Sample Handling Manual

Meso-ORIGINS

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1. Contact Information

Chief Investigator: **Prof Kevin Blyth**
Professor of Respiratory Medicine & Hon Consultant Respiratory Physician
NHS Research Scotland Senior Research Fellow
Glasgow Pleural Disease Unit
Queen Elizabeth University Hospital
1345 Govan Road
GLASGOW G51 4TF
☎ 0141 451 6163
✉ Kevin.blyth@glasgow.ac.uk

Project Manager: **Dr Alexandrea MacPherson**
Institute of Cancer Sciences
University of Glasgow
Wolfson Wohl
Garscube Estate
Bearsden G61 1QH
✉ Alexandrea.Macpherson@glasgow.ac.uk

Clinical Research Fellow: **Dr Mark Neilly**
Department of Respiratory Medicine
Glasgow Pleural Disease Unit
1345 Govan Road
GLASGOW G51 4TF
☎0141 451 6163
✉ Mark.Neilly@ggc.scot.nhs.uk

Glasgow Biorepository: **Clare Orange**
NHS GG&C Biorepository
Level 3 Laboratory Medicine /FM Building
Queen Elizabeth University Hospital
1345 Govan Road
Glasgow G51 4TF
☎0141 354 9490
✉ Biorepository.Research@ggc.scot.nhs.uk

Exhaled Breath Contact: **Dr Kevin Lamote**
University of Antwerp
Translational Research in Immunology and Inflammation
Universiteitsplein 1
2610 Wilrijk, BEL
☎ +32032652581
✉ kevin.lamote@uanterpen.be

When contacting, please include the following information:

- Study name (Meso-ORIGINS)
- Your name, email address and telephone number
- Your centre details
- Participant study ID (if applicable)

2. Introduction

The purpose of this manual is to describe the collection, processing, storage and transportation of translational samples for participants in Meso-ORIGINS.

3. Scope

This manual covers the processing, handling, storage and transportation of blood, pleural fluid, pleural biopsies and exhaled breath samples collected at study centres. The manual covers all samples collected across all sites.

Most samples are collected in every participant at every site, with certain exceptions. These include exhaled breath samples, which can be omitted at sites where facilities are not in place for acquisition or storage, or on grounds of participant preference. Pleural fluid samples should be collected in all participants at visits, *when available*. However, some types of fluid sample may not need to be generated in some centres, e.g., the unprocessed pleural fluid sample for cell line generation is only required at some centres. Site activities should be confirmed during site set-up and can be clarified at any time via the local PI or the PREDICT-Meso Project Manager: Alexandrea.Macpherson@glasgow.ac.uk

4. Responsibilities

The clinical staff at participating centres are responsible for ensuring that samples are collected, handled, processed and stored at their clinical centre in accordance with these instructions.

These samples should then be shipped to the NHSGG&C Biorepository at the Queen Elizabeth University Hospital in Glasgow in accordance with these instructions (see section 13 below).

Staff at the NHSGG&C Biorepository taking receipt of sample shipments are responsible for checking and resolving any inconsistencies between samples received and accompanying documentation.

Please read this manual carefully and contact the Clinical Research Fellow or Project Manager with any questions. Please ensure that you complete and return the declaration at the end of this document (Section 16) stating that you have received, read and understood this manual.

5. Related Documents

- Clinical Study Protocol: Meso-ORIGINS
- Meso-ORIGINS Biopsy Manual
- Meso-ORIGINS US Manual
- Meso-ORIGINS MRI Manual
- Meso-ORIGINS Exhaled Breath Sampling Manual
- Meso-ORIGINS Sample Log and Transfer form- blood and pleural fluid samples
- Meso-ORIGINS Sample Log and Transfer form- Exhaled Breath

6. Consumables and Equipment

6.1. **Equipment**

To be provided by the Clinical Site: Centrifuge (refrigerated), with bucket adapters suitable for processing of blood tubes and pleural fluid containers (30ml, then 5ml).

6.2. **Consumables**

The Project Manager will provide the following items:

Item	For sample
PAXgene tubes	Whole blood for germline DNA
DNase/RNase-free 1.5 ml cryovials (yellow caps)	Serum
DNase/RNase-free 1.5 ml cryovials (orange caps)	Plasma
DNase/RNase-free 5 ml cryovials (Green caps)	Processed Pleural Fluid Supernatant
DNase/RNase-free 5 ml cryovials (Blue caps)	Processed Pleural Fluid Cell Pellet
DNase/RNase-free 5 ml cryovials (Purple caps)	Unprocessed Pleural Fluid
30ml Universals	Pleural Fluid Collection
Exhaled breath sample apparatus- mouthpiece, filter, Tedlar bag, Tenax column sorbent tubes	Exhaled breath
Cryolabels	All
Plastic cryoboxes for cryovials <2ml	Serum, Plasma, and Pleural Fluid Cell Pellet
Cardboard cryoboxes cryovials and tubes >2ml	Whole Blood, Whole Blood for Germline DNA, Pleural Fluid Unprocessed, Processed Pleural Fluid Supernatant
Glass jar	Exhaled breath
Biopsy pots	FFPE Pleural Biopsies
Padded envelopes	FFPE Pleural Biopsy Blocks
Royal Mail SafeBoxes – **select sites only**	Unprocessed Pleural Fluid Samples

The clinical site will provide the following items:

Item	For sample
EDTA tube: VACUETTE® TUBE 6 ml K2EDTA, lavender cap	Whole blood
EDTA K3 tube, 9 ml Lavender capped	Blood collection for Plasma
SST Clot activator 5ml tube, yellow capped	Blood collection for Serum
Needles for research blood draw	Blood
Syringes for research blood draw	Blood
Bubble wrap	All
Indelible marker pen	All

6.3. **Study sample kit detail**

Study sample kits provided to each site will contain:

- Plastic cryoboxes for 1.5ml vials
- Cardboard cryoboxes for >1.5ml vials
- Padded envelopes
- Cryolabels will be supplied in each study bag for corresponding number of tubes for that activity (some will also have spares). Please take care when opening these bags not to lose any loose labels. An envelope with spare labels will also be provided.
- Study pack paper envelopes/bags with tubes for sample collection. Once collected and processed, tubes will be stored in the cryoboxes supplied and envelopes/bags can be disposed of (reuse/recycle where possible). Pack detail in table below:

Arm (visit)	Pack	Packs per participant	Additional info	Contains	Sample detail
Arm A **A1** (including remote observation)	**Medium** bag marked: *Meso-ORIGINS Arm A Visit A1*	1		1 x Paxgene tube	Germline blood
				8 x 1.5ml vials with yellow caps	Serum
				8 x 1.5ml vials with orange caps	Plasma
				19 x labels	For storage tubes in pack (17) plus 2 whole blood tubes (supplied by site)
Arm A **A1**	**Medium** bag marked: *Meso-ORIGINS Pleural fluid A1*	1		8 x 30ml universals	Pleural Fluid collection
				12 x 4.5ml tubes with purple caps	**Unprocessed** Pleural Fluid
				20 x 4.5ml tubes with green caps	**Processed** Pleural Fluid **supernatant**
				4 x 1.5ml tubes with blue caps	**Processed** Pleural Fluid cell **pellets**
				36 x labels	For storage tubes in pack (36)
Arm A **A3** or **A4** or **A5** or **A6**	**Medium** envelope/bag marked: *Meso-ORIGINS Arm A A3…A6*	4	As visit A3, A4, A5 and A6 all have the same sampling activities, these packs are marked A3..A6 and can be used for any of these visits.	8 x 1.5ml vials with yellow caps	Serum
				8 x 1.5ml vials with orange caps	Plasma
				18 x labels	For storage tubes in pack (16) plus 2 whole blood tubes (supplied by site)
Arm B **B1**	**small** bags marked: *Meso-ORIGINS Arm B (B1)*	1		1 x Paxgene tube	Germline blood
				8 x 1.5ml vials with yellow caps	Serum
				8 x 1.5ml vials with orange caps	Plasma
				19 x labels	For storage tubes in pack (17) plus 2 whole blood tubes (supplied by site)
Arm B **B2**	**large** bags marked: *Meso-ORIGINS Arm B visit B2*	1	These large bags will contain biopsy pots and a medium bag which contains pleural fluid collection tubes	Biopsy pots for Arm B research biopsies	Pleural biopsy (6 per participant)
				8 x 30ml universals	Pleural Fluid collection
				12 x 4.5ml tubes with purple caps	**Unprocessed** Pleural Fluid
				20 x 4.5ml tubes with green caps	**Processed** Pleural Fluid **supernatant**
				4 x 1.5ml tubes with blue caps	**Processed** Pleural Fluid cell **pellets**
				36 x labels	For storage tubes in pack (36)

As pleural fluid will not be available in all participants, fewer of these will be provided. Please contact the PM to request more if needed.

