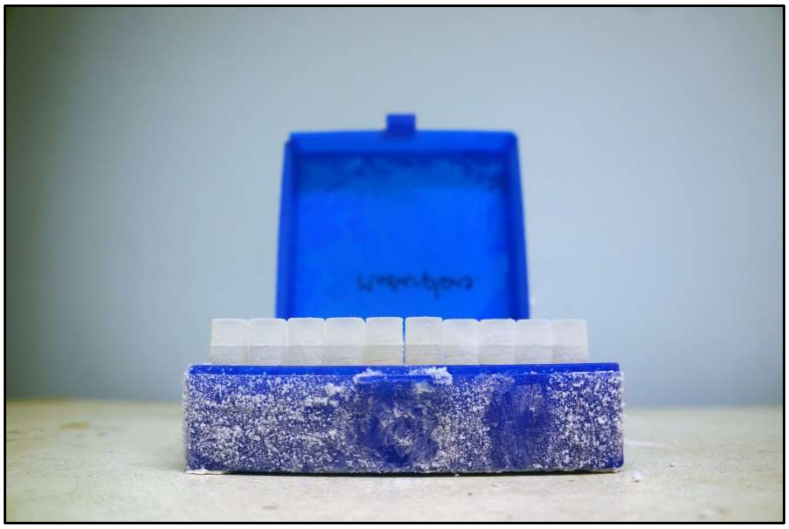
MANCHESTER 1824
The University of Manchester

MASTERplans
Maximizing SLE Therapeutic Potential by Application of Novel and Systematic Approaches



SOP reference no:	**PLANS-002 version 3.0**
SOP title	**Hub sample processing**

Index

1. Background

Prediction of Lupus treAtment respoNse Study (PLANS) is an open label observational study to identify predictors of response to rituximab (RTX) and mycophenolate (MMF) in patients with cutaneous or renal manifestations of systemic lupus erythematosus (SLE). Recruitment of 240 patients will take place at 20+ sites in England.

This Standard Operating Procedure (SOP) has been written to standardise processes across sample processing hubs.

Clinical sites will include well-resourced academic centres as well as district general hospitals. An important consideration was to reduce the amount of processing taking place at the clinical sites, so as less well-resourced sites can take part in PLANS. This in turn will make it easier to recruit participants quickly.

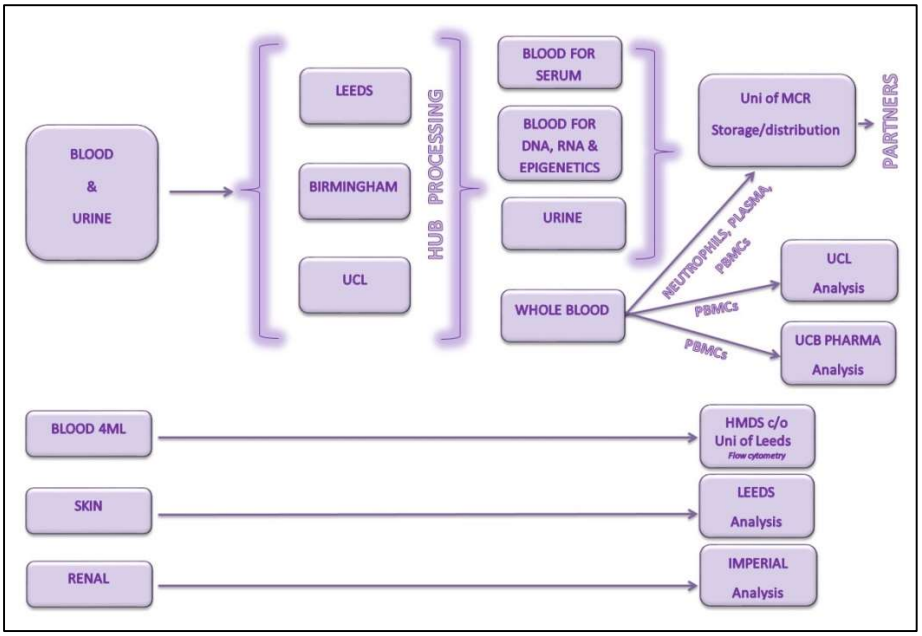
The decision was made to locate primary processing of samples at "sample processing hubs" at MASTERPLANS academic partner institutions in Leeds, Birmingham and London.

Blood, urine and possibly renal samples will be sent direct from the clinical site to the technical hubs. Processing can be extensive in addition to receipt, aliquoting, freezing and onward transport to University of Manchester (UoM) for storage and distribution among MASTERPLANS Partners.

Epidermal and/or skin biopsies will be sent directly from clinical sites to University of Leeds so this process is not included in this SOP.

Following hub processing most samples will be sent to UoM, where the samples will be biobanked and delivered periodically in bulk to MASTERPLANS partners for analysis.

Figure 1: MASTERPLANS sample movements



Blood and urine samples are collected from patients according to the following schedule of hospital visits:

Visit 1	V2	V3	V4	V5	V6	V7	V8	Flare after V6
Baseline 0	Baseline 1	Baseline 2	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up
Week -2 to 0	Week 0	Week 2	Week 4	Week 12	Week 26	Week 40	Week 52	After Wk 26
Blood Urine Kit A	Blood Urine Kit C	No samples	No samples	Blood Urine Kit C	Blood Urine Kit C	No samples	No samples	No samples

Kits A and C are the standard packs of blood and urine tubes arriving at hubs, Kit A being the small pack (1 or 3 blood tubes and a urine pot) and Kit C being the larger pack used for most visits. Kit B does not come to the hubs.

Please note that some hospitals are not collecting Kit A at all, and some are reversing the order of Kits A and C. The correct Visit number should always appear on labels, regardless of which Kit is received. If Kit C is received from Visit 1, the labels would show 'V1'. If Kit A is received in Visit 2, the labels would show 'V2'.

2. Purpose of this SOP

This document outlines the procedure for standardised processes concerning the receipt, processing and onward transport of blood and urine samples arriving from clinical sites. Processing of samples from the following tube types will be outlined in this SOP:

- Serum separation tubes
- Whole blood EDTA tubes (DNA; separation of PBMCs, neutrophils and plasma)
- RNA Tempus tubes
- Urine pots

This document does not cover the processing of skin or renal samples, which are sent direct to Leeds / Imperial. It also does not cover the processing of the 4 ml whole blood tube, which is sent direct from the clinic to Leeds.

