and remove the lid.

Check that the cassette contains all the expected tissue for that cassette number.

Using forceps move tissues from the cassette to the metal mould, arranging the tissues in the optimum configuration for sectioning (Appendix 1 - Orientation for embedding Biobank tissues).

Move the metal mould and tissues to the cold spot and allow the wax start to cool, fixing the tissues in place.

An alternative embedding method is to place the molten wax on to the cold spot briefly, allowing a thin wax of wax to cool before placing the tissues in to the mould.

Place the cassette on top of the metal mould and dispense enough molten wax fill the cassette.

Transfer the metal mould and cassette unit to the cold module to allow the wax to set.

Repeat this process for all tissue cassettes.

Once the wax has fully set, the blocks can be removed from the metal mould.

Completed blocks may have excess wax around the edges; this excess can be removed using the Para Trimmer on the embedding station or by using a solid scalpel or a blunt knife.

Switching off the embedding station

Once all tissues have the successfully embedded, the embedding station should be put in to “sleep” mode by pressing the “Sleeping” icon

Missing tissues

Any cassettes found to have a tissue missing should have the details recorded on the database.

Damaged or missing tissues are to be replaced and collections to be arranged if required.

Block archiving

Blocks should be sorted in to numerical order by cassette number for each Mouse Barcode.

Blocks are then kept according to the Necropsy Batch Number and are registered on the Mouse Database once all the expected cassettes have been embedded. The last tissues normally to be embedded are the bones (a week after the soft tissues are embedded.

For soft tissue, brains and hard tissue fill in processing information for each on database, including: Tissue Type, Fixation End time/date, Programme Start time/date and Programme Type.

Register blocks by scanning each individual barcode into database. Details can also be added manually.

If a block is missing, a comment should be made on the database.

Once all blocks have been registered, the excel sheet is updated indicating in what storage cabinet and drawer the batch can be found in. This allows for easy location of samples later on.

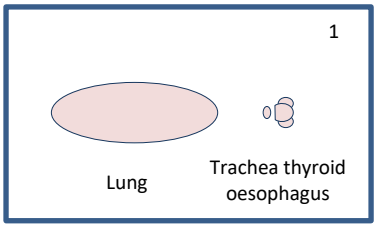
Cleaning, Waste Disposal and Maintenance

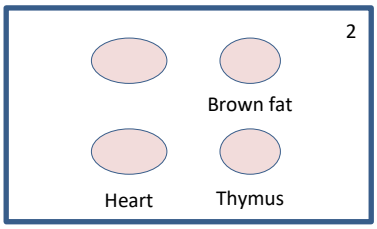
After every use of the embedding station:

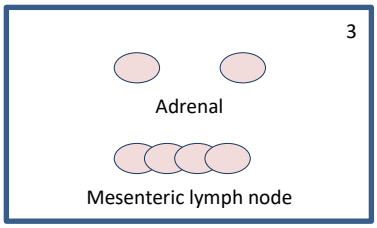
- The station should be cleared of excess wax on the hotspot and cold modules, a plastic scraper may be used if the wax has begun to set.
- Remove excess water/ice from the cold module.
- The wax drip tray, located under the embedding station, should be emptied and cleared of excess wax.
- The wax tank and tissue tank should be topped up with pastillated wax.
- The work area around the embedding station should be cleared of wax and the floors vacuumed.
- Transfer all waste to a yellow clinical waste bag or clearly labelled waste container. This is then transferred to a designated area in the Morgan for waste disposal.
- Cassette lids are placed in processor baskets to be cleaned as part of the Flush Cycle on the Tissue Processor.
- Once the Flush cycle is complete, cassette lids and processor baskets should be removed from the Tissue Processor and left to air dry. The Tissue Processor retort should be wiped with tissue to remove excess water.

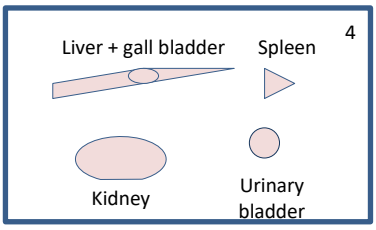
Appendix 1

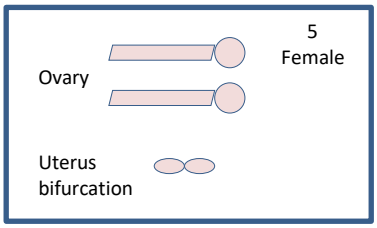
Orientation for embedding Biobank tissues.