7. Sample Collection Schedule

Whole blood, plasma, serum, exhaled breath, pleural tissue biopsies (including archived pleural biopsy FFPE block retrieval) and pleural fluid samples will be collected from participants according to the schedule of assessments outlined below.

7.1. **Arm A (including remote observation for A1 samples)**

Study Procedure	A1	A2	A3	A4	A5	A6
Plasma sample	X	X[a]	X	X	X	X
Serum sample	X	X[a]	X	X	X	X
Whole blood sample	X	X[a]	X	X	X	X
Whole blood sample for germline DNA	X	X[a]				
Pleural fluid sample **unprocessed** for Cell lines (room temp) *(participating sites only)*	X[b]	X[a]				
Pleural fluid sample **unprocessed** for banking (frozen)	X[b]	X[a]				
Pleural fluid sample **processed** for Cell Pellet and Supernatant Banking	X[b]	X[a]				
Previous FFPE pleural biopsy retrieval	X					
Exhaled Breath Sample *(participating sites only)*	X	X[a]				

[a] if not taken at visit A1

[b] if available- not all participants will have pleural fluid available at visit A1

7.2. **Arm B**

Study Procedure	B1	B2	B3	B4
Plasma sample	X	X[a]		
Serum sample	X	X[a]		
Whole blood sample	X	X[a]		
Whole blood sample for germline DNA	X	X[a]		
Pleural fluid sample; Unprocessed; for Cell lines		X		
Pleural Sample; Processed; for Cell Pellet and Supernatant Banking		X		
Pleural biopsies		X		
Exhaled Breath Sample *(participating sites only)*	X	X[a]		

[a] if not taken at visit B1

8. Sample Collection Notes

8.1. **Labelling conventions**

Printed labels will be supplied in each study bag for corresponding number of tubes for that activity (some will also have spares). Please take care when opening these bags not to lose any loose labels. An envelope with spare labels will also be provided.

Please complete participant study ID- Pt # (e.g., MO-001), visit (e.g., A1, A2) and Sample type:

WB – Whole Blood **G** – Whole Blood for Germline DNA **P** – Plasma

S – Serum **PP** – Pleural Pellet **PS** – Pleural Supernatant

PF- Pleural fluid (unprocessed)

Example label - blank

Pt #:
Visit:
Sample:

Example label - completed

Pt #: MO-001
Visit: A1
Sample: S

8.2. **Cryoboxes and Cryovials**

Numerous cryoboxes will be needed for sample storage.

Cryobox	For tubes/vials	sample
Plastic	<2ml	plasma, serum and pleural fluid pellet in 1.5ml cryovials
Cardboard	2ml-6ml	blood for germline DNA in 2.5ml PAXgene tube; unprocessed pleural fluid, pleural fluid supernatant in 4.5ml cryovials; whole blood in 6ml EDTA tube

Please label cryoboxes with:

- Meso-ORIGINS
- study site
- box # (sites can label boxes sequentially as used)
- sample types contained

If space in freezers is limited, sites may fill up cryoboxes with mixed sample types of matching cryovial sizes (e.g., serum and plasma) as long as tubes are properly labelled, cryobox templates are complete with sample type, and samples are easy to identify.

Different coloured caps will be used for different sample types cryovials:

Yellow		Serum
Orange		Plasma
Blue		Pleural Fluid Pellet
Green		Pleural Fluid Supernatant
Purple		Unprocessed Pleural Fluid

8.3. **Sample logs**

Two sample log and shipping excel forms will accompany this manual

- Meso-ORIGINS Sample log and transfer form
- Meso-ORIGINS Pleural Biopsy FFPE log and transfer form

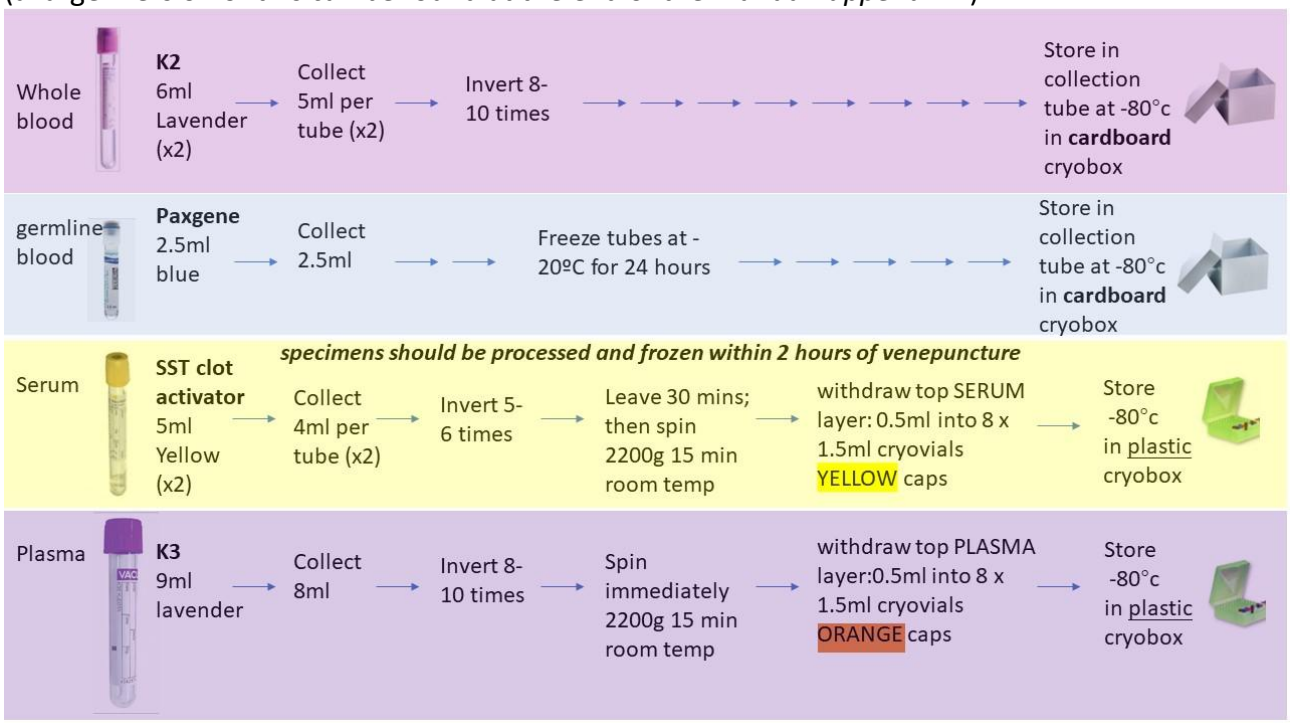
It is advised that various tabs are created on these files. Tab 1 for a whole sample log; Tab 2 for first shipment; Tab 3 for next shipment and so on. This allows this document to act as a sample log and a shipping log in one place. Instructions will be provided on these documents.

9. Research Blood Sample Processing, Storage and Shipment

As outlined above, research bloods will be collected and processed at Visit A1-A6 (including A1 for remote observation) and B1, to generate the following samples:

Sample Type	Blood Collection Volume /Tube Type	Visits	Manual
Whole blood	10ml of blood collected into 2 x 6ml EDTA tubes (5ml/tube)	A1, A3-A6 B1	Section 9.1
Whole blood for germline DNA	2.5ml blood collected into a PAXgene Blood DNA tube	A1, B1	Section 9.2
Serum	8ml blood collected into 2 x 5ml Serum Vacuette tubes (4ml/tube), processed into 8 x 1.5ml cryovials (0.5ml/cryovial) with yellow caps	A1, A3-A6 B1	Section 9.3
Plasma	8ml blood collected into 1 x 9ml EDTA tube (8ml/tube), processed into 8 x 1.5ml cryovials (0.5ml/cryovial) with orange caps	A1, A3-A6 B1	Section 9.4

Blood sampling processing is summarised in the infographic below:
(a larger version of this can be found at the end of the manual- *appendix 1*)



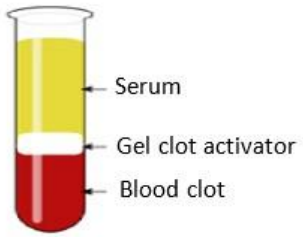
9.1. **Whole Blood**

- Samples to be collected at Visit A1-A6 and/or B1 as described. Samples stored in the collection tube
- 10ml of blood collected into 2 x 6ml EDTA tubes (5ml/tube). Samples are stored in the collection tube
- Check expiration date on 6ml EDTA tubes; if expired replace with new ones
- Collect approximately 5mls of venous blood into each 6ml EDTA tube
- Gently invert the tubes 8-10 times
- Complete the Meso-ORIGINS label (section 16) with an indelible pen and place label firmly onto tubes, ensuring that the bottom of the label is twisted at the base of the tubes
  Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere
- Immediately place the tubes into cardboard cryobox and place cryobox box into -80ºC (+/-10ºC) freezer until ready to ship (see section 13 for shipping instructions)
  - The samples can go into the same cardboard cryobox as the Whole Blood for Germline DNA samples (below) and pleural fluid samples (section 10) until the cryobox is full and a new cardboard cryobox needs to be started
- Complete sample info (participant study ID, visit, Sample type (WB)) and placement in cryobox on cryobox template (see section 14)
- Complete the whole blood worksheet (section 15.1) with: the visit, date and time of collection; time samples were frozen; box number; tube position in box; and the details of the operator
- Store the completed template and worksheet in the Investigator Site File until shipping (Section 13)
- Complete the sample details on the Meso-ORIGINS Sample log and transfer form- blood and pleural fluid.