3. Abbreviations

HRA	Health Research Authority – the body that approves clinical studies and amendments
MASTERPLANS	The MRC-funded project 'MAximizing SIe ThERapeutic PotentiaL by Application of Novel and Stratified approaches'
MRC	Medical Research Council
PBMCs	Peripheral blood mononuclear cell, a white cell component of blood, to be studied in MASTERPLANS by University College London and Industry partner

	3.
PLANS	Short title: Prediction of Lupus treAtment respoNse Study Long title: An open label observational study to identify predictors of response to rituximab and mycophenolate in patients with systemic lupus erythematosus including cutaneous or renal manifestations
SLE	Systemic lupus erythematosus; lupus
SOP	Standard Operating Procedure
UCL	University College London

4. Roles and responsibilities

Role	Responsibilities
Hub technician	• Source consumables and maintain a list of expenditure; send list periodically to the Project Manager • Receive and log incoming samples into eLABInventory • Process samples in accordance with this SOP. • Upload the Inventory Card to eLABInventory. • Prepare samples for onward shipment. • Liaise with Study Coordinator / Project Assistant concerning any problems or queries.
Courier	• Transport samples from clinical site to hub • Transport samples from hub to University of Manchester
Project manager	• Hub funding matters; changes to SOP; major issues
Study coordinator	• Provide aliquot tubes; primary contact for queries about samples arriving from clinical sites
Project assistant	• Logs and stores samples arriving at the University of Manchester from the hubs • Deals with any issues regarding samples being sent from the hubs to Manchester

5. Procedure

5.1 Receipt of samples at the hub

The following samples will arrive at the hub for each patient visit. The samples for each patient visit will arrive in a single box (either Kit A or Kit C) at ambient temperature. Because some biomarkers are highly perishable, the box contents must be processed immediately on arrival. Sometimes samples may be missing, in cases where the patient produced a sub-optimal volume of blood or urine.

BASELINE 0 (Visit 1) – **Kit A**:

	Contents	**Procedure required** *followed by freezing in all cases*	**No. of tubes**	**Tube volume**
1.	Blood (red top)	Serum extraction and aliquoting (Section 5.3)	1	10 ml
2.	Blood (purple top)	Plasma extraction and aliquoting (Section 5.4)	1 (adults only)	10 ml
3.	Urine	Centrifuge, filter and aliquot (Section 5.6)	1	125 ml (75 ml urine)
4.	Blood (Tempus)	Freezing only (RNA extraction at partners) (Section 5.8)	1 (adults only)	10 ml

BASELINE 1 (Visit 2) and follow-up visits – Kit C:

	Contents	**Procedure required** *followed by freezing in all cases*	**No. of tubes**	**Tube volume**
1.	Blood (red top)	Serum extraction and aliquoting (Section 5.3)	2	10 ml
2.	Blood (purple top)	PBMCs, plasma and neutrophils extraction and aliquoting (Section 5.5)	7	10 ml
3	Urine	Centrifuge, filter and aliquot (Section 5.6)	1	125 ml (75 ml urine)
4.	Blood (purple top)	Aliquot (Section 5.7)	1	4 ml
5.	Blood (Tempus; blue top)	Freezing only (RNA extraction at partners) (Section 5.8)	2	10 ml

Recording of samples

An Inventory Card will arrive with each sample (See Appendix C). The back of the card will be completed by the hub technician on the arrival and completion of each sample process.

Samples are added to eLABInventory on the same day as they are processed, as otherwise labels cannot be printed. It is preferable to “create” samples within eLABInventory after the clinical site has notified the hub that samples are on the way. Whilst spinning samples, labels are printed and added to tubes. Excess sample records can be deleted later in eLABInventory.

The only exception is PBMCs. These are added to eLABInventory after the PBMCs have been aliquoted and then labels are printed and attached.

The completed inventory card is scanned or photographed and then entered into eLABInventory in the file storage tab. The file name is saved as the ID, visit number and date the sample arrived at the hub. The inventory card can be uploaded the next day.

A separate guide to the use of eLabInventory is provided (SOP PLANS-006).

5.2 Order of processing of samples

The proposed order of processing of samples is given in Appendix C. MASTERPLANS partners have repeatedly emphasised that processing of the following samples is time-critical:

- Serum
- PBMCs
- Urine

Every effort should be made, including advance reservation of centrifuges, to ensure that these samples are taken to the point that they are stable as quickly as possible.

5.3 Serum (Kits A & C)

The tubes should be processed as soon as possible after delivery.

- Baseline 0 (Visit 1): 1x 10 ml red top
- All remaining visits: 2x 10 ml red top

1. Record the number of tubes, total volume and date in the 'Serum' section on the back of the Inventory Card.
2. Centrifuge at 2000g for 15 minutes at room temperature
    - Update the Inventory Card with the time of start of centrifugation.
3. Using a pipette, the serum layer should be carefully removed into 2 ml aliquot tubes immediately.

    Please note: be careful not to disturb the fine clot barrier between the serum and waste blood product.

    The amount of serum aliquoted into each tube will be as follows. Tubes will be filled in the priority order shown, leaving out partners lower down if there is not enough. All serum should be aliquoted, even if this means the final tube is only partially full.

Priority	Destination	No. of aliquot tubes	Volume per aliquot tube	Total volume
1	Bath	1	0.5 ml	0.5 ml
2	Liverpool	5	200 ul	1 ml
3	Birmingham	3	0.5 ml	1.5 ml
4	Industry partner 1	2	1 ml	2 ml
5	*Rest*	According to availability	1 ml	According to availability

4. Update eLabInventory, including the volume in each tube and the intended partner destination (there is no space on the Inventory Card for this information).
5. Print off the labels using the DYMO printer.
6. Label the aliquot tubes in the standard way.
7. Place the tubes in a freezer box for serum samples and store at -80°C immediately after aliquoting.
8. Update the Inventory Card with the time and temperature of freezing.

The serum aliquots will be sent in batches to the University of Manchester (see Section 8.1).

5.4 Whole blood for plasma (Kit A only; 'Plasma_A')

1x 10 ml EDTA tube will be received from adult patients at Visit 1. This volume is too small to be processed by the extraction method used for Kit C.

1. In the 'EDTA' section on the back of the Inventory Card, circle 'Kit A' and record the number of tubes, total volume and date.
2. Centrifuge at 2000g for 15 minutes at room temperature
    - Update the Inventory Card with the time of start of centrifugation.
3. Using a pipette, the plasma layer should be carefully removed into 2 ml aliquot tubes immediately.

    Please note: be careful not to contaminate the plasma with the waste blood product.

    The amount of plasma aliquoted into each tube will be as follows. All plasma should be aliquoted, even if this means the final aliquot tube is only partially full.
4. Discard the pellet.