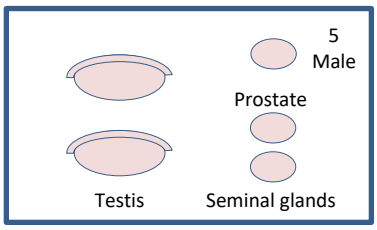


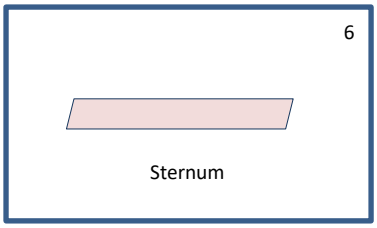


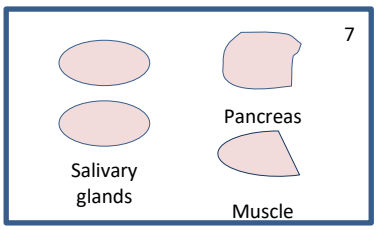


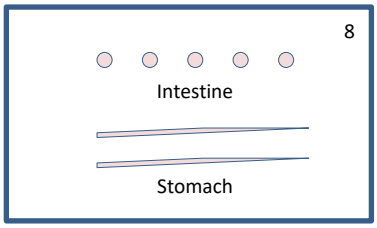


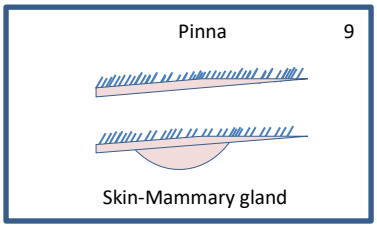


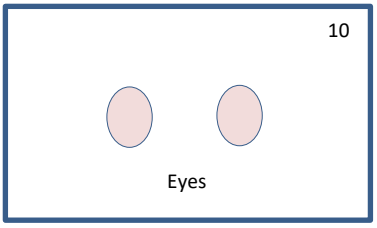


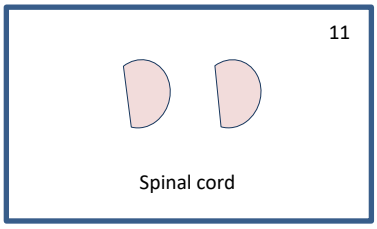


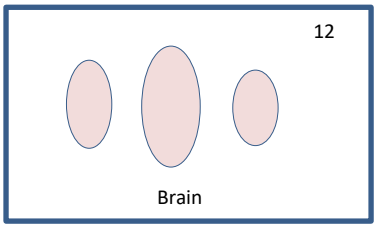


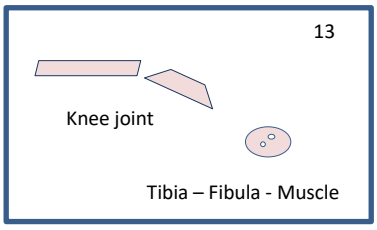


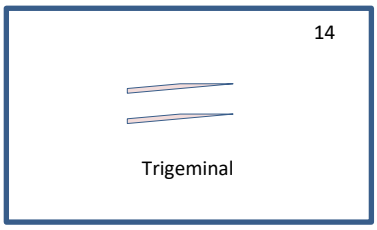


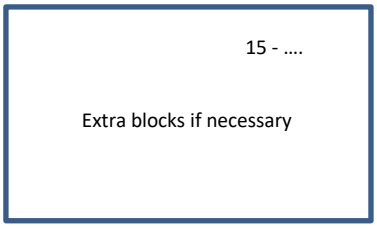












SANGER INSTITUTE

SUBJECT: Terminal collection training manual

INTRODUCTION:

Terminal collection is a blood and tissue collection from control and knockout adult mice (approx.16 weeks old).
Specimens are collected for both the Sanger Biobank and for collaborators.

Task	Number of males collected per line	Number of females collected per line
Sanger Biobank	2	2
3i ears for AH/FACS	2	2
3i Spleen for GG/3i CTL function	2	2
3i spleen for AH/FACs	3	3
3i RHL for AH/FACs	3	3
3i mesenteric lymph nodes for AH/FACS	3	3
LHL and tail / OBCD Bone Ethanol	0	6
Brain for BY	3	0
Eyes for VM	3	0

ASSOCIATED SOPS/DOCUMENTATION:

- SOP0017 Necropsy and tissue collection
- Mammalian Phenotype (MP) terms: http://www.informatics.jax.org/vocab/mp_ontology
- Mouse pathology (MPATH) terms: https://www.ebi.ac.uk/ols/ontologies/mpath
- *The Anatomy of the Laboratory Mouse*, Margaret J. Cook,

PROCEDURE:

Preparation of the terminal collection room

1. All work should be carried out wearing correct PPE.
2. Tubes and pots should be set up, filled and labelled in advance as required.
3. Set up the camera, ensure the lens is fitted correctly and the battery is charged.
4. Prepare the collection area with all required equipment;

    a. dissection board
    b. dissection tool kits and pen
    c. absorbent paper
    d. cadaver disposal bag
    e. 70% ethanol spray bottle
    f. PBS (pH 7.4) in a plastic container
    g. Overhead lighting can be used if required.
    h. Switch on balance and place petri dish on the scale bed.

