





Sample Handling Manual

Meso-ORIGINS



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Contents

1. CONTACT INFORMATION	3
2. INTRODUCTION	4
3. SCOPE	4
4 RESPONSIBILITIES	4
	тт Л
5. RELATED DOCOMENTS.	
6. CONSUMABLES AND EQUIPMENT	5
6.1. EQUIPMENT	5
6.2. CONSUMABLES	5 E
7.1 ADVA	/
7.1. ARM A 7.2 Δρ. Β	/ 7
	······ /
8. SAMIPLE COLLECTION NOTES	ة
8.1. LABELLING CONVENTIONS	8
8.3. SAMPLE LOGS	
9 RESEARCH BLOOD SAMPLE PROCESSING STORAGE AND SHIPMENT	9
9.1. WHOLE BLOOD	9
9.2. Whole Blood for Germline DNA	10
9.3. Serum	10
9.4. Plasma	11
10. PLEURAL FLUID	12
10.1. UNPROCESSED PLEURAL FLUID FOR CELL LINES – PARTICIPATING SITES ONLY	13
10.2. UNPROCESSED PLEURAL FLUID FOR BANKING	13
10.3. PROCESSED PLEURAL FLUID: CELL PELLET AND SUPERNATANT BANKING	14
11. PLEURAL BIOPSIES	15
11.1. RETRIEVAL OF FFPE BLOCKS ACQUIRED DURING CLINICAL CARE	16
11.2. PROCESSING AND RETRIEVAL OF FFPE BLOCKS ACQUIRED BY RESEARCH SAMPLING.	16
11.2.1. No Mesothelioma Evolution in Arm A	17
11.2.2. Multi-region Pleural Biopsies in Arm B	17
12. EXHALED BREATH	19
13. TRANSPORT OF PROCESSED SAMPLES	20
13.1. WHOLE BLOOD, SERUM, PLASMA, PROCESSED PLEURAL FLUID (SUPERNATANT AND CELL PELLETS), UNPROCESSED PLEURAL F	LUID
(FROZEN SAMPLES)	Z1
13.2. PLEUKAL BIOPSY FFPE BLOCKS	21 22
13.4 Exhaied Breath Samples	22
14 CRYOBOX TEMPIATES	21
	24 2C
15. WURKSHEE IS	20
15.1. MESO-ORIGINS WHOLE BLOOD WORKSHEET	20 27
15.3. MESO-ORIGINS SERUM WORKSHEET	
15.4. Meso-ORIGINS Plasma Worksheet	29
15.5. Meso-ORIGINS Pleural Fluid Worksheet - UNPROCESSED Fluid – ROOM TEMPERATURE	30
15.6. Meso-ORIGINS PLEURAL FLUID WORKSHEET - UNPROCESSED FLUID FOR BANKING - (FROZEN)	31
15.7. Meso-ORIGINS PROCESSED PLEURAL FLUID WORKSHEET - SUPERNATANT AND CELL PELLET FOR BANKING	32
15.8. MESO-ORIGINS PLEURAL BIOPSY FFPE BLOCKS WORKSHEET	33
15.9. Meso-ORIGINS Exhaled Breath Sample Worksheet	34
16. DECLARATION	35
APPENDIX 1: BLOOD SAMPLING INFOGRAPHIC	36
APPENDIX 2: PLEURAL FLUID SAMPLING INFOGRAPHIC	37



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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: <u>Alexandrea.Macpherson@glasgow.ac.uk</u>