Priority	Destination	No. of aliquot tubes	Volume per aliquot tube	Total volume
1	Manchester (for allocation to partners later	Up to 8	0.5 ml	Up to 4 ml

5. Update eLabInventory, including the volume in each tube.
6. Print off the labels using the DYMO printer, taking care to use sample type 'Plasma_A'.
7. Label the aliquot tubes in the standard way.
8. Place the tubes in a freezer box for serum samples and store at -80°C immediately after aliquoting. Update the Inventory Card with the time and temperature of freezing.

The plasma aliquots will be sent in batches to the University of Manchester (see Section 8.1).

5.5 Whole blood (Kit C only): separation of PBMCs, plasma and neutrophils

7x10ml EDTA tubes (purple top) will be processed as soon as possible after delivery.

The following procedure should be performed using aseptic technique in a Biosafety Level 2 cabinet.

1. In the 'EDTA' section on the back of the Inventory Card, circle '7x10 ml' (even if there are less tubes). Record the number of tubes, total volume and date.
2. Leucosep tubes should be kept in a fridge at 4°C. Remove from the fridge approximately two hours before the samples are expected to arrive.
3. Warm up the prefilled Leucosep tube to room temperature.
4. Pipette the blood from the blood tubes and divide equally into 2 Leucosep tubes.
5. Measure the total volume of blood from the 7x 10 ml tubes (e.g. using the volume markers on the Leucosep tubes) and record this on the Inventory Card.
6. Centrifuge at 800g for 15minutes in a swinging-bucket rotor without brake at room temperature.
    - Update the Inventory Card 'EDTA' section with the time of start of first centrifugation.
7. After centrifugation the sequence of layers is from top to bottom:
    1. Plasma
    2. Enriched cell fraction of PBMCs
    3. Separation medium with porous barrier in the middle

4. Red cell pellet

Plasma:

1. Collect the top layer containing plasma using a pipette to a remnant of 5 to 10 mm in height above the PBMC interphase.
2. Pipette the plasma into 15 ml Falcon tubes.
3. Centrifuge at 1500g for 15 minutes to pellet the platelets and create platelet-poor plasma (PPP).
    - Update the back of the Inventory Card's 'Plasma' section with the time of start of centrifugation.
    - Print off labels whilst spinning samples.
4. Place the plasma (ca. 30 – 40 ml) in aliquots as follows into 2 ml aliquot tubes.

Destination	No. of aliquot tubes	Volume per aliquot tube	Total volume
Aeirtec	1	0.5 ml	0.5 ml
Liverpool	5	0.5 ml	2.5 ml
Industry partner 2	10	0.5 ml	5 ml
Imperial	1	1.8 ml	1.8 ml
Manchester	1	1.8 ml	1.8 ml
Spare	According to availability	1.8 ml	According to availability

5. Mark the volume and partner destination of the tubes on eLabInventory.
6. Put in the freezer box for plasma samples and freeze at -80^{o} C immediately.
7. Delete excess aliquots in eLABInventory.

The plasma aliquots will be sent in batches to the University of Manchester (see Section 8.1).

PBMCs:

1. Using a Pasteur pipette, collect the PBMCs and platelets (layer 2) from each Leucosep tube and combine into a fresh 50ml tube. Top up the volume to 50ml with 1X PBS.
2. Centrifuge at 600g for 10 minutes, at 4^{o} C with the brake on.
    - Update the back of the Inventory Card 'PBMCs' section with the time of start of centrifugation.

3. Decant the supernatant into a waste bucket. (A red blood cell layer may be observed on top of the white blood cell pellet).
4. Resuspend the pellet by gently pipetting in 2ml of RBC lysis buffer,
5. Incubate cells for 10minutes at room temperature. Top up the volume to 20ml with 1xPBS and centrifuge at 350g for 10 minutes, at 4° C with brake on.
6. Decant the supernatant into a waste bucket.
7. Resuspend the pellets by gently pipetting in 1ml of PBS. Top up volume to 10ml with PBS.
8. Count cells as shown below (Section 5.5.1 Kova / 5.5.2 Neubauer).
    - Calculate the total number of cells (the sum of all the PBMCs in all tubes processed for PBMCs).
    - Update the back of the Inventory Card 'PBMCs' section with total number of cells.
9. Top up the volume to 15 ml with PBS and centrifuge at 400g for 10 minutes at 4° C.
10. Determine the number of aliquot tubes needed according to the schedule below:

Total cell count	Number of vials	Vials to UCL	Vials to Industry partner 3
<19 x 10^6	1	1	0
20-34 x 10^6	2	2	0
35-44 x 10^6	3	3	0
45-80 x 10^6	4	3	1
81-100 x 10^6	5	4	1
101-140 x 10^6	6	5	1
141-180 x 10^6	7	5	2
181-200 x 10^6	8	6	2
201-220 x 10^6	9	6	3
221-240 x 10^6	10	7	3

Each vial should have 1ml of cell suspension and have an equal number of cells place in it.

For example, if the PBMC count is 52 million cells, the PBMC will be stored in 4 aliquots of 13 million cells/ml each.

11. Add samples and aliquots to eLABInventory. For PBMCs the total cell count is also added, as well as the individual cell count.
12. Print off labels using the DYMO printer.

13. Label the required number of aliquot tubes in the standard way.
14. Ensure the following is entered on the back of the Inventory Card and later in eLabInventory: number of cells based on the PBMC count; tube destination (UCL / Industry partner 3). This is in addition to the information on date / time of processing.
15. Prepare the freezing medium (0.5 ml) per vial: room temperature FCS and 20% DMSO *(for example, 1.5ml of freezing medium is required for 3 vials. 1.5 ml of freezing medium would comprise 1.2 ml FCS and 0.3 ml DMSO*).
16. After the centrifuge cycle is complete, decant the supernatant in to a waste bucket and resuspend cells in 0.5 ml of room temperature 100% FCS per vial *(for example, 1.5ml of resuspended cells is required for 3 vials)*.
17. Add the freezing medium dropwise to the cell suspension in a 1:1 ratio.
18. Aliquot 1ml of cell suspension into each labelled vial.

Preparing Mr Frosty

• Remove the high-density polyethylene vial holder and foam insert from the polycarbonate unit.

• Add 250 mL of 100% isopropyl alcohol to the fill line in the fume hood. DO NOT OVERFILL. Avoid slopping the isopropyl alcohol on the labels because the alcohol can cause the ink to run.

• Replace alcohol after every fifth use and document this reagent change.

• Replace foam insert and vial holder.

19. Place the cryovials into holes in the vial holder in a Mr. Frosty™ Freezing Container (Mr. Frosty will be at room temperature). Fill all empty cells of the Mr. Frosty – do not leave any empty.
20. Store at -80^{o} C for up to 72 hours.
    - Update the back of the Inventory Card 'PBMCs' section with the time of start of freezing at -80^{o}C.
21. Within 72 hours (ideally within 48 hours) remove the vials from Mr.Frosty™, transfer to a freezer box for PBMC samples and store at -150^{o} C or below.
    - Update the back of the Inventory Card 'PBMCs' section with the time of start of freezing at -150^{o}C.