Movement within Terminal collection

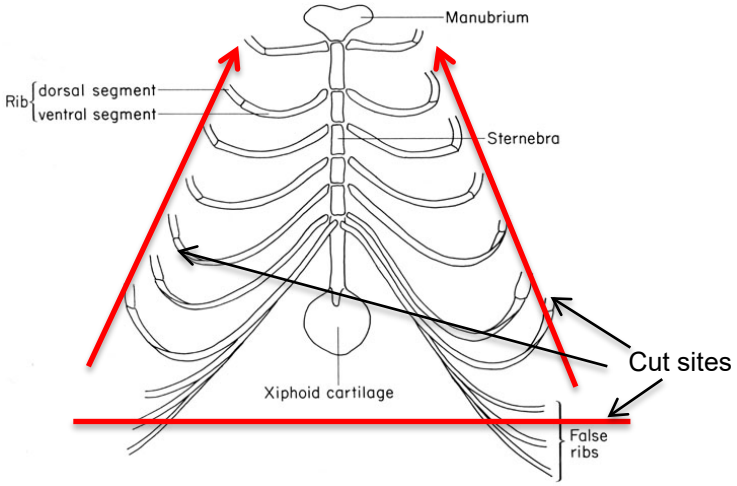
1. Every mouse has an associated necropsy card providing details of the mouse ear clip ID, sex, mouse barcode and assigned tasks (if any).
2. Mice are brought in to the terminal collection room, weighed and anaesthetised.
3. Anaesthetised mice are bled via a retro-orbital bleed and an ear clip and tail clip are taken for genotyping. Cervical dislocation and/or femoral cut is then carried out.
4. Tissue collection tubes are placed in a collection tube rack.
5. Mice, necropsy cards and tubes (in racks) are passed, in a basket, for tissue collection to begin.

Confirmation of mouse ID and samples to be collected – carried out on all mice where tissues are to be collected:

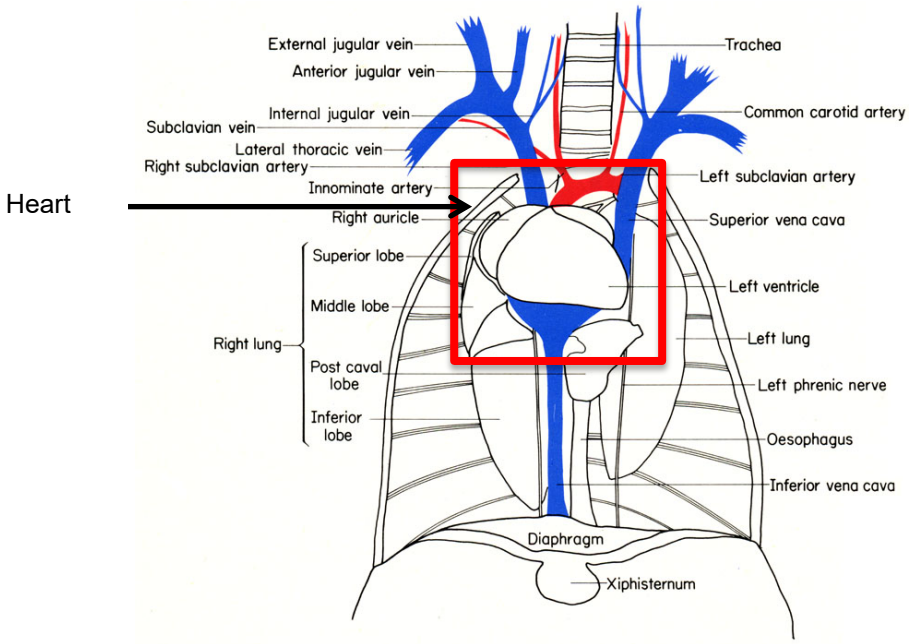
1. Check the mouse ear clip ID and sex match those stated on the necropsy card.
2. Check the mouse barcode on any collection tube labels match the mouse barcode on the necropsy card.
3. Confirm genotyping samples have been collected (1 x ear clip and 1 X tail clip).

Confirmation of death, removal and weight of the heart – carried out on all mice:

1. Check the pedal reflex by pinching a rear paw with forceps, there should be no reaction.
2. Spray the mouse with 70% ethanol to dampen the fur to aid dissection.
3. Make an incision at the base of the sternum (Xiphoid cartilage) and extend the incision following the line of the rib cage.
4. Cut up either side of the rib cage, from the base of the ribs cage towards the head, to fully expose the heart and lungs.