9.2. **Whole Blood for Germline DNA**

- Samples to be collected at Visit A1 (including remote observation) and B1 as described. Samples stored in the collection tube
- Check expiration date on PAXgene Blood DNA tube; if expired replace with new one
- Collect approximately 2.5mls of venous blood into a PAXgene Blood DNA tube
- Complete the Meso-ORIGINS label with an indelible pen and place label firmly onto tube, ensuring that the bottom of the label is twisted at the base of the tube
- Freeze tubes at -20ºC for 24 hours before placing the tubes into the cardboard cryobox and place cryobox box into -80ºC (+/- 10ºC) freezer until ready to ship (see section 13 for shipping instructions)
    - The samples can go into the same cardboard cryobox as the Whole Blood samples (above) and pleural fluid samples (below section 10) until the cryobox is full and a new cardboard cryobox needs to be started
- Complete sample info (participant study ID, visit, Sample type (G)) and placement in cryobox on cryobox template (see section 14)
- Complete the whole blood for Germline DNA worksheet (section 15.2) with: the visit, date and time of collection; time samples were frozen; box number; tube position in box; and the details of the operator
- Store the completed template and worksheet in the Investigator Site File until shipping (Section 13)
- Complete the sample details on the Meso-ORIGINS Sample log and transfer form- blood and pleural fluid.

9.3. **Serum**



Centrifugation of clotted blood causes separation of blood cells from the serum. Serum moves to the top of the tube and forms the supernatant. The gel layer in the vacutainer serves to separate the blood clot from the serum after centrifugation (see diagram). This top layer of serum can then be carefully removed with a pipette and stored at -80ºC.
Centrifugation should occur as soon as possible after blood has clotted and all specimens should be processed and frozen within **2 hours** of venepuncture.

Method

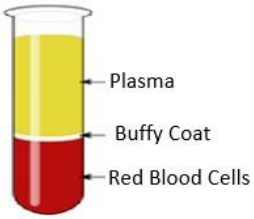
- Samples to be collected at Visit A1-A6 (including A1 remote observation) and/or B1 as described. Samples are stored after processing into aliquots in 1.5ml cryovials
- 8ml blood collected into 2 x 5ml Serum Vacuette tubes (4ml/tube)
- Check expiration date on yellow vacutainers; if expired replace with new ones
- Collect approximately 4ml of venous blood into 2 yellow vacutainer tubes containing SST clot activator (approximately 8 ml in total)
- Gently invert samples 5-6 times
- Record sample collection date and time on serum worksheet
- Allow the samples to clot for 30 minutes at room temperature before centrifugation
- Centrifuge at 2200g for 15 minutes at room temperature
- Record centrifugation time on serum worksheet
- Carefully withdraw the top layer using a pipette and dispense 0.5ml aliquots into the DNase/RNase-free 1.5 ml cryovials with **yellow caps.** There should be enough serum for 8 cryovial tubes. Do not overfill these tubes
- Complete the Meso-ORIGINS labels with an indelible marker and stick them securely onto tubes, ensuring that the bottom of the label is twisted around the base of the tube.

Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere

- Label the top of the tubes using an indelible marker with the participant study ID, visit, S
- Immediately place the tubes into a plastic cryobox and place cryobox box into -80ºC (+/- 10ºC) freezer until ready to ship (see section 13 for shipping instructions)
  - The samples can go into the same plastic cryobox as the plasma samples (below) and the pleural pellets (section 10) until the cryobox is full and a new plastic cryobox needs to be started
- Complete sample info (participant study ID, visit, Sample type (S)) and placement in cryobox on cryobox template (see section 14)
- Complete the Serum worksheet (section 15.3) with: the visit, date and time of collection; centrifuge start time; time samples were frozen; box number; number of tubes frozen; tube position in box; and the details of the operator
- Store the completed template and worksheet in the Investigator Site File until shipping (Section 13)
- Complete the sample details on the Meso-ORIGINS Sample log and transfer form- blood and pleural fluid.

9.4. **Plasma**

Centrifugation of un-clotted blood causes separation of blood cells from plasma. A clear layer of plasma will form the supernatant and can then be carefully removed using a pipette.



The white cells and platelets will form a layer underneath the plasma - this is known as the buffy coat layer. The red blood cells form a layer underneath the buffy coat (see diagram).

Method

- Samples to be collected at Visit A1-A6 (including A1 remote observation) and/or B1 as described. Samples are stored after processing into aliquots in 1.5ml cryovials
- Check expiration date on 9ml EDTA tube; if expired replace with new one
- Collect 8mls of venous blood into a purple EDTA tube
- Gently invert sample 8-10 times and leave upright prior to centrifugation
- Record the sample collection time on the plasma laboratory worksheet
- Centrifugation should be done **immediately** with these samples as they do not need to clot
- Centrifuge at 2200g for 15 minutes at room temperature
- Record the time of centrifugation on the plasma laboratory worksheet
- Carefully withdraw upper plasma layer using a pipette
- Transfer 0.5 ml aliquots of plasma into DNase/RNase-free 1.5 ml cryovials with **orange caps** and discard the pellet and any remaining plasma. There should be sufficient plasma for 8 cryovial tubes. Do not overfill the tubes
- Complete the Meso-ORIGINS labels using an indelible pen and stick them onto the tubes, ensuring that they are secure, and the bottom of the label is twisted around the end of the microfuge tube
  Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere
- Label the top of the tubes using an indelible marker with the participant study ID, visit, P
- Immediately place the tubes into a plastic cryobox and place cryobox box into -80ºC (+/- 10ºC) freezer until ready to ship (see Section 13 for shipping instructions)
  - The samples can go into the same plastic cryobox as the serum samples (above) or pleural pellets (section 10) until the cryobox is full and a new plastic cryobox needs to be started

- Complete sample info (participant study ID, visit, Sample type (P)) and placement in cryobox on cryobox template (see section 14)
- Complete the Plasma worksheet (section 15.4) with: the visit, date and time of collection; centrifuge start time; time samples were frozen; box number; number of tubes frozen; tube position in box; and the details of the operator
- Store the completed template and worksheet in the Investigator Site File until shipping (Section 13)
- Complete the sample details on the Meso-ORIGINS Sample log and transfer form- blood and pleural fluid.

10. Pleural Fluid

At Visits A1 (including remote observation) and B2 pleural fluid will be collected (where available) and handled to generate different types of samples, each for specific purpose.

At each applicable visit, 6 pleural fluid samples to be collected in 6 x 30ml universal container (180ml in total) *At **participating sites only**, an additional 2 x 30ml universal unprocessed samples will be collected for generation of cell lines (see section 10.1). These sites will collect 8 x 30ml universals of pleural fluid total (240ml). Sites will be informed in advance by PM.*

Sample Type	Collection Volume /Tube Type	Section
Unprocessed	2 x 30ml Unprocessed send to Cambridge for cells lines	10.1
Unprocessed	2 x 30ml Unprocessed sample for banking	10.2
Processed	4 x 30ml Processed sample for Cell Pellet/Supernatant Banking	10.3

Samples at Visit A1 will only be available in participants with an indwelling pleural catheter in-situ. In this setting, a total of 180ml should be aspirated from the IPC drainage bottle or removed using an IPC drainage line attached to a 50 ml syringe.

At Visit B2, 180 ml (6 x 30ml) of pleural fluid will be obtained during thoracoscopy.

At sites participating in the generation of cell lines, 240ml (the above 180ml plus 2 additional 30 ml) should be obtained

Once the fluid samples are obtained:

- ***At participating sites only*** *- The unprocessed sample (2 x 30ml) for generation of cell lines (section 10.1) should be stored at room temperature and be shipped within 5 hours (see Section 13.3). DO NOT CHILL THIS SAMPLE*
- **At all sites**- The unprocessed sample (2 x 30ml) for banking (section 10.2) should be kept cold (in the fridge or on ice) and must be aliquoted into 4.5ml cryovials and frozen within 2 hours of sampling
- **At all sites**- The samples (4 x 30ml) for processing (pellet and supernatant) (section 10.3) should be kept cold (in the fridge or on ice) and must be processed and frozen within 2 hours of sampling.