The PBMCs will be sent in batches to the University of Manchester (see Section 8.1), or optionally direct to UCL and / or Industry partner 3 (see Section 8.2).

Red cell pellet for neutrophils:

Neutrophils will be extracted from one of the two Leucosep tubes only. Neutrophils are extracted from the red cell pellet (layer 4 from centrifugation of the Leucosep tubes). The Leucosep barrier is pierced using long scissors; these will normally be autoclaved though some disposable scissors will be provided.

1. Autoclave a pair of long scissors to sterilise them.
2. Following removal of the top layers of plasma and PBMCs, pierce the porous layer (layer 3) with the long scissors.
3. Add 20 ml RBCs lysis buffer (ammonium chloride), inverting the tube or with gentle vortex transversely to mix.
4. Transfer the contents of the tube into 4x Falcon 50 ml tubes.
5. Top up the tubes with 1x RBC lysis buffer and put in a 37^{o}C water bath for 5 minutes.
6. Centrifuge at 400g for 10 minutes in swinging bucket rotor at room temperature with the brake on to create a cell pellet.
    - Update the back of the Inventory Card 'PBMCs' section with the time of start of centrifugation.
7. Remove and discard supernatant. Resuspend the pellet in 1 ml PBS.
8. Transfer the resuspended pellet to one Falcon tube and add 46 ml PBS to neutralise the ammonium chloride pH.
9. Centrifuge the isolated neutrophils at 1000g for 5 mins at 4C with the brake on.
10. Remove and discard the supernatant.
11. Add 1ml TRizol in the fume hood. Mix thoroughly by pipetting 5 times and leave at room temperature for 5 minutes.
12. Transfer to one aliquot tube, put in a freezer box for neutrophil samples and put in the -80^{o}C freezer.
    - Update the back of the Inventory Card 'Neutrophil' section with the time of start of freezing.

The neutrophil samples will be sent in batches to the University of Manchester with the other samples destined for Manchester (see Section 8.1).

5.5.1 Counting cells (Kova v3.0)

N.B. The procedure for counting cells differs depending on equipment. For this reason we have included two procedures in this SOP.

1. Mix 10ul of cell suspension with 90ul of trypan blue in PBS.
2. Fill one grid of the slide by touching the pipette tip at the dent of the grid chamber. (see figure A). Capillary action draws the cell suspension in to fill the chamber. Avoid air bubbles and do not reuse a grid chamber once used.
3. Use a phase contrast microscope (40x objective) and count the cells in 9 squares only (see figure B).
4. Aim to count between 25-100 cells in the 9 squares.
5. Follow the formula below to calculate the cell count:

- Number of cells in 9 squares x dilution factor x 10000 = cells/ml
- Total number of cells= cells/ml x total volume

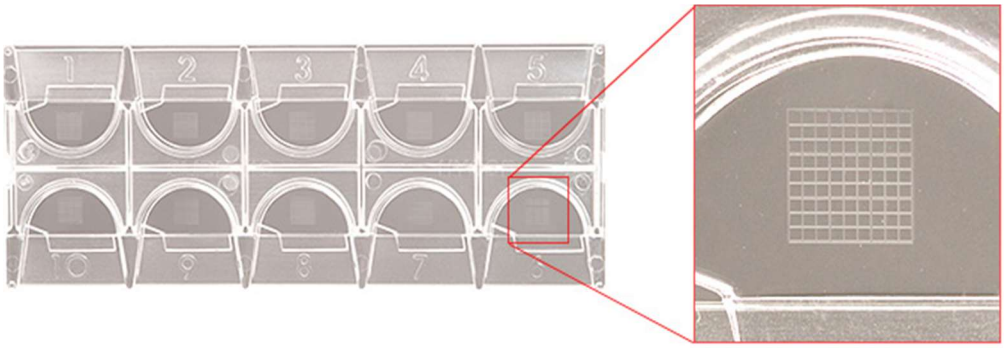


Figure A

A KOVA Glasstic slide 10 with quantitative grid

Figure B

A quantitative grid

5.5.2 Counting cells (Neubauer v3.0)

1. Mix 10ul of cell suspension with 90ul of trypan blue in PBS in a small tube.
2. Press cover glass onto the slide until diffraction (rainbow) rings appear.
3. Fill one side of the counting chamber by touching the pipette tip at the interface of the slide and cover glass. Capillary action draws the cell suspension in to fill the chamber. Ensure none of the cell suspension enters the surrounding channels and avoid air bubbles.
4. Use a phase contrast microscope (40x objective) and count the cells in the 25 triple lined central squares (see figure A).
5. Count cells which touch the upper and right border but not those which touch the lower and left border (see figure B).
6. Aim to count between 25-100 cells in the 25 squares.
7. Follow the formula below to calculate the cell count:
    - Number of cells in 25 squares x dilution factor= cells x 10^4/ml
    - Total number of cells= cells/ml x total volume

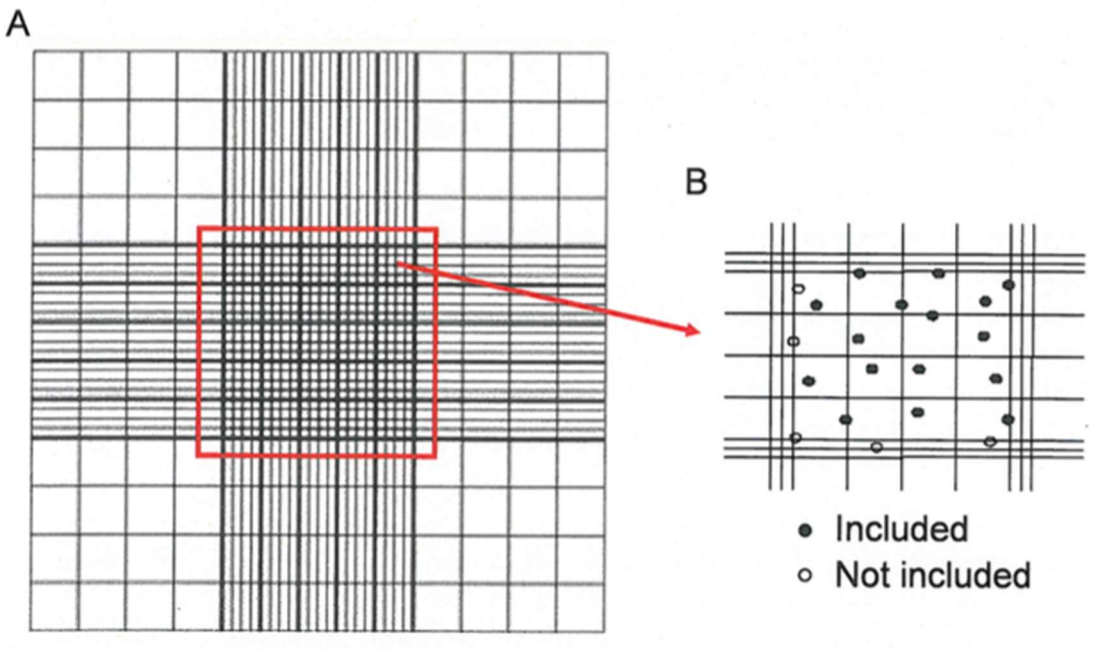


Figure A: Neubauer counting chamber. The red box shows the 25 triple lined central squares.