5. Pick up the heart using forceps and dissect from beneath. Avoid damaging the lungs and thymus.

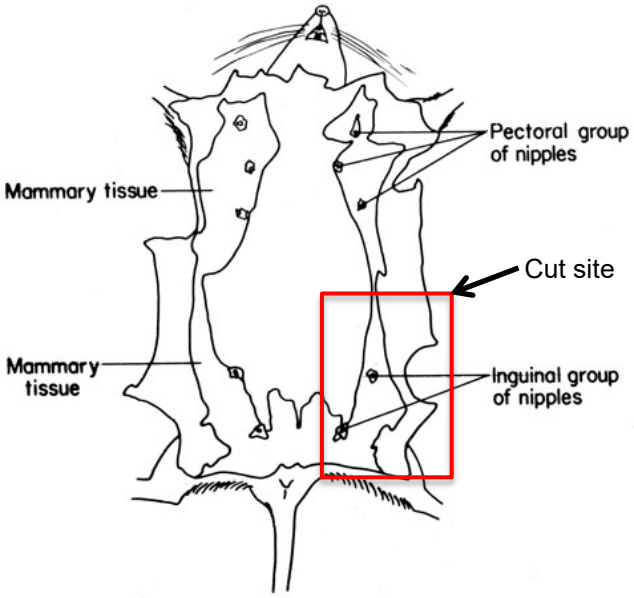


6. If required, the heart should be weighed; this is carried out on all mice where tissues are to be collected. Trim any excess tissue/fat and remove excess blood by blotting the heart on to absorbent tissue.
7. Place the heart in a petri dish to be weighed. Record the heart weight on the Phlebotomy DCF on the database; using the data report button on the scales and write the weight on the necropsy card.
8. The heart should either be placed in a cadaver bag or placed in to the required collection pot

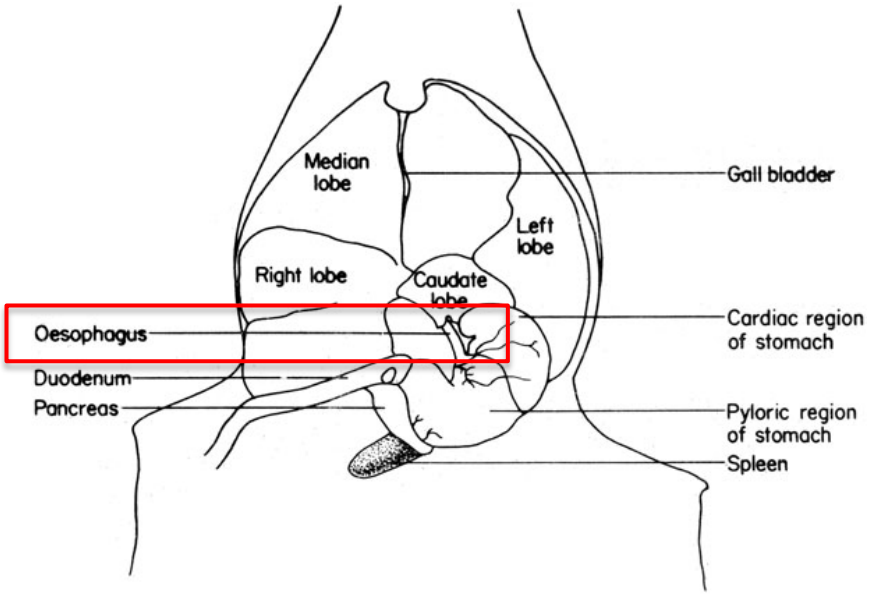
Collecting tissue for the Biobank, full necropsy

- ❖ All tissues collected for the Biobank are collected in 300ml of formalin (except the eyes which are collected in Davidson's fluid).
- ❖ All tissues should be examined for abnormalities. Abnormalities should be recorded on the database using the correct MP term and an image taken if required.
- ❖ Refer to Appendix 1 for a Biobank tissue collection check list.
- ❖ Organs are not required to be collected in the order described.

1. Open the peritoneal cavity by making a single midline incision through the abdominal wall from sternum to pubis. Be careful not to cut the bowel loops below.
2. The peritoneum can be separated from the skin by gently pulling the tissues apart.
3. Inspect for enlarged superficial lymph nodes and inguinal mammary glands with overlying skin before collecting a skin sample, containing mammary tissue.



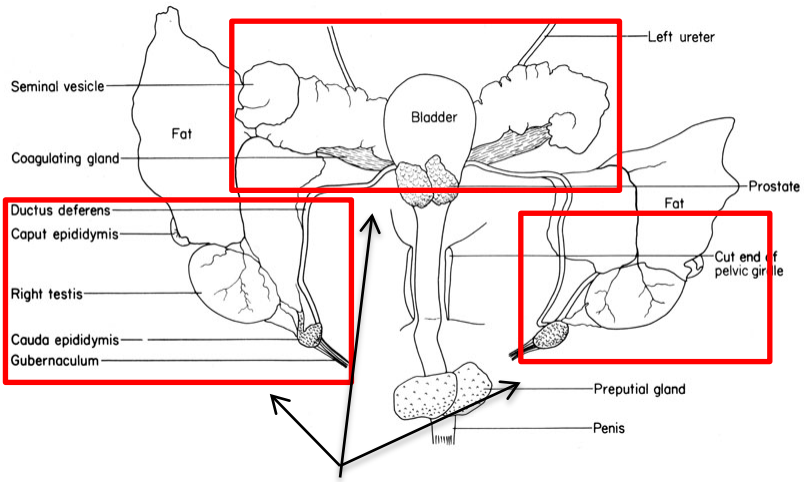
4. Identify the stomach and cut the oesophagus just before it enters the stomach. Gently remove the entire GI tract, making a final cut at the rectum.