10.1. Unprocessed Pleural Fluid for Cell lines – participating sites only

2 x 30 ml samples in 30ml universal tubes should **only be collected at participating sites**. Site activities should be confirmed during site set-up and can be clarified at any time via the local PI or the PREDICT-Meso Project Manager: Alexandrea.Macpherson@glasgow.ac.uk

- Keep unprocessed pleural fluid at room temperature

- Label the top of the 2 x 30ml universals using an indelible marker with the participant study ID, visit, Sample type (PF)
- Complete the Meso-ORIGINS pleural fluid labels with an indelible marker and stick them securely onto tube
- Complete the Pleural Fluid- unprocessed (room temp) worksheet (section 15.5) with the visit, date and time of collection and details of operator
    - Photocopy/save an electronic copy, storing the copy complete worksheet in the Investigator Site File
- Send the original complete worksheet with the unprocessed pleural fluid sample at room temperature in the royal mail safe boxes provided to Prof. Marion MacFarlane at MRC Toxicology Unit, Cambridge (see Section 13.3) for detailed instructions on transportation).

10.2. **Unprocessed Pleural Fluid for Banking**

This sample (2 x 30ml) should be generated for all participants, where possible, at all sites at Visits A1 and B2.

- Keep unprocessed pleural fluid cold (on ice or in the fridge) until aliquoting.
- Carefully dispense 4.5ml aliquots into 12 x 4.5ml cryovial tubes with purple caps. Do not overfill these tubes
- Complete the Meso-ORIGINS labels with an indelible marker and stick them securely onto tubes, ensuring that the bottom of the label is twisted around the base of the tube. Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere
- Label the top of the tubes using an indelible marker with the participant study ID, visit, PF
- Immediately place the tubes into a cardboard cryobox and place cryobox box into -80ºC (+/- 10ºC) freezer until ready to ship (see section 13 for shipping instructions)
    - The samples can go into the same cardboard cryobox as the processed supernatant (below) until the cryobox is full and a new cardboard cryobox needs to be started
- Complete sample info (participant study ID, visit, Sample type (PF)) and location in the cryobox on the cryobox template (see section 14)
- Complete the Pleural Fluid- unprocessed fluid for banking worksheet (section 15.6) with: visit, date and time of collection; time samples were frozen; box number; number of tubes frozen; tube position in box; and the details of the operator
- Store the completed template and worksheet in the Investigator Site File until shipping (Section 13)
- Complete the sample details on the Meso-ORIGINS Sample log and transfer form- blood and pleural fluid.

10.3. **Processed Pleural Fluid: Cell Pellet and Supernatant Banking**

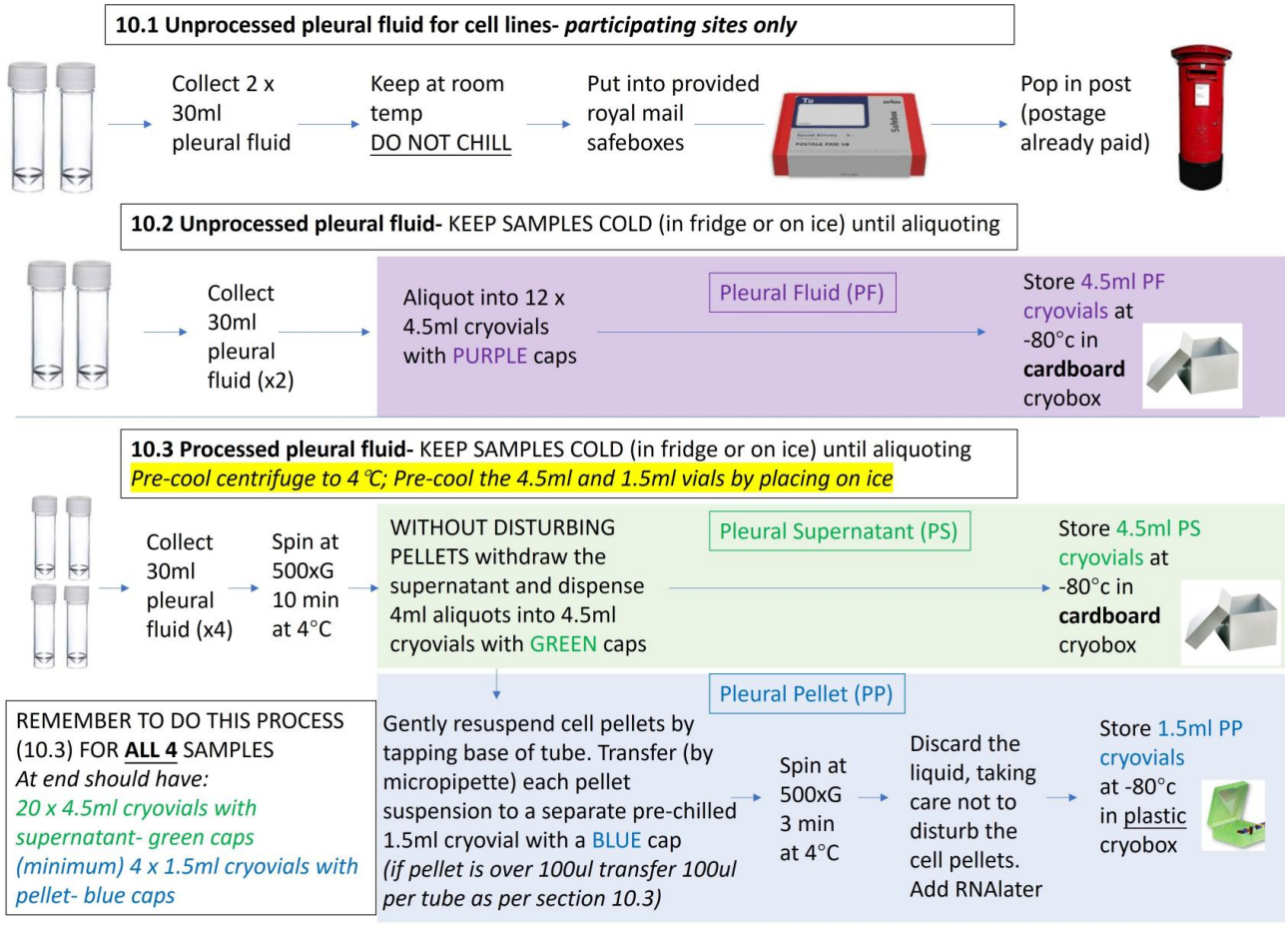
These samples (4 x 30ml) should be generated for all participants, where possible, at all sites at Visits A1 and B2.

The processing detail below is **PER 30ML SAMPLE.** Please remember to treat both samples as below.

- Pre-cool the centrifuge to 4ºC Keep all samples on ice throughout procedure
- Pre-cool the 4.5ml and 1.5ml vials by placing on ice
- Spin 30ml samples at 500xG for 10 minutes at 4ºC
- Complete the Meso-ORIGINS labels with an indelible marker and stick them securely onto final storage tubes (1-2 x 1.5 ml cryovial for cell pellet with a **blue cap** and 5 x 4.5ml cryovials for supernatant with **green caps** **PER 30ML SAMPLE**), ensuring that the bottom of the label is twisted around the base of the tubes. Please ensure the labels are placed on all tubes before they are chilled, otherwise labels will not adhere

- Record centrifugation time on Processed Pleural Fluid worksheet (section 15.7)
- WITHOUT DISTURBING THE PELLET carefully withdraw the supernatant using a sterile pipette and dispense 4ml aliquots into the 4.5ml cryovials. There should be enough supernatant per sample for 5 x 4.5ml tubes. Keep on ice/at 4ºC. Do not overfill these tubes
  - Note: the number of vials will vary per sample based on cellular make up of fluid and pellet size. We acknowledge that some samples will only have enough fluid for 4 vials of supernatant. If any supernatant remains, please discard, **taking care not to disturb pellet**
- **Process the cell pellets FIRST before continuing to label/process the supernatant. The supernatant can remain on ice while the pellets are processed. Note that short time to freezing is critical to maintain sample quality, so please freeze pellets as quickly as possible following processing. Keep pellets on ice when not actively handling. When handling pellets please use finger and thumb (not closed fist) at top of tube to minimise pellet being exposed to heat**
- Gently resuspend the cell pellet by tapping the base of the 30ml tube, then use a P-1000 micropipette to transfer cell suspension to pre-chilled labelled cell pellet 1.5ml cryovial(s)
- *If the pellet size exceeds 100ul, split pellet into multiple cryotubes with no more than 100ul pellet (no more than 2 vials are needed per 30ml sample, remaining pellet can be discarded)*
- Spin the cryovial in the centrifuge, using an appropriately sized bucket adapter, at 500xG for 3 minutes at 4oC. (If a suitably sized bucket is not available, this sample can be processed in separate chilled microcentrifuge).
- Gently discard the liquid, taking care not to disturb the cell pellet. A small amount (e.g. one drop) of remaining liquid is not a problem
- Add RNAlater solution (which will be provided) drop by drop to the pellet – approximately 3-5 times the pellet volume up to a max of 500ul (there should be more RNAlater than pellet but do not fill tube completely)
- **Do not be concerned if the drops disturb the pellet**
- Label the top of the tubes using an indelible marker with the participant study ID, visit (e.g., B2) and PP (pleural pellet)
- **Immediately place the tubes into a plastic cryobox and place cryobox box into -80ºC** (+/- 10ºC) freezer until ready to ship (see Section 13 for shipping instructions).
  - The samples can go into the same plastic cryobox as the serum and plasma samples until the cryobox is full and a new plastic cryobox needs to be started
- Continue supernatant processing- label the top of the tubes using an indelible marker with the participant study ID, visit (e.g., B2) and PS (pleural supernatant)
- Immediately place the tubes with supernatant into cardboard cryobox on ice or freezer
  - The samples can go into the same cardboard cryobox as the unprocessed fluid (above) until the cryobox is full and a new cardboard cryobox needs to be started
- Complete sample info (participant study ID, visit, Sample type (PS)) and placement in cryobox on cryobox template (see section 14)
- Complete sample info (participant study ID, visit, Sample type (PP)) and placement in cryobox on cryobox template (see section 14)
- Complete the Processed Pleural Fluid worksheet (section 15.7) with: the visit, date and time of collection; centrifugation start time and the details of the operator. For both pellets and supernatant complete separately: number of tubes frozen; time samples were frozen; box number; tube positions in box
- Store the completed template and worksheet in the Investigator Site File until shipping (Section 13)
- Complete the sample details on the Meso-ORIGINS Sample log and transfer form- blood and pleural fluid.