Figure B: An example of cells to include and not include in one triple lined central square.

5.6 Urine (Kits A & C)

One 125 ml pot containing urine will be received by the hub for processing. The sample should be processed as soon as possible after delivery.

We have ethical approval to collect a maximum of 75 ml urine, so discard any excess urine.

1. Record the number of tubes, total volume and date in the 'Urine' section on the back of the Inventory Card.
2. Pour urine into suitable centrifuge tubes, e.g. 6 – 7 12 ml universal tubes or 2x 50ml tubes.
3. Spin at 2000g for 10 minutes at room temperature.
    - Update the back of the Inventory Card 'Urine' section with the time of start of centrifugation.
4. Draw up the spun urine into a 20ml syringe leaving any pellet undisturbed.

    If the pellet is disturbed, the sample needs to be re-spun to be collected and to avoid clogging the filter.

5. Filter the urine using a 0.2µm Minisart filter, which fits on the end of the syringe, into fresh tubes. If necessary, use a second filter.

6. Using a pipette, the filtered urine should be split into the following aliquots in the **standard** 2 ml aliquot tubes in the following order:

Destination	No. of aliquot tubes	Volume per aliquot tube	Total volume
Aeirtec	1	500 ul	500 ul
Birmingham	3	1.8 ml	5.4 ml
Industry partner 1	3	1.8 ml	5.4 ml
Manchester	6	1.8 ml	10.8 ml
Liverpool	12	1.8 ml	21.6 ml

- Update eLabInventory with the tube numbers and partner destination.

7. Put 10 ml urine for Industry partner 2 in the 1.5 ml **Sarstedt** tubes.
   - Update eLabInventory with the tube numbers and partner destination.

Destination	No. of aliquot tubes	Volume per aliquot tube	Total volume
Industry partner 2 (1)	10	1.0 ml	10.0 ml

8. Put the remaining 20 ml urine for Industry partner 2 in **2x 15 ml Falcon** tubes.
   - Update eLabInventory with the tube numbers and partner destination.

Destination	No. of aliquot tubes	Volume per aliquot tube	Total volume
Industry partner 2	2	10.0 ml	20.0 ml

9. Put the tubes in a freezer box for urine samples and freeze immediately at -80°C.
   - Update the back of the Inventory Card ‘Urine' section with the time of start of freezing.

The aliquots will be sent in batches to the University of Manchester (see Section 8.1).

5.7 Blood tube (4 ml) for DNA / epigenetics (Kit C only)

1x 4 ml blood tubes will be received at the hub for processing.

1. Record the number of tubes, total volume and date in the ‘DNA’ section on the back of the Inventory Card.
2. Prepare 1 ml aliquots (i.e. up to 4 aliquot tubes).
3. Put in a freezer box for DNA / epigenetics samples and freeze at -80C.
    - Update the back of the Inventory Card ‘DNA’ section with the time of start of freezing.

The tubes will be sent in batches to the University of Manchester (see Section 8.1).

5.8 RNA Tempus tubes (Kits A & C)

1. VISIT 1: 1x 10ml Tempus tubes will be received at the hub for processing.

   VISIT 2 & FOLLOW-UPs: 2x 10 ml Tempus tubes will be received.

   Record the number of Tempus tubes, total volume and date in the ‘Tempus’ section on the back of the Inventory Card.
2. Vortex the tubes.
3. Put in a freezer box for Tempus tubes and freeze at -80C.
    - Update the back of the Inventory Card ‘Tempus’ section with the time of start of freezing.

The tubes will be sent in batches to the University of Manchester (see Section 8.1).

6. Update of eLabInventory

eLabInventory should be updated on the same day as samples are processed, preferably before the arrival of samples, as excess aliquots can easily be deleted from eLABInventory. This only excludes PBMCs as the aliquots of PBMCs are not known until the cell count has been completed. To note:

- The completed inventory card should be scanned, and the scanned document should be entered into eLabInventory.
- The inventory card can be uploaded into eLABInventory the next day.
- After the samples have been sent to the University of Manchester / UCL / Industry partner 3, the recipient at the institution receiving the samples will update the location of samples on arrival.

7. Freezer failure / freeze thaw cycles

Every effort should be made to avoid the possibility of freeze thaw cycles. A note should be made in eLabInventory of the sample number of any samples affected.

8. Transport from the hub to other partners

8.1 Transport of samples to the University of Manchester for biobanking

The hub will send the following samples in batches of aliquots to the University of Manchester for storage:

- Serum
- Plasma
- PBMCs (unless sent direct to UCL / Industry partner 3 – see Section 8.2)
- Neutrophils
- Urine
- Blood for DNA / epigenetics
- Blood for RNA (Tempus tube)

Batches will be sent on dry ice and all available aliquot tubes can be transported together. The frequency with which the samples are sent will depend on how much storage capacity there is at the hubs; the frequency should be agreed with the SPTOG owing to the high cost of couriers.

Shipping details:

- Liaise with the MASTERPLANS Project Assistant in Manchester to select a suitable shipment date.
- Ensure availability of dry ice. If necessary, order dry ice.
- Book the courier.
- Pack the samples in accordance with Appendix C (Sample shipping between MASTERPLANS partners).

To ship to Manchester please use the following address:

<Contact>
<Address>
<Email>
<Phone>

- Update eLabInventory to indicate the samples to be consigned. All samples to be sent are to be added into virtual boxes on eLABInventory that match the physical box being consigned.
- A document describing the contents and sample IDs must be included in the package, e.g. a report from eLabInventory.
- Once ready to be shipped, please contact the PLANS project administrator (details below) stating the nature and number of samples to be shipped, with confirmation that the samples have been packaged and labelled as described above. **If you have any**

questions or concerns about packaging and labelling, contact the project administrator BEFORE preparation for shipping.