5. Remove the Reproductive organs

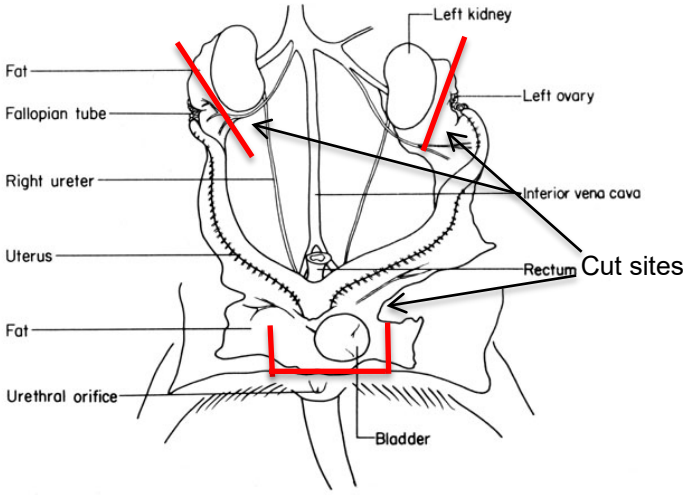
   a. Male

      i. Collect both testes individually by gently lifting the testes out of the body and cutting the Ductus deferens.
      ii. Collect the seminal vesicles and bladder together by gently lifting out of the carcass and cutting from underneath.

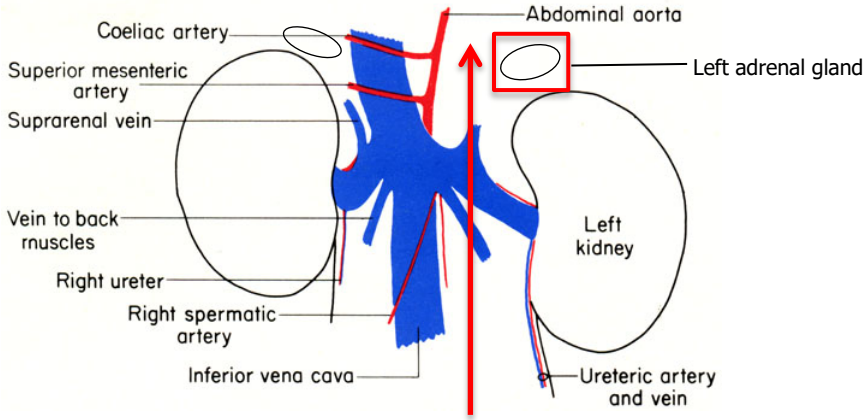


Cut sites

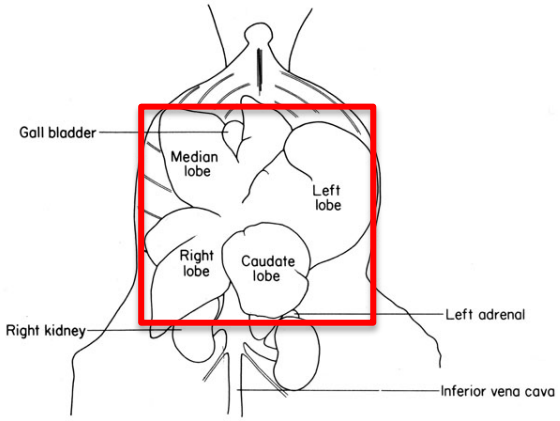
b. Female - Collect the uterus, ovaries and bladder together.
    i. Lift the ovaries and cut away from the surrounding fat.
    ii. Cut below the uterus, close to the cervix and gently lift out the reproductive tract.



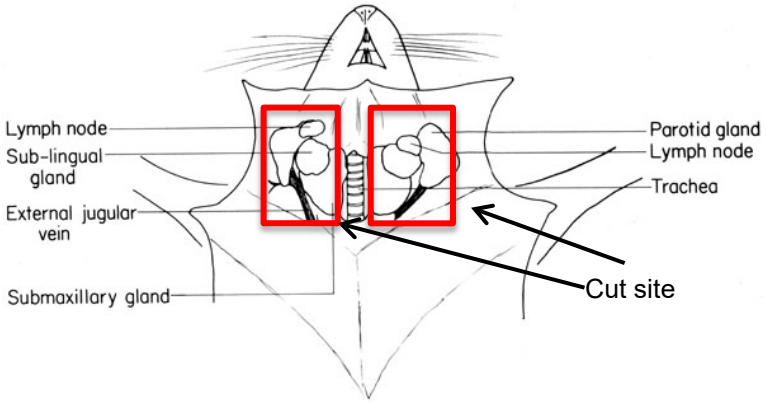
6. Collect both kidneys and detach the adrenal glands. Place the adrenal glands in to a biopsy cassette. The right kidney should be cut to aid identification during tissue trimming.