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,	Exhaled breath
filter, Tedlar bag, Tenax column sorbent tubes	
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	Medium bag marked:	1		1 x Paxgene tube	Germline blood
<u>A1</u>	Meso-ORIGINS			8 x 1.5ml vials with yellow caps	Serum
	Arm A Visit A1			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	Medium bag marked:	1		3 x 30ml universals	Pleural Fluid collection
<u>A1</u>	Meso-ORIGINS			6 x 5ml tubes with purple caps	Unprocessed Pleural Fluid
	Pleural fluid A1			10 x 5ml tubes with green caps	Processed Pleural Fluid supernatant
				2 x 1.5ml tubes with blue caps	Processed Pleural Fluid cell pellets
				18 x labels	For storage tubes in pack (18)
Arm A	Medium envelope/bag	4	As visit A3, A4, A5 and A6 all	8 x 1.5ml vials with yellow caps	Serum
<u>A3</u> or	marked:		have the same sampling	8 x 1.5ml vials with orange caps	Plasma
<u>A4</u> or <u>A5</u> or A6	Image:	18 x labels	For storage tubes in pack (16) plus 2 whole blood tubes (supplied by site)		
Arm A	Medium bags marked:	1	These packs will follow later	1 x biopsy pot	Pleural biopsy
<u>A8</u>	Meso-ORIGINS		for A8 visits to ensure biopsy	3 x 30ml universals	Pleural Fluid collection
	Pleural biopsy and fluid		pots are within expiry dates	6 x 5ml tubes with purple caps	Unprocessed Pleural Fluid
	Arm A (A8)			10 x 5ml tubes with green caps	Processed Pleural Fluid supernatant
				2 x 1.5ml tubes with blue caps	Processed Pleural Fluid cell pellets
				18 x labels	For storage tubes in pack (18)
Arm B	small bags marked:	1		1 x Paxgene tube	Germline blood
<u>B1</u>	Arm B (B1)			1 x label	
Arm B	large bags marked:	1	These large bags will contain	Biopsy pots for Arm B research biopsies	Pleural biopsy
<u>B2</u>	B2Meso-ORIGINSArm B visit B2	biopsy pots and a	biopsy pots and a medium bag	(6 per participant)	
			which contains pieural fluid	3 x 30ml universals	Pleural Fluid collection
			conection tubes	6 x 5ml tubes with purple caps	Unprocessed Pleural Fluid
				10 x 5ml tubes with green caps	Processed Pleural Fluid supernatant
				2 x 1.5ml tubes with blue caps	Processed Pleural Fluid cell pellets
	1			18 x labels	For storage tubes in pack (18)

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

Study Procedure	A1	A2	A3	A4	A5	A6	A7	A8	A9
Plasma sample	x	Xa	х	х	х	х			
Serum sample	x	Xa	х	х	х	х			
Whole blood sample	x	Xa	х	х	х	х			
Whole blood sample for germline DNA	x	Xa							
Pleural fluid sample unprocessed for Cell lines (room temp- participating sites only)	Xp	Xa						x	
Pleural fluid sample unprocessed for banking (frozen)	Xp	Xa						x	
Pleural fluid sample processed for Cell Pellet and Supernatant Banking	Xp	Xa						x	
Additional Pleural biopsies taken as part of study								x	
Previous FFPE pleural biopsy retrieval	x								
Exhaled Breath Sample (participating sites only)	x	Xa							

^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
Whole blood sample for germline	v	Va	
DNA	^	^	
Pleural fluid sample; Unprocessed;		v	
for Cell lines		×	
Pleural Sample; Processed; for Cell		v	
Pellet and Supernatant Banking		×	
Pleural biopsies		х	

^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 using the key below:

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

Example label - completed

Pt #:	
Visit:	
Sample:	

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 in 1.2ml cryovials
Cardboard	2ml-6ml	blood for germline DNA in 2.5ml PAXgene tube; unprocessed
		pleural fluid, pleural fluid pellet, pleural fluid supernatant in 5ml
		cryovials; whole blood in 6ml EDTA tube

Please label cryoboxes with:

- o Meso-ORIGINS
- o study site
- box # (sites can label boxes sequentially as used)
- o 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, 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	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	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	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 as described. <u>Samples stored in the collection tube</u>
- 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 <u>Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere</u>



- Immediately place the tubes into cardboard cryobox and place cryobox box into -80°C (+/-10°C) 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 <u>section 14</u>)
- 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 and B1 as described. <u>Samples stored in the collection tube</u>
- 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
- Immediately place the tubes into the cardboard cryobox and place cryobox box into -80°C (+/- 10°C) 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 <u>section 14</u>)
- 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 -80°C.