Pleural fluid sampling processes are summarised in the infographic below
(a larger version of this can be found at the end of the manual- appendix 2)



11. Pleural Biopsies

Collection and retrieval of pleural biopsies is **core** study activity. This will happen via two pathways within Meso-ORIGINS:

1. Retrieval of FFPE blocks generated within clinical care, including post-mortem (Visit A1, Visit Ax*, and visit Apm**)
2. Collection of research specific biopsies within the study (Visit B2).

**Visit Ax- biopsies taken within NHS care, performed with clinical suspicion of mesothelioma evolution, are NOT study specific activity and therefore will have no study visit number. These biopsies are designated visit Ax.*

***Visit Apm- Post-mortem biopsies taken within NHS care, performed with clinical suspicion of mesothelioma in participants whom repeat biopsy during life was not possible due to insufficient fitness or technical factors, are NOT study specific activity and therefore will have no study visit number. These biopsies are designated visit Apm.*

At each time point it is essential we retrieve all available FFPE Blocks and send to the RTB with appropriate labelling and packaging. See section 11.1 and 11.2 for further detail.

Pleural biopsy FFPE blocks should be stored at room temperature before shipping and sent to the PREDICT-Meso Research Tissue Bank (RTB) as soon as possible after collection. See section 13 for

shipping details. Details of FFPE block(s) should be completed on the Pleural Biopsy FFPE block worksheet (section 15.8) and the Meso-ORIGINS Pleural Biopsy FFPE log and transfer form.

11.1. **Retrieval of FFPE blocks acquired during Clinical Care**

Retrieval of FFPE Blocks acquired during clinical care will be central study activity at Visit A1, and Visit Ax if this occurs. Appropriate labelling of the retrieved FFPE blocks is integral, described below:

FFPE blocks from the diagnosis of **BENIGN PLEURAL INFLAMMATION** that led to inclusion in the study (**visit A1**), should be labelled with Study ID and visit number (**e.g. MO-001, A1**).

All further biopsies taken within NHS care will be performed with **clinical suspicion of MESOTHELIOMA EVOLUTION**. (As noted- these biopsies are NOT study specific activity, have no study visit number, and should be designated **visit Ax)**.

Such biopsies may reveal a diagnosis of BENIGN PLEURAL INFLAMMATION or MESOTHELIOMA. These blocks should be labelled with Study ID, Ax, and confirmation of diagnosis: BN = Benign, M = Mesothelioma e.g. (**MO-001, Ax, BN** or **MO-002, Ax, M**).
If there is more than one Ax biopsy time point with a benign diagnosis, please label with sequential numbers (BN, BN2, BN3…) e.g. (**MO-001, Ax, BN; MO-001, Ax, BN2).**

Post-mortem biopsies taken with **clinical suspicion of MESOTHELIOMA EVOLUTION** should be designated **visit Apm)**. As above such biopsies may reveal a diagnosis of BENIGN PLEURAL INFLAMMATION or MESOTHELIOMA. These blocks should be labelled with Study ID, Apm, and confirmation of diagnosis: BN = Benign, M = Mesothelioma e.g. (**MO-001, Apm, BN** or **MO-002, Apm, M**).

Pleural biopsy FFPE block(s) corresponding to A1 visit, all subsequent Ax visits and any Apm visits should be retrieved from the local pathology department and transported to the PREDICT-Meso RTB as per shipping instructions in Section 13. **These samples should be sent to the RTB as soon as possible after retrieval.** No study specific sampling processing is required.

Details of FFPE block should be completed on the Pleural Biopsy FFPE block worksheet (section 15.8) and the Meso-ORIGINS Pleural Biopsy FFPE log and transfer form.

This task is the responsibility of the PI, executed by Research Nurse staff or local biorepository staff depending on local arrangements. (Local responsibilities/delegates will be recorded in the site delegation log).

Visit	Diagnosis- block detail	label	example
A1	benign pleural inflammation - pre study that led to study inclusion	Study ID and visit number (A1)	**MO-001, A1**
Ax	Biopsies taken within NHS care - performed with clinical suspicion of mesothelioma evolution.	Study ID, Ax, and confirmation of diagnosis: BN = Benign or M = Mesothelioma If there is more than one Ax biopsy time point with a benign diagnosis, please label with sequential numbers (BN1, BN2, BN3…)	**MO-001, Ax, BN** or **MO-002, Ax, M** **MO-001, Ax, BN1; MO-001, Ax, BN2; MO-001, Ax, BN3**
Apm	Post-mortem biopsies performed with clinical suspicion of mesothelioma where repeat biopsy during life was not possible	Study ID, Apm, and confirmation of diagnosis: BN = Benign or M = Mesothelioma	**MO-001, Apm, BN** or **MO-002, Apm, M**

11.2. Processing and Retrieval of FFPE blocks acquired by Research Sampling

Further information regarding the different biopsy procedures that may be used to acquire the biopsies can be found in the Biopsy Manual.

Once the pleural tissue biopsy samples are obtained, they should be fixed in 10% formalin and prepared as standard protocols for FFPE preservation.

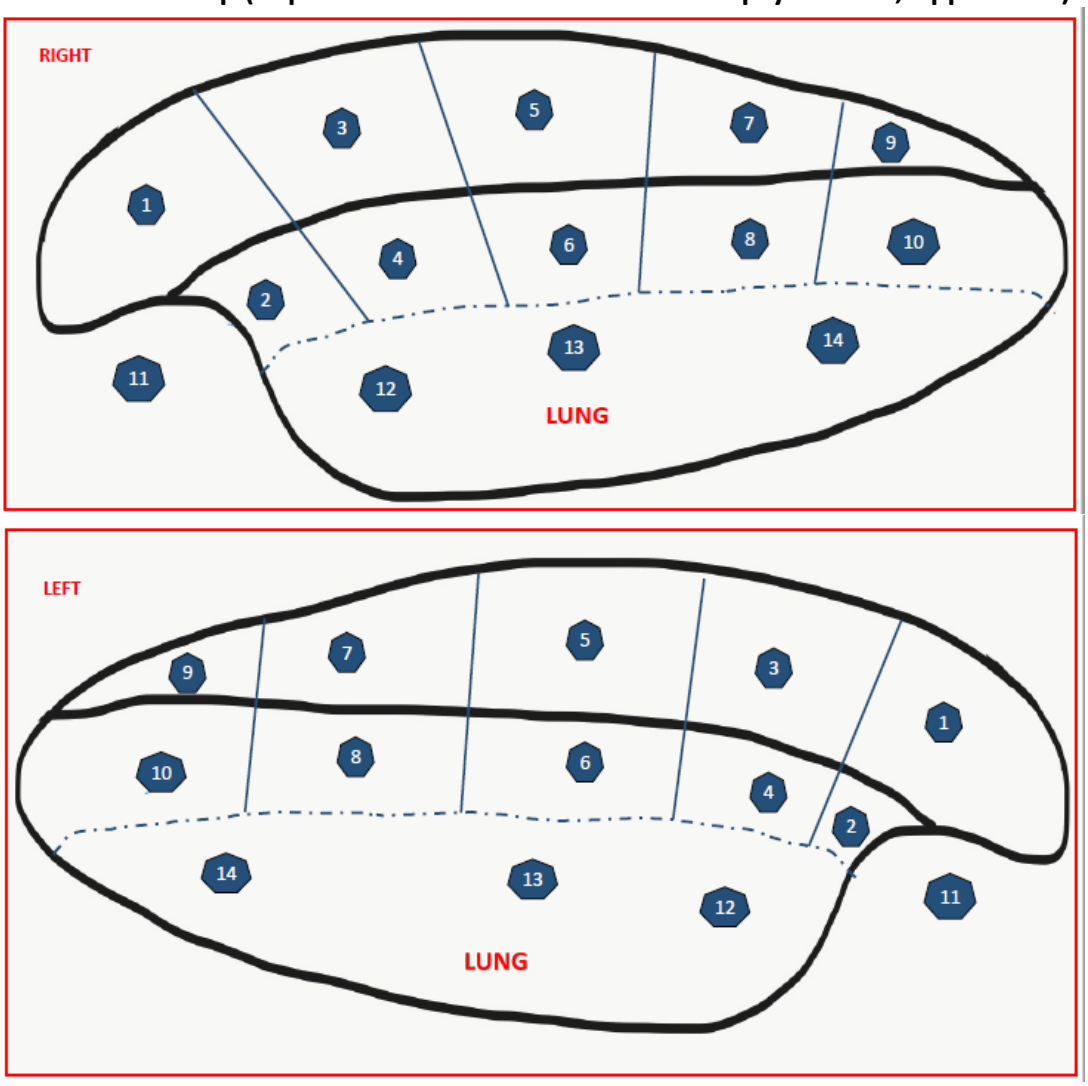
11.2.1. Multi-region Pleural Biopsies in Arm B