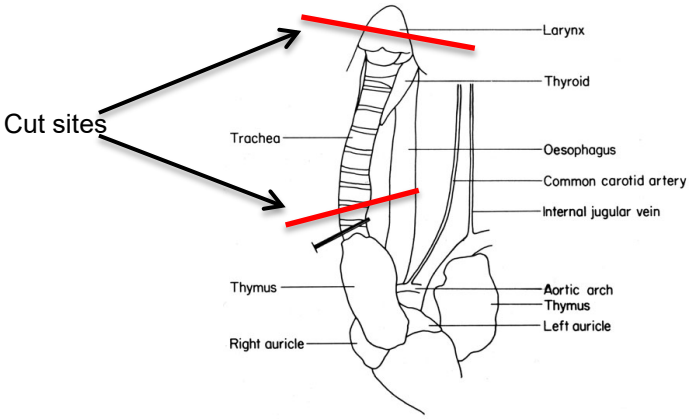
7. Collect all lobes of the liver, including the gall bladder.



8. Expose the salivary glands by making an incision between the head and the ribcage. Gently lift and collect the salivary glands together, exposing the trachea, thyroid glands and oesophagus below.



9. Gently pull the trachea to allow a cut close to the larynx, to ensure the thyroids are collected. A second cut is made as the trachea descends behind the manubrium (top of the sternum). The trachea should be placed in a biopsy cassette.



10. Loosen the thymus and collect the lungs and thymus together.

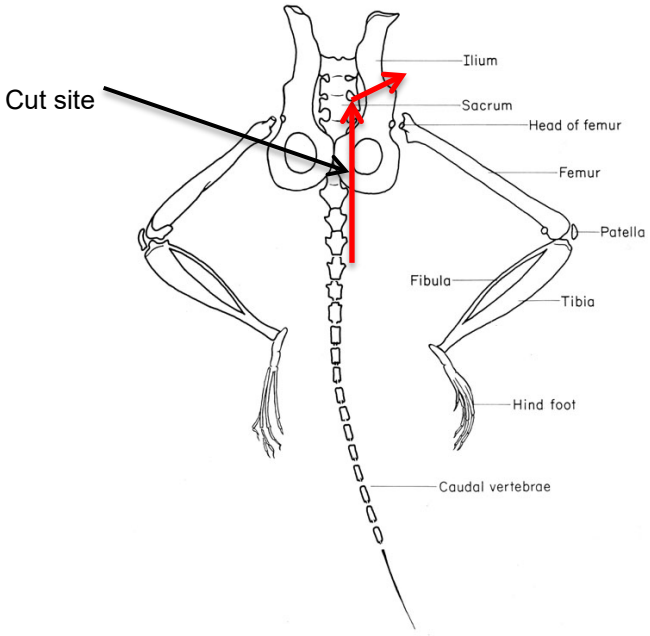
11. Remove the ribcage by cutting through the manubrium. The internal cavity should now be clear of organs.

12. Remove the left ear (pinna), collecting the loose skin only.

13. Collect both eyes using fine tip forceps to lift out the eye ball from the eye socket. Eyes should be placed in Davidson's fluid.

14. Collect the brown fat, remove the skin from between the shoulder blades, lift the brown fat with forceps and dissect out.

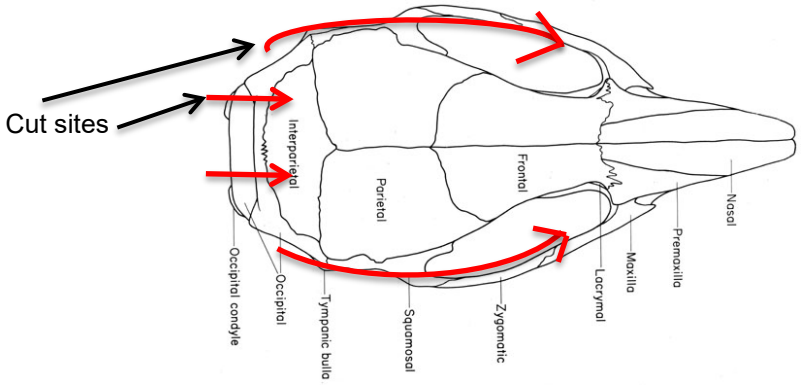
15. Identify the right hind limb and trim away the skin to expose the leg and muscle. Cut up from the tail, close to the spine to avoid damaging the femoral head, until the limb had been removed.



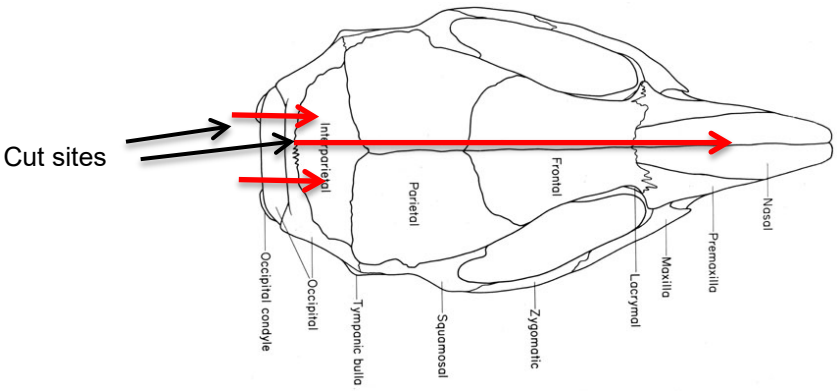
16. Collect the lumbar region of the spine by cutting the spinal column below the ribs and above the pelvis.