- Once the package has been collected by the courier, please email the courier tracking number / details to the PLANS project administrator.
- The project assistant in Manchester will update the location of the virtual box(es) on eLABInventory to the Manchester freezer.

8.2 Optional transport of PBMCs direct to UCL and / or Industry partner 3

Originally the intention was to send all PBMCs direct to UCL and Industry partner 3, though both partners have now agreed that samples should be routinely sent to the University of Manchester for storage (see Section 8.1). Transfer direct to Manchester is now possible following approval of HRA Substantial Amendment SA3 (October 2017). However, the option of sending PBMCs direct to UCL and Industry partner 3 remains, as follows:

PBMCs can be transported on dry ice to UCL and / or Industry partner 3 in batches according to the hub's capacity to store PBMCs at below -150ºC and periodic requests for samples from UCL / Industry partner 3.

Shipping details:

- Liaise with the named contacts at UCL and Industry partner 3 to select a suitable shipment date. This will probably be a different date for the two organisations.
- Ensure availability of dry ice. If necessary, order dry ice.
- Book the courier.
- Pack the samples in accordance with Appendix C (Sample shipping between MASTERPLANS partners).

To ship to UCL, please use the following address:

<Contact>
<Address>
<Email>
<Phone>

To ship to Industry partner 3, please use the following address:

<Contact>
<Address>
<Email>
<Phone>

- Update eLabInventory to indicate the samples to be consigned. All samples to be sent are to be added into virtual boxes on eLABInventory that match the physical box being consigned.
- A document describing the contents and sample IDs must be included in the package, e.g. a report from eLabInventory.
- Once ready to be shipped, please contact the UCL / Industry partner 3 contact (details above) stating the nature and number of samples to be shipped, with confirmation that the samples have been packaged and labelled as described above. **If you have any questions or concerns about packaging and labelling, contact the UCL / Industry partner 3 contact or the PLANS project administrator BEFORE preparation for shipping.**
- Once the package has been collected by the courier, please email the courier tracking number / details to the UCL / Industry partner 3 contact.
- **Transfers to UCL**: When the samples arrive at UCL, the UCL hub technician will update the location of the virtual box(es) on eLABInventory to the UCL freezer.
- **Transfers to Industry partner 3**: After Industry partner 3 has confirmed the arrival of the samples, the hub technician will update the location of the virtual box(es) on eLABInventory to the Industry partner 3 freezer. (NB The hub remains responsible for doing this as Industry partner 3 does not have access to eLABInventory).

9. Communication channels between the hubs and the University of Manchester

The SPTOG group will meet periodically to share and review experiences and resolve issues. Potential changes to SOPs should be notified to the Project Manager, who will put them on the next SPTOG agenda.

Hubs will contact the Study Coordinator for replenishment of certain consumables (see Appendix A).

Hubs will contact the Project Assistant regarding consignments of samples being sent to the University of Manchester for storage.

Hubs will contact the Project Manager concerning financial matters, changes to process and significant risks and issues.

10. Governance and changes to this SOP

The MASTERPLANS Principal Investigator has responsibility for compliance, risk and research integrity with regard to this SOP and provides final approval.

Changes to this SOP will be referred via the MASTERPLANS Project Manager (email: masterplans@manchester.ac.uk) to the PLANS Sample Processing Technical Operations Group (SPTOG) for first level approval. Changes to the SOP that, in the view of the Chair of the Sample Processing Group or the MASTERPLANS Project Manager, require a strategic operational decision, will be referred to the MASTERPLANS Project Steering Group (PSG)

for further input and approval. The Chairs of the SPTOG and PSG (if involved) will sign the SOP to signify the approvals of these Groups.

11. Other SOPs referenced

SOP	Title of SOP
PLANS-006	Use of eLabInventory

APPENDIX A: Reagents, tubes and equipment needed

This section lists the consumables requirements.

Procured by the University of Manchester and sent to hubs

Tubes/Equipment	Company	Cat no
2ml Cryovial tubes	Greiner	122263
Instramed Mayo Scissors 17 cm length single use disposable scissors	Northumbrian Medical Supplies	S42-2016
Cryo Labels for DYMO 18 mm x 38 mm	LabTag EU	ED1F/EF1F-073
Industry partner 2 urine aliquot tubes: Sarstedt 1.5 ml microtubes, conical base	Sarstedt	72.692.005

Procured by University of Birmingham and sent to hubs

Tubes/Equipment	Company	Cat no
Minisart 0.2 um filters	Sartorius	16534K

Procured by the individual hubs

These items will be funded through additional funding to hub universities from the University of Manchester (process to be clarified). Hubs will send an itemised account of items purchased to the University of Manchester at dates to be agreed.

Reagents	Company	Cat no	Storage/Usage
1X Dulbecco's Phosphate Buffered Saline Modified, without calcium chloride and magnesium chloride, liquid, sterile-filtered, suitable for cell culture	Sigma	D8537-24X500ML	Fridge (2°C - 4°C) Keep at room temperature after use
Cell lysis reagent (ALFA AESAR, Mfr. Part No. J62990.AP) *Owing to the high cost of lysis buffer, a decision was made to make this up after the initial supplies have been exhausted. See below.*	Alfa Aesar or Fischer Scientific or VWR	J62990.AP or 15475269 J62990.AP	
Lysis buffer ingredients[1]: • Ammonium chloride NH_4CL (1 Kg) • Potassium bicarbonate $KHCO_3$ (1 Kg) • Ethylenediaminetetraacetic acid EDTA (100g)	 Sigma Sigma Sigma	 A9434-1KG 60339-1KG E6758-100G	Fridge (2°C - 4°C)
Foetal Bovine Serum, South American origin 500 ml (LabTech branded) (FCS)	LabTech	FCS-SA /500	Freezer (-15 °C - -20°C) After defrost, keep in fridge (2°C - 4°C)
DMSO (Dimethyl sulfoxide)	Sigma	D8418	Room Temperature

[1] Lysis buffer is used as 1x but can be made up periodically in 10x batches. Both 10x and 1x will last for up to one month if autoclaved.

Use the following ingredients for 10x and autoclave as soon as it has been made up.

- 82.9 g ammonium chloride
- 10.0 g potassium bicarbonate
- 0.37 g EDTA
- Make up to 1 litre with distilled water

Make up batches of 1x as required by diluting the 10x and autoclaving immediately.