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 as described. <u>Samples are stored after processing into aliquots in 1.5ml cryovials</u>
- 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 <u>yellow caps.</u> There should be enough serum for 8 cryovial tubes. <u>Do not overfill these tubes</u>
- 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 -80°C (+/-10°C) 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 <u>section 14</u>)
- 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 as described. <u>Samples are stored after processing into aliguots in 1.5ml cryovials</u>
- 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 <u>orange caps</u> and discard the pellet and any remaining plasma. There should be sufficient plasma for 8 cryovial tubes. <u>Do not overfill the tubes</u>
- 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 -80°C (+/-10°C) freezer until ready to ship (see <u>Section 13</u> 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 <u>section 14</u>)
- 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, A8 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, 3 pleural fluid samples to be collected in 3 x 30ml universal container (90ml in total) generating the following:

Sample Type	Collection Volume /Tube Type
Unprocessed	1 x Unprocessed sample for banking
Processed	2 x Processed sample for Cell Pellet and Supernatant Banking

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 5 x 30ml universals of pleural fluid total. Sites will be informed in advance by PM.

Samples at Visit A1 will only be available in participants with an indwelling pleural catheter in-situ. In this setting, a total of 90ml should be aspirated from the IPC drainage bottle or removed using an IPC drainage line attached to a 50 ml syringe. 150 ml (90 ml plus 2 additional 30 ml) should be obtained at sites **participating in the generation of cell lines**.

At Visits A8 and B2, 90 ml (3 x 30ml) of pleural fluid will be obtained during thoracoscopy. 150 ml (90 ml plus 2 additional 30 ml) should be obtained at sites **participating in the generation of cell lines.**

Once the fluid samples are obtained:

• At participating sites only - The unprocessed sample (2 x 30ml) for generation of cell lines (<u>section 10.1</u>) should be stored at room temperature and be shipped within 5 hours (<u>see Section 13.3</u>). <u>DO NOT CHILL THIS SAMPLE</u>



- At all sites- The unprocessed sample (1 x 30ml) for banking (section 10.2) should be kept cold (in the fridge or on ice) and must be aliquoted into 5ml cryovials and frozen within 2 hours of sampling
- At all sites- The two samples (2 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: <u>Alexandrea.Macpherson@glasgow.ac.uk</u>

- Keep unprocessed pleural fluid at <u>room temperature</u>
- 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 (<u>section 15.5</u>) 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 (1 x 30ml) should be generated for all participants, where possible, at all sites at Visits A1, A8 and B2.

- Keep unprocessed pleural fluid cold (on ice or in the fridge) until aliquoting.
- Carefully dispense 5ml aliquots into 6 x 5ml cryovial tubes with purple caps. <u>Do not overfill</u> 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.
 <u>Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere</u>
- 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 -80°C (+/-10°C) 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 <u>section 14</u>)
- 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 (2 x 30ml) should be generated for all participants, where possible, at all sites at Visits A1, A8 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 5ml and 1.5ml vials by placing on ice
- Spin 30ml samples at 400xG for 10 minutes at 4ºC
- Complete the Meso-ORIGINS labels with an indelible marker and stick them securely onto final storage tubes (1 x 1.5 ml cryovial for cell pellet with a <u>blue cap</u> and 5 x 5ml cryovials for supernatant with <u>green caps</u> PER 30ML SAMPLE), ensuring that the bottom of the label is twisted around the base of the tubes. <u>Please ensure the labels are placed on all tubes before</u> <u>they are chilled</u>, <u>otherwise labels will not adhere</u>
- 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 5ml cryovials. There should be enough supernatant per sample for 5 x 5ml tubes. Keep on ice/at 4°C. <u>Do not overfill these tubes</u>
 - 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**.
- Label the top of the tubes using an indelible marker with the participant study ID, visit (e.g., A8) 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 <u>section 14</u>)
- Gently resuspend the cell pellet by tapping the base of the 30ml tube, then use a P-1000 micropipette to transfer cell suspension to the pre-chilled labelled cell pellet 1.5ml cryovial
- Spin the cryovial in the centrifuge, using an appropriately sized bucket adapter, at 400xG 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
- Label the top of the tubes using an indelible marker with the participant study ID, visit (e.g., A8) and PP (pleural pellet)
- Immediately place the tubes into a plastic cryobox and place cryobox box into -80°C (+/-10°C) freezer until ready to ship (see <u>Section 13</u> 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
- Complete sample info (participant study ID, visit, Sample type (PP)) and placement in cryobox on cryobox template (see <u>section 14</u>)
- 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 <u>core</u> study activity. This will happen via two pathways within Meso-ORIGINS:

- 1. Retrieval of FFPE blocks generated within clinical care (Visit A1 and Visit Ax*)
- 2. Collection of research specific biopsies within the study (Visit A8 and 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.

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 <u>section 13</u> for shipping details. Details of FFPE block(s) should be completed on the Pleural Biopsy FFPE block worksheet (<u>section 15.8</u>) 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**).

Pleural biopsy FFPE block(s) corresponding to A1 visit and all subsequent Ax visits should be retrieved from the local pathology department and transported to the PREDICT-Meso RTB as per shipping instructions in <u>Section 13</u>. **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 (<u>section</u> <u>15.8</u>) and the Meso-ORIGINS Pleural Biopsy FFPE log and transfer form.

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	Study ID, Ax, and confirmation of diagnosis: BN = Benign or M = Mesothelioma	MO-001, Ax, BN or MO-002, Ax, M MO-001, Ax, BN1;
	evolution.	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, BN2; MO-001, Ax, BN3

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).

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. No Mesothelioma Evolution in Arm A

Research Biopsies are collected at Visit A8, following completion of at least 18 months of reassuring (benign) clinical follow-up. A single 25 ml formalin-filled biopsy pot is provided for this purpose in the visit pack. See Meso-ORIGINS Biopsy Manual for full detail.

Method

- Biopsy samples can be acquired by LAT or image-guided (CT or US) biopsy, as per the Meso-ORIGINS Biopsy Manual)
- Complete the relevant worksheet from the Meso-ORIGINS biopsy manual: Appendix 1 for Thoracoscopy or Appendix 2 for Image-guided biopsy.
- The research biopsy pot provided in the visit pack is not sterile, and should only be introduced into the sterile field in a sterile container, e.g. a gallipot
- The research biopsy pot 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 or needle using a sterile needle, tweezers or other suitable method, before placement into the pot.
- The biopsy forceps or needle should not be dipped into the biopsy pot, unless they are rinsed to remove formalin before reinsertion into the patient
- Once sampling at each site has been completed, the lid on the pot should be secured tightly.
- The pot can then be lifted out of the sterile gallipot by a non-sterile assistant
- The biopsy pot should be placed inside the sealable plastic bag provided in the visit pack, alongside a local pathology department request form highlighting that these are Research Biopsies, **NO DIAGNOSTIC STUDIES ARE TO BE PERORMED, 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 <u>section 13</u> for shipping details. This retrieval activity is part of Visit A9
- Both the Research Biopsy Pot and corresponding FFPE block(s) should be labelled with Study ID and Study Visit (e.g. MO-001, A8)

11.2.2. 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 <u>section 13</u> 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 exhaled breath will be collected to generate the following samples:

Sample Type	Collection Volume /Tube Type
Exhaled Breath	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:

- 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 (<u>Section 13</u>)
- Complete the sample details on the Meso-ORIGINS log and transfer form- Exhaled Breath.