17. Remove the head from the body, trim away any fur and soft tissue to expose the skull. Remove the brain from the skull using either method described:

    a. Using fine scissors make a vertical incision through the left and right side of the bone protecting the cerebellum (occipital bone) and remove. Gently cut along both sides of the cranium, towards the nose and peel the cranium gently away from the brain. The brain can be removed using a fine spatula.



    b. Using fine scissors make a vertical incision through the left and right side of the bone protecting the cerebellum (occipital bone) and remove. Gently cut along the midline of the brain towards the nose and peel away the cranium. The brain can be removed using a fine spatula.



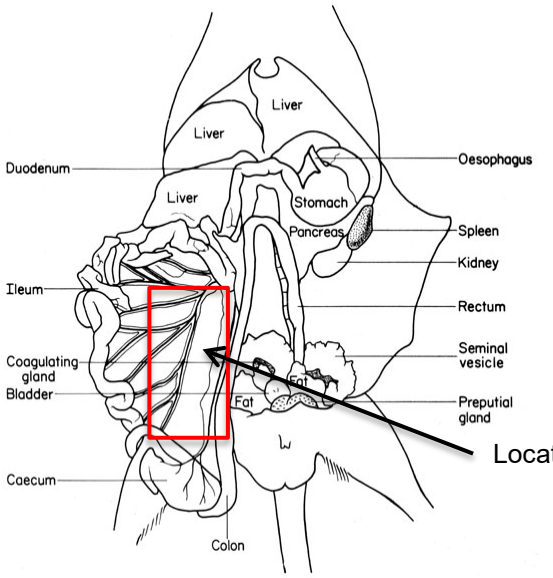
18. Place the skull, containing the trigeminal nerves, the brain and the remaining carcass in to the collection pot and note the completion time on the pot lid (fixation start time).

- **Task - Spleen for 3i CTL function (green eppendorf) and Spleen for 3i FACS (blue eppendorf) – 1ml HBSS**

1. Open the peritoneal cavity and locate the spleen.
2. Collect the spleen, avoiding damage, remove excess fat and place in to the collection tube.

- **Task – Mesenteric lymph nodes for 3i FACS (Red eppendorf) – 1ml HBSS**

1. Open the peritoneal cavity.
2. Locate the mesenteric lymph nodes. The mesenteric lymph nodes are located within the folds of the Ileum, Caecum and Jejunum. Dissect out the lymph nodes, removing as much fat as possible and place in to the collection tube.

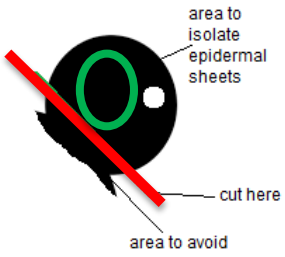


- **Task - R-HL (bone marrow) for 3i FACS – Purple top tube – 40ml HBSS**

1. Identify the right hind limb and remove the skin.
2. Cut up from the tail, close to the spine; avoid damaging the femoral head and spinal column, until the limb had been removed.
3. Rinse in tap water to remove stray fur
4. Wash in PBS (pH 7.4) before placing into the collection tube.

❖ **Task – Ears for 3i tissue immune cells (Clear eppendorf) – 1ml PBS (pH7.4)**

1. Collect both ears by cutting the loose pinnae only, avoid collecting any connective tissue found at the base of the ear and place in to the collection tube.



Collections for OBCD strategic award

❖ **Task – LHL and tail/OBCD bone ethanol – White top tube – 24ml 70% ethanol**

1. Identify the left hind limb.
2. Cut up from the tail, close to the spine; avoid damaging the femoral head and the spinal column, until the limb had been removed.
3. Remove the tail from the body, ensuring there are at least 4 sections of vertebrae attached.
4. Slice the skin at the base of the tail and remove the tail skin.
5. Place both the LHL and the tail in to the collection tube.

❖ The base of the tail, including the vertebrae, is used for compression testing; ensure the whole tail is fully immersed in the 70% ethanol.

❖ **Task – OBCD Joint/ LHL – Green top tube – 24ml 10% NBF**

1. Identify the left hind limb.
2. Cut up from the tail, close to the spine; avoid damaging the femoral head and the spinal column, until the limb had been removed.
3. Place both the LHL in to the collection tube.

Collection of Brains – blue lid falcon tube – 15ml NBF

1. The brain should be removed, as described in the Biobank collection method, avoid any damage.
2. Once removed, the brain should be weighed and the weight recorded on the necropsy card and the Phlebotomy DCF.
3. Place the brain into collection tube.

Collection of Eyes – glass vials – Zinc fix

1. Collect both eyes using fine tip forceps to lift out the eye ball from the eye socket.
2. Each eye should be placed in the correct, individually labelled collection tube.

Collections for QC – orange lid – 60ml NBF

1. Collect the brain, liver and kidney as described in the Biobank collection.

Recording terminal collections on the database.

Every mouse that has at least a heart weight measured is recorded on the mouse database.

