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1. Purpose

1.1. The purpose of this SOP is to collect and freeze high quality tissue in a consistent manner for contribution to the ShARM aged murine biorepository. The SOP is written for two operators (**Operator 1** and **Operator 2**) but may be carried out by a single operator. Any deviances from this protocol must be reported to the ShARM technician (info@sharmuk.org).

2. Scope

- 2.1.** This protocol is to be followed by individuals who are trained and competent in performing the procedures described.
- 2.2.** Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during the procedure, should be addressed to the relevant departmental lead and to the ShARM-UK technician (info@sharmuk.org).

3. Safety requirements

- 3.1.** Adhere to all local health and safety rules and regulations including the use of laboratory coats and gloves
- 3.2.** Staff must have read and understood the relevant COSHH and Risk Assessments
- 3.3.** Staff must be competent in the use and care of sharp instruments
- 3.4.** Staff must wear appropriate PPE when handling liquid nitrogen (LN2) and be trained and competent in its use

4. Quality control

- 4.1.** ShARM will only accept tissues from freshly sacrificed animals. If it is to be collected, the brain and gut must be frozen within 3 minutes of the death of the animal. All other tissues must be frozen within 10 minutes of the death of the animal.
- 4.2.** Please collect as many tissues (including lobes and regions dissected as indicated) as possible within the allotted time
- 4.3.** If only a single lobe or region of a tissue is to be collected, where possible, please collect the lobe/region specified, if this is not possible specify on the necropsy card which lobe/region has been collected
- 4.4.** Record all deviations and important information on the necropsy card provided below (also available at www.ShARMUK.org)

5. Equipment and consumables required

- 5.1.** Equipment for blood collection including sterile eppendorfs
- 5.2.** Clean dissection kit (also cleaned between mice with dilute trigene or similar)
- 5.3.** Prelabelled cryovials (max. capacity 2ml); labels are provided by ShARM and must be positioned along the length of the tube (NOT wrapped around)
- 5.4.** Gloves
- 5.5.** LN2 in a suitable container for transportation
- 5.6.** Cryobox for holding 2ml cryovials
- 5.7.** A polystyrene box (with lid) large enough to hold a cryobox

6. Preparation prior to procedure

6.1. Prepare your working area

6.2. Gather and clean equipment

6.3. Prelabel all vials for sample collection

6.4. Prepare freezer setup described below which provides a fast and efficient method to freeze all tissues (except serum).

- Place an empty cryobox into a polystyrene box filled with enough LN2 to surround the cryobox as shown in Fig. 6.1, but not so much that the samples will float

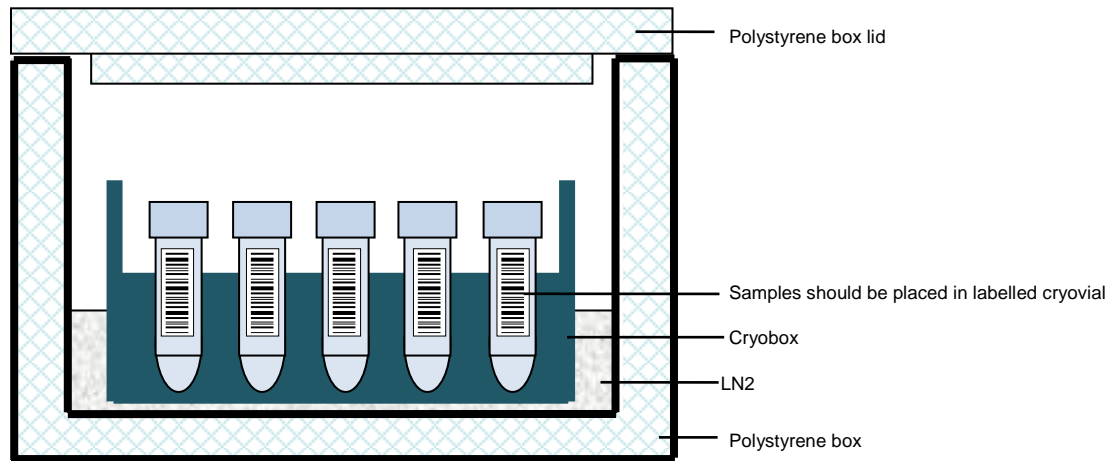


Figure 6.1.: A cut away diagram showing the setup required to freeze tissue samples in cryovials in LN2