13. Transport of Processed Samples

Sample Type	Collection Volume /Tube	Detail	Temp
	Туре		
Whole blood	10 x 6ml EDTA tubes	2 x 6ml per visit;	-80°C
	(5ml/tube)	5 visits- A1, A3-A6	
Whole blood-	1 x 2.5ml tube with whole	1 x 2.5ml tube; 1 visit- A1	-80°C
germline DNA	blood for germline DNA	1 x 2.5ml tube; 1 visit- B1	
	Up to 40 Serum samples	8 x 0.5ml in 1.5ml cryovials per	-80°C
Serum	with yellow caps in 1.5ml	visit; 5 visits- A1, A3-A6	
	cryovials		
	Up to 40 Plasma samples	8 x 0.5ml in 1.5ml cryovials per	-80°C
Plasma	with orange caps in 1.5ml	visit; 5 visits- A1, A3-A6	
	tubes		
participating sites	Arm A: 2 x 30ml universals	2 x 30ml at Visit A1 (if IPC in-situ	ambient
only	Arm B: 2 x 30ml universals	and fluid available), Visit A8,	
Pleural Fluid:		Visit B1	
Unprocessed			
sample for cell			
lines			
	Unprocessed Pleural fluid	1 x 30ml aliquoted into 6 x 5ml	-80°C
Pleural Fluid:	(5ml cryovials with purple	at Visit A1 (if IPC in-situ and	
Unprocessed	caps)	fluid available), Visit A8 (if fluid	
sample for banking	Arm A: 0-12 vials	available), Visit B2	
	Arm B: 6 vials		
	Effusion cell pellets (1.5ml	Arm A: 1x1.5ml pellet cryovial	-80°C
Pleural Fluid:	cryovials with blue caps)	per sample per visit from up to	
Processed for Cell	Arm A: Up to 4 vials	2 visits: Visit A1 (if IPC in-situ	
Pellet		and fluid available), Visit A8,	
	Arma D. D. viala	2 samples per visit	
	Arm B: 2 viais	Arm B: Visit B2; 2 samples	0000
	envoyiele with green cons)	Arm A: 5 X 5mi cryoviais per	-80°C
Pleural Fluid:	Arm A: 0.20 vials	sample per visit from up to 2	
Processed for	ATTILA. 0-20 VIAIS	fluid available) Visit A9, 2	
Supernatant		complex per visit	
	Arm B: 10 vials	Arm B: Visit B2: 2 camples	
Plaural tissue	FEDE blocks	archived samples and any new	ambient
hionsies		samples taken as part of study	uniblent
01043163	500ml exhaled broath	1 x 500ml column: 1 visit- A1	amhient
Exhaled breath	sample in column in glass		unibient
	iar		
	Jui .		

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

The following complete **<u>original</u>** documents should accompany the shipped samples:

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

Sites should keep a **<u>copy</u>** 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 <u>Alexandrea.macpherson@glasgow.ac.uk</u>

- These samples must all be stored at -80°C (+/-10°C) 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
 - \circ 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 <u>Biorepository.research@ggc.scot.nhs.uk</u> copying in <u>Clare.Orange@ggc.scot.nhs.uk</u>

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
- 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 <u>Biorepository.Research@ggc.scot.nhs.uk</u> to let them know to expect samples and copy in <u>Mark.Neilly@ggc.scot.nhs.uk</u>
- 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 <u>es899@mrc-tox.cam.ac.uk</u> and Xiao-Ming Sun <u>xms21@mrc-tox.cam.ac.uk</u> copying in Marion MacFarlane <u>mm2312@mrc-tox.cam.ac.uk</u> 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:

- <u>These samples can be stored and transported under ambient conditions</u>
- 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 <u>kevin.lamote@uantwerpen.be</u> copying in <u>Alexandrea.macpherson@glasgow.ac.uk</u>
- 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 - <u>New template to be printed per box</u>

Study: Meso-ORIGINS Site:

Samples:

box#:

Please note the following for each sample:

- o Participant study ID
- o Participant initials
- Visit (eg A1 or B2)
- S (<u>Serum</u>) or P (<u>P</u>lasma) or PP (<u>P</u>leural <u>P</u>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

Meso-ORIGINS Sample Handling Manual Version 1.9 09May23



For <u>CARDBOARD</u> cryobox holding 2.5ml PAXgene tubes whole blood for germline DNA; 5ml vials for pleural fluid supernatant, and unprocessed pleural fluid; 6ml tubes for whole blood - <u>New</u> <u>template to be printed per box</u>

Study: Meso-ORIGINS Site:

Samples:

box#:

Please note the following for each sample:

- o Participant study ID
- o Participant initials
- Visit (eg A1 or B2)
- PS (<u>P</u>leural <u>S</u>upernatant); PF (unprocessed Pleural <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
			- 10		10	- 10		
37	38	39	40	41	42	43	44	45
40	47	40	40	50	54	50	50	54
40	47	48	49	50	51	52	53	54
EE.	56	57	EQ	50	60	61	60	62
55	50	57	50	59	00	01	02	03
64	65	66	67	68	69	70	71	72
	00		01	00	00	10		12
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 B2)	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: _____



15.2. Meso-ORIGINS Whole Blood for Germline DNA Worksheet

Participant Study ID:_____

Participant Initials:_____

Centre Name: _____

Visit (e.g. <i>,</i> A1 or B2)	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: ______



15.3. Meso-ORIGINS Serum Worksheet

Participant Study ID:_____

Participant Initials:_____

Centre Name: _____

Date and ollection Time	Centrifugation start time	Time Frozen
Box positions	Number of tubes frozen	Operator (Print Name and Sign)
3	Date and Illection Time	Date and Centrifugation start billection Time time ox positions Number of tubes frozen

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



15.4. Meso-ORIGINS Plasma Worksheet

Participant Study ID:_____

Participant Initials:_____

Centre Name: _____

Date and Collection Time	Centrifugation start time	Time Frozen
Box positions	Number of tubes frozen	Operator (Print Name and Sign)
	Date and Collection Time Box positions	Date and Collection TimeCentrifugation start timeBox positionsNumber of tubes frozen

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



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

Participant Study ID:_____

Participant Initials:_____

Centre Name: _____

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



15.6. Meso-ORIGINS Pleural Fluid Worksheet - unprocessed fluid for banking - (Frozen)

Participant Study ID: _____ Partic

Participant Initials:_____

Centre Name: _____

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



15.7. Meso-ORIGINS Processed Pleural Fluid Worksheet - supernatant and cell pellet for banking

Participant Study ID:_____

Participant Initials:	
-----------------------	--

Centre Name: _____

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

Pellets

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

Supernatant

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



15.8. Meso-ORIGINS Pleural Biopsy FFPE blocks Worksheet

Participant Study ID:_____

Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or 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)



15.9. Meso-ORIGINS Exhaled Breath Sample Worksheet

Participant Study ID:_____

Participant Initials:_____

Centre Name: _____

Visit (e.g., A1 or B2)	Date and Collection Time	Collection tube ID
Volume	Sample Type (background or breath)	Operator (Print Name and Sign)



16. Declaration

I confirm that I have received, read and understood this manual (sample handling manual v1.8 28Nov22)

Site:_____

Site PI Name:______

Signature: ______

Date:	

Please return this declaration to the Project Manager <u>Alexandrea.macpherson@glasgow.ac.uk</u>



Appendix 1: Blood sampling infographic

1		Ť	T,
re in ection e at -80°c ardboard bbox	e in ection e at -80°c ardboard box	Store -80°c in <u>plastic</u> cryobox	Store -80°c in <u>plastic</u> cryobox
Stol coll in c	Stor colle tube in ca	1	A
 	↑ ↑ ↑	 hours of venepuncture withdraw top SERUN layer: 0.5ml into 8 x 1.5ml cryovials YELLOW caps 	withdraw top PLASM layer:0.5ml into 8 x 1.5ml cryovials ORANGE caps
 ↑	†	within 2 lins;	≥ iE _
†	 ↑	<i>l frozen</i> ave 30 m en spin 00g 15 n om temp	in mediate 00g 15 n om temp
Î	The second se	ed and Lee 22 roo	t Sp 23 ii Sp
vert 8-) times	↑ ♠	process rt 5- les	rt 8- mes
t 19 19	 ↑	uld be Invei 6 tim	Invei 10 ti
(2)		ns sho	Î
Collect 5ml pe tube (y	Collect 2.5ml	specime Collect 4ml pe tube (x	Collect 8ml
der) J	ot tor	der 1
K2 6ml Laven (x2)	Paxge 2.5ml blue	SST clo activa 5ml Yellow (x2)	K3 9ml lavenc
		O de sea D	
Whole blood	germlin blood	Serum	Plasma

Meso-ORIGINS Sample Handling Manual Version 1.9 09May23



Appendix 2: Pleural Fluid sampling infographic