Phlebotomy DCF
The phlebotomy DCF is used to record the anaesthetic used, mouse weight, blood volume collected, heart weight and brain weight.

Necropsy DCF
The necropsy DCF is used to record all the tasks assigned to each mouse, collection or damage of a task, abnormalities, general collection comments, the technician involved with completing the tasks, date and time the tasks were carried out.

- For each mouse processed the task statuses should be amended as appropriate in the Necropsy DCF to reflect the status of each sample collected e.g. Collected, Damaged, etc.

General comments, abnormal tissues and imaging

Any comments regarding the mouse and organs should be noted in the general comments section of the DCF.

MP terms should be stated, where possible, for abnormal tissues using the Necropsy Vocabulary List. If no MP term is available a concise comment should be made.

Abnormal tissues may require an image to be taken.

Post terminal collection

- Both the Phlebotomy DCF and the Necropsy DCF should be checked to ensure all hearts have been entered and all task statuses are marked appropriately.
- Once all heart weights, brain weights and collection tasks are complete, the mouse necropsy cards can be discarded.
- A visual check should be carried out to ensure all collection tubes have been used.

Post collection cleaning

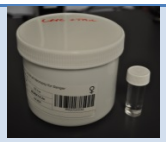
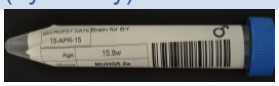
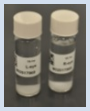
1. Dissection tools must be cleaned with warm water and detergent regularly throughout the terminal collection session
2. Dissection boards must be cleaned with warm water and detergent regularly throughout the terminal collection session
3. Collection tube racks and baskets must be cleaned with Trigene or alcohol wipes
4. Downflow tables must be cleaned with warm water and detergent.
5. Cadaver bags should be placed in the freezer.
6. Plastic pots containing PBS should be rinsed out.
7. Petri dishes and absorbent tissue should be discarded in the yellow clinical waste bag.
8. The balance should be cleaned with alcohol wipes.
9. Computer mouse, keyboard and barcode scanners should be wiped with alcohol wipes.

TROUBLESHOOTING:

Damaged, missing tissue or tissue placed in incorrect fixative during terminal collection

Task	Action during collection session
Biobank	❖ Use appropriate collection status ❖ Add a comment on necropsy ❖ Annotate the collection pot
QC samples	
Eyes	• Use appropriate collection status • Add a DCF comment • Email contact • Mark the collection tube
Spleen and mesenteric lymph nodes for 3i	❖ Use appropriate collection status ❖ Add a DCF comment ❖ Email contact ❖ Mark the collection tube
RHL for 3i FACS	
Ears for 3i FACS	
LHL and tail / OBCD Bone ethanol	❖ Use appropriate collection status ❖ Add a DCF comment ❖ Email contact ❖ Mark the collection tube
OBCD Joint / LHL	
Brain	❖ Use appropriate collection status ❖ Add a DCF comment ❖ Annotate the collection pot

Post collection sample handling

Task	Fixative and collection tube	Post collection destination
Biobank	 300ml NBF and 3ml Davidson's fluid (eyes only)	Transport to the lab at RT using a metal transport container.
Brain	 15ml NBF	Transport to the lab cold room using a metal transport container.
Eyes	 Zinc fix	Transport to the lab at RT using a metal transport container. Store in a lockable cabinet
Spleen for 3i CTL function **Spleen for 3i FACS** **Mesenteric lymph nodes for 3i FACS**	Spleen for CTL – Green eppendorf  Spleen for FACS – Blue eppendorf  Mesenteric lymph nodes – Red eppendorf  All tubes 1ml HBSS	Transport to the lab on ice using a polystyrene igloo.
RHL for 3i FACS	 40ml HBSS	Transport to the lab on ice using a polystyrene igloo.
Ears for 3i FACS	 1ml PBS – clear eppendorf	Transport to the lab at RT.
LHL and tail / OBCD Bone ethanol	 24ml 70% ethanol – white capped tube	Transport to the lab cold room using a metal transport container.
OBCD Joint - LHL	 24ml 10% NBF – green capped tube	Transport to the lab cold room using a metal transport container.
QC samples	 60ml NBF	Transport to the lab at RT using a metal transport container.

Appendix 1: Biobank tissue checklist

1	Heart
2	Skin with mammary gland
3	Stomach and GI tract (Duodenum, Jejunum with Peyer's patch, Ileum, colon, rectum and Mesenteric Lymph nodes)
4	Spleen
5	Pancreas
6	Reproductive organs and urinary bladder • Female - ovary and uterus • Male - testes, prostate, seminal glands
7	Kidneys and Adrenals. • Adrenal glands should be placed in a biopsy cassette
8	Liver + Gall Bladder
9	Trachea, Thyroid, Oesophagus placed in a biopsy cassette
10	Salivary glands
11	Thymus
12	Lungs
13	Sternum
14	Left Pinna
15	Eyes
16	Brown Fat
17	Right hind limb with muscle
18	Spinal column (lumbar region)
19	Brain
20	Trigeminal ganglion
21	Carcass