Multiple research biopsies (4-6) will be collected during LAT or VATS thoracoscopy in Arm B patients at Visit B2. The Thoracoscopy Findings Worksheet (Appendix 3 in the Meso-ORIGINS Biopsy Manual) includes a zonal map of the pleural space, which should be used to identify and record the anatomical location of each research sampling site. This map is reproduced overleaf for easy reference. It is of **critical importance** to the heterogeneity studies planned using this material that:

- Research and clinical biopsies are recorded separately and put into different biopsy pots
- **Research samples from a single site are put into a single, numbered research biopsy pot** (#1, #2, #3, #4, #5, #6)

For example: 6 research sites might be sampled, plus 5 clinical biopsies (as directed by the operator's normal judgement). This should generate 7 separate biopsy pots at the end of procedure: 6 research biopsy pots, each one containing samples from a single research biopsy site, and 1 clinical biopsy pot containing all 5 clinical biopsy samples.

Pleural Zone Map (Reproduced from Meso-ORIGINS Biopsy Manual, Appendix 1)



Method

- Biopsy samples should be acquired by LAT or VATS, as per the Meso-ORIGINS Biopsy Manual)
- The six research biopsy pots provided in the visit pack are not sterile, and should only be introduced into the sterile field in a sterile container, e.g. a gallipot
- Research biopsy pot #1 should be opened and placed inside this gallipot (or other suitable vessel) within reach of the operator acquiring the biopsies
- Tissue samples should be removed from biopsy forceps using a sterile needle, tweezers or other suitable method, before placement into research biopsy pot #1; this pot number should be recorded against the appropriate pleural zone on the Meso-ORIGINS Arm B Thoracoscopy Worksheet (Appendix 3 of the Meso-ORIGINS Biopsy Manual).
- The biopsy forceps should not be dipped into the biopsy pot, unless they are rinsed to remove formalin before the scope is inserted back into the patient
- Once sampling at the first research site has been completed, the lid on pot #1 should be secured tightly.
- The pot can then be lifted out of the sterile gallipot by a non-sterile assistant and replaced with research biopsy pot #2, which should be opened, ready to accept samples
- The process should be repeated until up to 6 research sites have been sampling, filling up to 6 research biopsy pots and recording each sampling site against the appropriate pleural zone on the Meso-ORIGINS Arm B Thoracoscopy Worksheet
- Worksheet records should include any visible pleural abnormalities, the sites that research biopsies were taken from and the pots used for each site. The form also records the number of clinical biopsies taken
- All biopsy pots containing research samples (up to 6) should be placed back inside the sealable plastic bag provided in the visit pack, alongside a local pathology department request form.
- This form must state clearly that these are Research Biopsies and NO DIAGNOSTIC ANALYSES ARE TO BE PEFORMED – ONLY FFPE BLOCK CREATION.
- The FFPE blocks generated should be stored at room temperature before shipping and sent to the PREDICT-Meso Research Tissue Bank (RTB) as soon as possible after collection. See section 13 for shipping details. This retrieval activity is part of Visit B3.
- **Both the Research Biopsy Pots and corresponding FFPE blocks should be labelled with Study ID, Study Visit and Pot Number (e.g. MO-001, B2, #3).** This is an essential step and of critical importance to the heterogeneity studies.

12. Exhaled Breath

At Visit A1 (or A2 if not collected at A1) and B1 (or B2 **BEFORE THORACOSCOPY** if not collected at B1) exhaled breath will be collected to generate the following samples:

Sample Type	Collection Volume /Tube Type
1 x background sample 1 x exhaled breath sample	2 x 500ml sorbent tube (3.5” long, 0.25” outer diameter) filled with 200mg Tenax®GR

Please see the exhaled breath sampling manual for detail

In summary:

- Take a background sample (see exhaled breath sampling manual for detail)
- Ask the participant to breathe calmly for 5 minutes through a mouthpiece that is connected to an inspiratory silica filter via a non-rebreathing valve

- After 5 minutes, the participant is asked to inhale maximally and hold their breath
- While the participant is holding their breath, attach a 10L Tedlar bag is to the expiratory outlet and ask the participant to exhale a full expiratory capacity at a slow rate
- When maximally exhaled, the Tedlar bag is closed and within 3 minutes connected to a TenaxGR-column via a pump
- From the bag, 500 ml (5 min at 100ml/min) of exhaled air is transferred on the column, which is tightly capped and stored in a glass jar at room temp until analysis.
- Label the top of the jar using an indelible marker with the participant study ID
- Place into box labelled using indelible marker with the following information:
    - Study Name (Meso-ORIGINS)
    - Recruiting Centre
    - Participant study ID
    - Participant initials
- Leave box at room temp until ready to ship (see section 10 for shipping instructions)
- Complete the exhaled breath worksheet and store the completed worksheet in the Investigator Site File until shipping (Section 13)
- Complete the sample details on the Meso-ORIGINS log and transfer form- Exhaled Breath.

13. Transport of Processed Samples

At the end of the study, each participant should have the following samples:

Sample Type	Collection Volume /Tube Type	Detail	Temp
Whole blood	10 x 6ml EDTA tubes (5ml/tube)	2 x 6ml per visit; 5 visits in Arm A- A1, A3-A6 1 visit in Arm B- B1	-80°C
Whole blood-germline DNA	1 x 2.5ml tube with whole blood for germline DNA	1 x 2.5ml tube; 1 visit- A1 1 x 2.5ml tube; 1 visit- B1	-80°C
Serum	Up to 40 Serum samples with yellow caps in 1.5ml cryovials	8 x 0.5ml in 1.5ml cryovials per visit; 5 visits in Arm A- A1, A3-A6; 1 visit in Arm B- B1	-80°C
Plasma	Up to 40 Plasma samples with orange caps in 1.5ml tubes	8 x 0.5ml in 1.5ml cryovials per visit; 5 visits in Arm A- A1, A3-A6; 1 visit in Arm B- B1	-80°C
Pleural Fluid: Unprocessed sample for cell lines *(participating sites only)*	Arm A: 2 x 30ml universals Arm B: 2 x 30ml universals	2 x 30ml at Visit A1 (if IPC in-situ and fluid available), Visit B1	*ambient*
Pleural Fluid: Unprocessed sample for banking	Unprocessed Pleural fluid (4.5ml cryovials with purple caps) Arm A: 0-24 vials Arm B: 12 vials	2 x 30ml aliquoted into 12 x 4.5ml at Visit A1 (if IPC in-situ and fluid available), Visit B2	-80°C
Pleural Fluid: Processed for Cell Pellet	Effusion cell pellets (1.5ml cryovials with blue caps) Arm A: Up to 4 vials Arm B: 4 vials	Arm A: Visit A1 (if IPC in-situ and fluid available) 1x1.5ml pellet cryovial per sample (x4), Arm B: Visit B2; 4 samples	-80°C

Sample Type	Collection Volume /Tube Type	Detail	Temp
Pleural Fluid: Processed for Supernatant	Effusion supernatant (4.5ml cryovials with green caps) Arm A: 0-20 vials Arm B: 20 vials	Arm A: Visit A1 (if IPC in-situ and fluid available), 5 x 4.5ml cryovials per sample (x4) Arm B: Visit B2; 4 samples	-80°C
Pleural tissue biopsies	FFPE blocks	archived samples and any new samples taken as part of study	*ambient*
Exhaled breath	2 x 500ml exhaled breath samples in columns, in glass jar. 1 x background; 1 x sample	2 x 500ml column; 1 visit- A1 (or A2); B1 (or B2)	*ambient*