7. Procedure

7.1. Euthanize the animal according to local or licence rules - record the date and time of death and method used on the necropsy card

7.2. **Operator 1: Serum:**

- If it is to be collected, collect the blood and record the method used on necropsy card
- Pass carcass to **Operator 2**.
- Decant the blood into a sterile eppendorf and allow to stand for 30-60mins at room temperature
- Centrifuge the eppendorfs at 5000G at 4°C for 10 mins
- Aliquot the serum in volumes of 50µl into prelabelled vials
- Freeze at -80°C

7.3. **Operator 2: Brain:**

- If the brain is to be collected, remove the head and pass the carcass to **Operator 2**.
- Remove the skin and skull – ensure the meninges have been removed
- Gently remove brain and cut in 2 pieces (orientation of cut is not important)
- Place both halves in a single cryovial and freeze as below.

7.4. **Freezing procedure**

- Once the tissue is collected, place in the appropriate, prelabelled cryovial and close
- Place the cryovial in the relevant position in the cryobox in the LN2
- Ensure the lid of the polystyrene box is replaced to prevent the LN2 from evaporating
- Top up LN2 if required

7.5. **Operator 1: Collection of abdominal viscera**

- Expose and remove the abdominal viscera and pass carcass to **Operator 2**
- Gut (duodenum):
- If the gut is to be collected gently separate the gut from the connective tissue and cut under the stomach.
- Collect a 2 cm section of the gut (duodenum) from just under the stomach (or note on the necropsy card which region was taken)
- Place in vial and freeze as described in 7.4.

- For the following tissues; separate the desired tissue from surrounding connective tissue, dissect as below and freeze as described in 7.4.
- Pancreas – Collect whole
- Spleen – Collect whole
- Liver – Collect the left lobe or note on the necropsy card which lobe has been collected

7.6. Operator 2: Collection of kidneys and thoracic viscera

- For the following tissues; separate the desired tissue from surrounding connective tissue, dissect as below and freeze as described in 7.4.
- Kidney – Collect left kidney or note on the necropsy card if the right has been collected
- Remove the heart, thymus and lung together, pass carcass to **Operator 1**
- Heart – Collect whole
- Thymus – If present - collect whole
- Lung – Collect whole

7.7. Operator 1 – Bone, muscle, adipose and breast tissue collection

- Muscle – Note on the necropsy card which muscle has been provided
- Bone – Collect the tibia (or note on the necropsy card if another bone is collected), ensure the heads of the bone, cartilage and growth plates are intact where appropriate, remove soft tissue, freeze and note on the necropsy card which bones have been provided
- White and brown adipose tissue – Separate the fat from the surrounding tissue, freeze and note on the necropsy card the location of the fat removed.
- Breast tissue – Cut a small flap of skin around the mammary fat pad, separate from the skin and freeze, note on the necropsy card the origin of the fat pad

7.8. Dispose of the remaining carcass according to your institutions regulations

7.9. Store samples at -80°C until ready for shipping

7.10. To ship, please follow shipping protocol

8. ShARM Necropsy card –

- See below

ShARM necropsy card

Please collect lobe / region indicated by * or indicate which region was collected

Colony ID		DOB				
Strain		Source		MGI no.		
Mouse ID no. (institute)			Mouse ID no. (ShARM)			Male/Female
Sacrifice	Method used	Cerv. Dis./Overdose/Exsang.	Date		Time	AM/PM
Tissue	Collected (✓)	Observations				
Serum						
Brain						
Gut:						
Duodenum *						
Jejunum						
Ileum						
Cecum						
Colon						
Pancreas:						
Spleen						
Liver:						
Left lobe *						
Median lobe						
Right lobe						
Caudate lobe						
Kidney:						
Left *						
Right						
Lung:						
Heart						
Thymus						
Muscle						
Bone						
Femur						
Breast tissue						
Brown adipose						
White adipose						
Welfare issues			Deviations from SOP			

Please send a pathology report if available.

Please sign to confirm that the serum, brain and gut were collected within 3 mins
and all other tissues within 10 mins of death.

Signed _____

Name _____