The following complete **original** documents should accompany the shipped samples:

- Complete sample worksheets
- Cryobox template
- Sample transfer form/ FFPE block transfer form

*Sites should keep a **copy** of these worksheets for their own records.*

13.1. Whole Blood, Serum, Plasma, Processed Pleural Fluid (Supernatant and Cell Pellets), Unprocessed Pleural Fluid (Frozen samples)

Whole Blood, Serum, Plasma, Processed Pleural Fluid (Supernatant and Cell Pellets) and some unprocessed Pleural Fluid (Frozen Samples) will be shipped to the NHSGGC biorepository annually, with room for flexibility upon contacting Alexandrea.macpherson@glasgow.ac.uk

- These samples must all be stored at -80°C (+/-10ºC) and transported on dry ice
- Sample tubes must be stored and transported in the cryoboxes provided
- Box number and tube position within the cryobox must be completed on the provided Meso-ORIGINS Sample log and transfer form, prior to shipping
  - As noted in section 8.3, Meso-ORIGINS Sample log and transfer form is an excel file supplied separately from this manual. It is advised that various tabs are created on this file. Tab one for a whole sample log; Tab 2 for first shipment; Tab 3 for next shipment and so on
  - This allows this document to act as a sample log and a shipping log in one place
- Each shipment should have a **NEW** Sample log and transfer form, but we ask that sites keep a copy/ record of all shipping forms so that a cumulative log of samples shipped is available
- Complete original worksheets, cryobox templates and Sample Log and Transfer forms should be packaged with the samples (in addition please email a copy to Biorepository.Research@ggc.scot.nhs.uk)
- *Please keep copies of the sample worksheets and transfer forms in your ISF for your own site records*
- Please ship all samples Monday-Thursday ONLY
- Samples should be sent to the PREDICT-Meso Research Tissue Bank (RTB) (under care of Glasgow Biorepository) at:

FAO NHSGGC Biorepository (PREDICT-Meso RTB)
L3/B/009
Laboratory Medicine Building
Queen Elizabeth University Hospital
Govan Road

Glasgow G51 4TF

- The PREDICT-Meso Project Manager will contact each site to advise when samples are to be shipped and will provide courier instructions including details of contact for dry ice shipment and account number
- Samples must be packed securely to avoid breakage during transit and meet P650 guidelines. Samples should be packed with sufficient dry ice to prevent thawing for at least 2 days to allow for any delays in transport or delivery (2.3 – 4.5 kg per 24 hours). Dry ice and transportation box will be provided by the courier at the time of sample collection
- For queries relating to the transfer of samples to the Glasgow Biorepository, please contact Biorepository.research@ggc.scot.nhs.uk copying in Clare.Orange@ggc.scot.nhs.uk

13.2. **Pleural Biopsy FFPE Blocks**

These samples can be stored and transported under ambient conditions

Pleural Biopsy FFPE Blocks should be prepared for shipping as follows:

- Wrap each block individually in paper towel or similar to protect the tissue surface.
- Place all wrapped blocks into the provided sealable bag. Ensure they are lying flat in the bag.
- Please use paper towels to pad out any space in the bag so there is no movement of blocks in transit
- Seal the bag with tape if possible
- Place the sealed bag into the padded envelope provided (with UN3773 label)
- send to the NHSGGC Biorepository at:

  FAO NHSGGC Biorepository (PREDICT-Meso RTB)
  L3/B/009
  Laboratory Medicine Building
  Queen Elizabeth University Hospital
  Govan Road
  Glasgow G51 4TF

- Please email Biorepository.Research@ggc.scot.nhs.uk to let them know to expect samples and copy in Mark.Neilly@ggc.scot.nhs.uk
- Please ship all samples Monday-Thursday ONLY
- At the same time as posting the material, the site must complete the appropriate Pleural Biopsy FFPE worksheet (section 15.8) and the "Meso-ORIGINS Pleural Biopsy FFPE block transfer Form" and sent these with the blocks
  - As noted in section 8.3, Meso-ORIGINS Pleural Biopsy FFPE block transfer Form is an excel file supplied separately from this manual. It is advised that various tabs are created on this file. Tab one for a whole sample log; Tab 2 for first shipment; Tab 3 for next shipment and so on
  - This allows this document to act as a sample log and a shipping log in one place
- Each shipment should have a **NEW** Sample log and transfer form, but we ask that sites keep a copy/ record of all shipping forms so that a cumulative log of samples shipped is available
- Complete original worksheets, and Sample Log and Transfer forms should be packaged with the samples
- *Please keep copies of the sample worksheets and transfer forms in your ISF for your own site records.*

13.3. **Unprocessed Pleural Fluid- at room temperature (at participating sites)**

To be shipped within 5 hours of collection

- These samples can be stored and transported under ambient conditions
- 2 x 30ml universal with pleural fluid tube should be securely packaged using the provided Royal Mail SafeBox. This will include postage, packaging, absorbent wadding, a self-seal bag with an 'O' ring to ensure inner container is watertight.
- Remember to include the original complete worksheet
- SafeBoxes should be sent to the MacFarlane lab at MRC Toxicology Unit in Cambridge:

  MacFarlane lab
  MRC Toxicology Unit
  Gleeson Building
  Tennis Court Road
  Cambridge CB2 1QR
- Please call ahead to let the lab know that they should expect samples so that someone is there to receive them. Please call 07500126479 (if no answer use, 01223 334576)
- If no-one answers please email Emily Self es899@mrc-tox.cam.ac.uk and Xiao-Ming Sun xms21@mrc-tox.cam.ac.uk copying in Marion MacFarlane mm2312@mrc-tox.cam.ac.uk to alert them to expect samples
- Please do not ship samples on Fridays as there is no one to receive these samples until Monday. Please ship all samples Monday-Thursday.
- Please contact the PREDICT-Meso Project Manager when your supply of safeboxes is running low so that these can be replenished.
- *For sites local to Cambridge, safeboxes will not be used. Instead, standard boxes and a taxi courier will be organised. Please call 07500126479 (or 01223 334576) to organise.*

13.4. **Exhaled Breath Samples**

Shipping every 3-6 months, see detail below:

- These samples can be stored and transported under ambient conditions
- Conditioned tubes are used for exhaled breath collection and storage
- 6-month supply of tubes will be sent at the beginning of every project year by the PREDICT-Meso Project Manager or Dr Lamote.
- **NB:** All tubes, including those containing samples and empty tubes, need to be returned to the University of Antwerp at least every 6 months and new conditioned tubes requested. This is because passive diffusion of environmental compounds could accumulate in tubes older than 6 months causing contamination
- Exhaled breath samples should therefore be stored at room temperature and sent directly to University of Antwerp every 3-6 months (no later than 6 months), depending on the number of participants recruited and the need for fresh (empty) tubes
- Liaison regarding dispatch and return of sample tubes should be with Antwerp directly via contact kevin.lamote@uantwerpen.be copying in Alexandrea.macpherson@glasgow.ac.uk
- This includes any requirement for *additional tubes* to be sent, depending on recruitment rate
- Remember to include the original complete worksheet and Meso-ORIGINS Sample log and transfer form- Exhaled Breath.

14. Cryobox templates

For PLASTIC cryobox holding 1.5ml vials for serum, plasma and pleural pellet

- ***New template to be printed per box***

Study: Meso-ORIGINS Site: Samples: box#:

Please note the following for each sample:

- Participant study ID
- Participant initials
- Visit (eg A1 or B2)
- S (**S**erum) or P (**P**lasma) or PP (**P**leural **P**ellet)

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

For <u>CARDBOARD</u> cryobox holding 2.5ml PAXgene tubes whole blood for germline DNA; 4.5ml vials for pleural fluid supernatant, and unprocessed pleural fluid; 6ml tubes for whole blood - *<u>New template to be printed per box</u>*

Study: Meso-ORIGINS Site: Samples: box#:

Please note the following for each sample:

- Participant study ID
- Participant initials
- Visit (eg A1 or B2)
- PS (**<u>P</u>**leural **<u>S</u>**upernatant); PF (unprocessed **P**leural **<u>F</u>**luid); WB (**<u>W</u>**hole **<u>B</u>**lood); G (Whole blood for **<u>G</u>**ermline DNA)

1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26	27
28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54
55	56	57	58	59	60	61	62	63
64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	81

15. Worksheets

15.1. **Meso-ORIGINS Whole Blood Worksheet**

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B1)	Date and Collection Time	Time Frozen
Box #	**Box position**	**Operator (Print Name and Sign)**

Record whether blood was drawn using peripheral venous access device (e.g., butterfly) or central venous access device (CVAD) here: _____

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.2. **Meso-ORIGINS Whole Blood for Germline DNA Worksheet**

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B1)	Date and Collection Time	Time Frozen
Box #	**Box position**	**Operator (Print Name and Sign)**

Record whether blood was drawn using peripheral venous access device (e.g., butterfly) or central venous access device (CVAD) here: _____

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.3. **Meso-ORIGINS Serum Worksheet**

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B1)	Date and Collection Time	Centrifugation start time	Time Frozen
Box #	**Box positions**	**Number of tubes frozen**	**Operator (Print Name and Sign)**

Record whether blood was drawn using peripheral venous access device (e.g., butterfly) or central venous access device (CVAD) here: _____

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.4. **Meso-ORIGINS Plasma Worksheet**

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B1)	Date and Collection Time	Centrifugation start time	Time Frozen
Box #	**Box positions**	**Number of tubes frozen**	**Operator (Print Name and Sign)**

Record whether blood was drawn using peripheral venous access device (e.g., butterfly) or central venous access device (CVAD) here: _____

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.5. **Meso-ORIGINS Pleural Fluid Worksheet - unprocessed fluid – Room temperature**

(For sending to Cambridge. Manual Section 101.)

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B2)	Date and Collection Time	Operator (Print Name and Sign)

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.6. **Meso-ORIGINS Pleural Fluid Worksheet - unprocessed fluid for banking - (Frozen)**
(Manual Section 10.2)

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B2)	Date and Collection Time		Time Frozen
Box #	**Box position**	**Number of tubes frozen**	**Operator (Print Name and Sign)**

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.7. **Meso-ORIGINS Processed Pleural Fluid Worksheet - supernatant and cell pellet for banking – (Frozen)**

(Manual Section 10.3)

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B2)	Date and Collection Time	Centrifugation start time	Operator (Print Name and Sign)

Pellets

Pellets- Number of tubes frozen	Time frozen	Box #	Box positions

Supernatant

Supernatant- Number of tubes frozen	Time frozen	Box #	Box positions

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.8. **Meso-ORIGINS Pleural Biopsy FFPE blocks Worksheet**

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Visit (e.g.B2)	Biopsy date *	Date of retrieval
Number of blocks	**Date of transport to RTB**	**Operator (Print Name and Sign)**

*Acknowledged that the date of biopsy may pre-date the date of visit (any pleural biopsy within 1 year)

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

15.9. **Meso-ORIGINS Exhaled Breath Sample Worksheet**

Participant Study ID:_____ Participant Initials:_____

Centre Name: _____

Background samples

Visit (e.g., A1 or A2)	Date and Collection Time	Collection tube ID
Volume	**Sample Type**	**Operator (Print Name and Sign)**
	Background	

Breath Sample

Visit (e.g., A1 or B1)	Date and Collection Time	Collection tube ID
Volume	**Sample Type**	**Operator (Print Name and Sign)**
	Breath	

Please describe any deviations from the laboratory manual or issues below:

_____

_____

_____

_____

16. Declaration

I confirm that I have received, read and understood this manual (sample handling manual v2.4 04 Sep24)

Site:_____

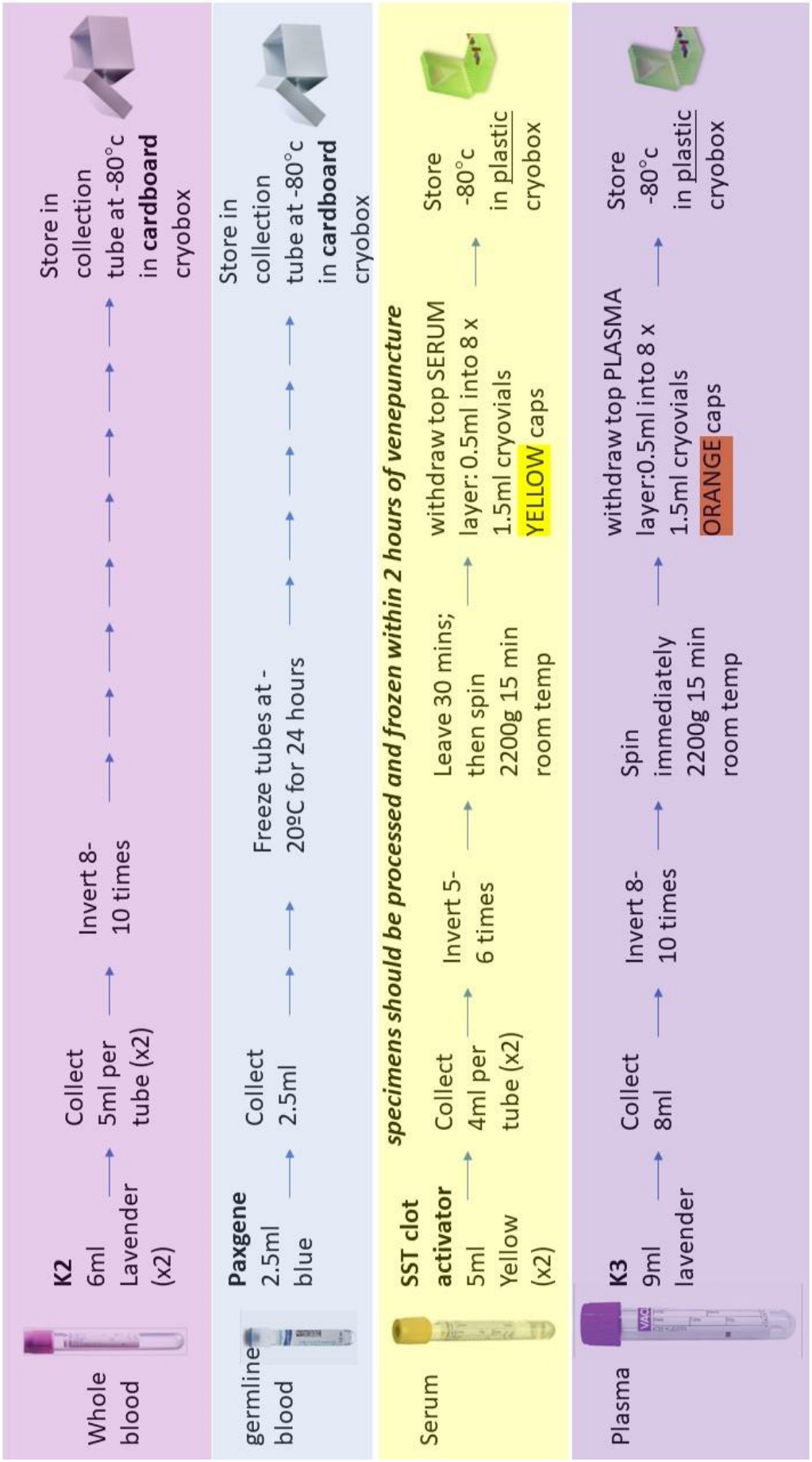
Site PI Name:_____

Signature: _____

Date: _____

Please return this declaration to the Project Manager Alexandrea.macpherson@glasgow.ac.uk

Appendix 1: Blood sampling infographic



Appendix 2: Pleural Fluid sampling infographic

10.1 Unprocessed pleural fluid for cell lines- *participating sites only*

Collect 2 x 30ml pleural fluid → Keep at room temp DO NOT CHILL → Put into provided royal mail safeboxes

→ Pop in post (postage already paid)

10.2 Unprocessed pleural fluid- KEEP SAMPLES COLD (in fridge or on ice) until aliquoting

Collect 30ml pleural fluid (x2) → Aliquot into 12 x 4.5ml cryovials with PURPLE caps → Pleural Fluid (PF) → Store 4.5ml PF cryovials at -80°c in **cardboard** cryobox

10.3 Processed pleural fluid- KEEP SAMPLES COLD (in fridge or on ice) until aliquoting
Pre-cool centrifuge to 4 ℃; Pre-cool the 4.5ml and 1.5ml vials by placing on ice

Collect 30ml pleural fluid (x4) → Spin at 500xG 10 min at 4°C → WITHOUT DISTURBING PELLETS withdraw the supernatant and dispense 4ml aliquots into 4.5ml cryovials with GREEN caps → Pleural Supernatant (PS) → Store 4.5ml PS cryovials at -80°c in **cardboard** cryobox

Pleural Pellet (PP)

REMEMBER TO DO THIS PROCESS (10.3) FOR **ALL 4** SAMPLES
At end should have:
20 x 4.5ml cryovials with supernatant- green caps
(minimum) 4 x 1.5ml cryovials with pellet- blue caps

Gently resuspend cell pellets by tapping base of tube. Transfer (by micropipette) each pellet suspension to a separate pre-chilled 1.5ml cryovial with a BLUE cap *(if pellet is over 100ul transfer 100ul per tube as per section 10.3)* → Spin at 500xG 3 min at 4°C → Discard the liquid, taking care not to disturb the cell pellets. Add RNAlater → Store 1.5ml PP cryovials at -80°c in plastic cryobox