Reagents	Company	Cat no	Storage/Usage
0.4% Trypan Blue	Sigma	T8154	Room Temperature
Isopropanol	ThermoFisher	67-63-0	Room Temperature - use in fume hood
TRIzol® Reagent	ThermoFisher	15596018	Fridge (2°C - 4°C) - use in fume hood
Leucosep Tubes pre-filled 50ml LEUCOSEP TUBE, 50 ML, PP, 30/115 MM, CONICAL BOTTOM, POROUS BARRIER, NATURAL, ASEPTICALLY PROD., PRE-FILLED WITH LEUCOSEP SEPARATION MEDIUM, 25 PCS./BOX	Greiner BioOne	227288	Fridge (2°C - 4°C) – bring to room temperature before use
Tubes/Equipment *(suppliers are suggested but equipment can be bought from any reasonable source)*	**Company**	**Cat no**	
Dymo LabelWriter 450 Turbo S0838860 (one per hub)	Chariot Office Supplies	ES83881	
Mr.Frosty™ (two per hub)	SLS	CRY8400	
3ml Pasteur Pipettes	Starstedt	86.1171.001	
15ml Centrifuge tube, conical base	Greiner	188271	
50ml Centrifuge tube, conical base	Greiner	227261	
20ml syringes	BD	SYR6009	
Neubauer Improved C-Chip Disposable Haemocytometer	Depends on method NANOENTEK	DHC-N01	
100 place hinged plastic box for 2.0 ml tubes	Starlab	I2310-5848	

APPENDIX B: Requested sample allocation (Kit C)

Collection priority	Sample	Number of tubes	Processing at hub	Requested allocation (aliquots may be larger)
	BLOOD			
1.	Blood for serum – red top	2x 10 ml	Centrifuge, aliquot, freeze and send to UoM for storage.	1 ml to Liverpool
				1.5 ml to Birmingham
				2 ml to Industry partner 1
				0.5 ml to Bath
2.	Blood for PBMCs, plasma and neutrophils – purple top	7x 10 ml	Extract PBMC and plasma fractions; prepare PBMCs for same day onward transport.	**PBMCs:** PBMCs from 70 ml to UCL and Industry partner 3 in an approximately 5:1 ratio
			Aliquot, freeze and send to UoM for storage.	**Plasma:** 1 ml to Imperial
				1 ml to Liverpool
				1 ml to Aeirtec
				5 ml to Industry partner 2
				1 ml to Manchester
			Extract neutrophils	**Red cells – neutrophils** to Liverpool
3.	Blood for DNA / epigenetics	1x 4 ml	Freeze at hub	Allocation to be determined
4.	Whole blood for RNA – Tempus tubes (blue top)	2x 10 ml	Freeze at hub	Allocation to be determined
	OTHER SAMPLES			
4.	**Urine**	72 – 75 ml in 150 ml tubes	Centrifuge; remove and filter supernatant and aliquot. Freeze and send to UoM for storage.	**Supernatant:** 500 ul to Aeirtec
				3 – 5 ml to Birmingham
				5 ml to Industry partner 1
				10 ml to UoM (epigenetics)
				20 ml to Liverpool
				10 ml to Industry partner 2

APPENDIX C: Instructions for shipping biological samples

When sending samples from the processing hubs to Manchester, UCL or Industry partner 3, please follow the packaging and labelling instructions below:

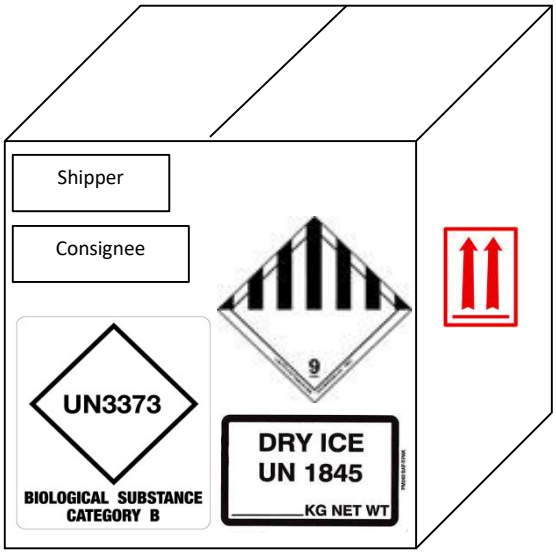
Biological Substance, category B

- Blood and blood products, such as serum, plasma, urine, and PBMCs are considered dangerous goods and fall under UN shipping number UN3373 Biological Samples category B. As such, these samples MUST be packaged in accordance with IATA packing instruction 650, described as:

  - The packaging shall consist of three components:
    (a) a primary receptacle,
    (b) a secondary packaging, and
    (c) a rigid outer packaging

  - The primary receptacle(s) shall be leak-proof; and must not contain more than 1 litre;
  - The secondary packaging shall be leak-proof;
  - If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them;
  - Absorbent material shall be placed between the primary receptacle(s) and the secondary packaging. The absorbent material shall be in quantity sufficient to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
  - The primary receptacle or the secondary packaging shall be capable of withstanding, without leakage, an internal pressure of 95 kPa (0.95 bar).
  - At least one surface of the outer packaging must have a minimum dimension of 100 mm × 100 mm.
  - The completed package shall be capable of successfully passing a drop test in at a height of 1.2 m. Following the appropriate drop sequence, there shall be no leakage from the primary receptacle(s) which shall remain protected by absorbent material, when required, in the secondary packaging.

- Samples should be shipped frozen on dry ice. Sufficient dry ice should be used to ensure that the samples remain frozen throughout transit.
- The samples and dry ice should be packed and sealed in a thick, polystyrene box. The box MUST be labelled as shown:

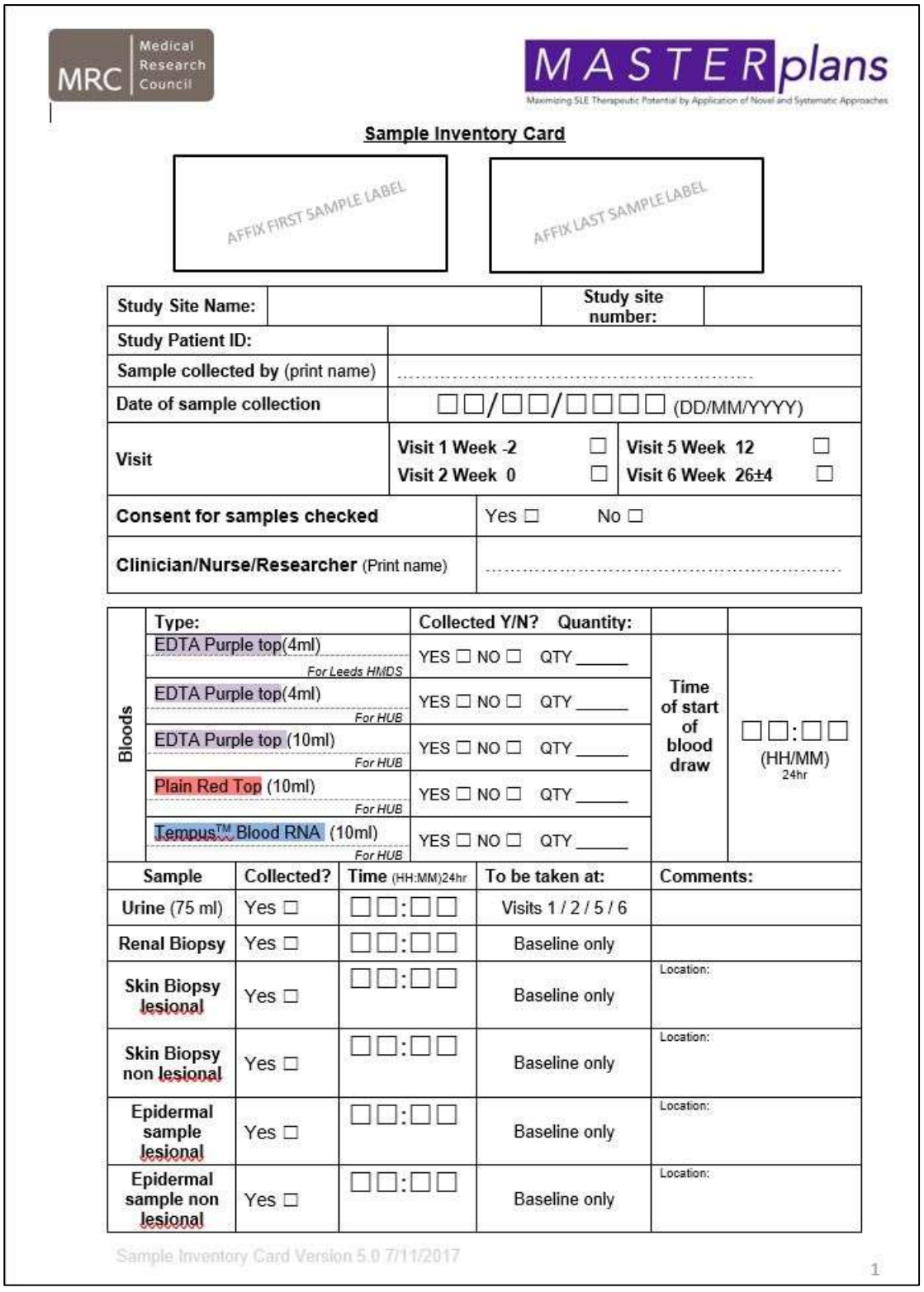


- The amount of the dry ice used (kg) must be shown on the package label.
- The correct packaging labels (as shown) should be purchased by the hub.

Shipping address

- The 'Shipper' information shown on the package should include your name, address and a contact telephone number.
- The 'Consignee' information should be UCL / Industry partner 3 / MASTERPLANS University of Manchester (addresses in Sections 8.1 and 8.2 above). As well as the address, include the phone number of the consignee.

APPENDIX D: Inventory Card



MRC Medical Research Council

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Maximizing SLE Therapeutic Potential by Application of Novel and Systematic Approaches

Sample Inventory Card

AFFIX FIRST SAMPLE LABEL

AFFIX LAST SAMPLE LABEL

Study Site Name:		**Study site number:**	
Study Patient ID:			
Sample collected by (print name)	.....		
Date of sample collection	☐☐/☐☐/☐☐☐☐ (DD/MM/YYYY)		
Visit	**Visit 1 Week -2** ☐ **Visit 2 Week 0** ☐	**Visit 5 Week 12** ☐ **Visit 6 Week 26±4** ☐	
Consent for samples checked	Yes ☐ No ☐		
Clinician/Nurse/Researcher (Print name)	.....		

	Type:	**Collected Y/N? Quantity:**		
Bloods	EDTA Purple top(4ml) *For Leeds HMDS*	YES ☐ NO ☐ QTY _____	**Time of start of blood draw**	☐☐:☐☐ (HH/MM) 24hr
	EDTA Purple top(4ml) *For HUB*	YES ☐ NO ☐ QTY _____		
	EDTA Purple top (10ml) *For HUB*	YES ☐ NO ☐ QTY _____		
	Plain Red Top (10ml) *For HUB*	YES ☐ NO ☐ QTY _____		
	Tempus™ Blood RNA (10ml) *For HUB*	YES ☐ NO ☐ QTY _____		

Sample	**Collected?**	**Time** (HH:MM)24hr	**To be taken at:**	**Comments:**
Urine (75 ml)	Yes ☐	☐☐:☐☐	Visits 1 / 2 / 5 / 6	
Renal Biopsy	Yes ☐	☐☐:☐☐	Baseline only	
Skin Biopsy lesional	Yes ☐	☐☐:☐☐	Baseline only	Location:
Skin Biopsy non lesional	Yes ☐	☐☐:☐☐	Baseline only	Location:
Epidermal sample lesional	Yes ☐	☐☐:☐☐	Baseline only	Location:
Epidermal sample non lesional	Yes ☐	☐☐:☐☐	Baseline only	Location:

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****For sample processing hub use only****

Hub name:		Patient ID:	
Technician name (please print)	.....		
Date & time of arrival at hub:	☐☐/☐☐/☐☐☐☐ (DD/MM/YYYY) ☐☐:☐☐ (HH:MM)24hr		

Urine	Initial volume: _____ ml	Processed same day? YES ☐ NO ☐ If no record date ☐☐/☐☐/☐☐☐☐ (DD/MM/YYYY)
Time of start of step 3 (centrifugation)	☐☐:☐☐ (HH:MM)24hr	Time of freezing ☐☐:☐☐ (HH:MM)24hr — Temp _____ °C

EDTA	Kit A /or Kit C *(pls circle)* Number of tubes _____ Total volume: _____ml	Processed same day? YES ☐ NO ☐ If no, record date: ☐☐/☐☐/☐☐☐☐ (DD/MM/YYYY)		
EDTA first centrifugation Kit A: Step 2 2000g / Kit C: Step 6 800g	☐☐:☐☐ (HH:MM) 24hr	PLASMA A / C:		
*PLASMA Kit C: step 3 (second) centrifugation 1500g	☐☐:☐☐ (HH:MM) 24hr	Time of freezing	☐☐:☐☐ (HH:MM) 24hr	Temp _____ °C
*PBMC step 2 (second) centrifugation 600g	☐☐:☐☐ (HH:MM) 24hr	Time of freezing	☐☐:☐☐ (HH:MM) 24hr	Temp -80 °C
PBMC Freezing < -150°C	Date ☐☐/☐☐/☐☐☐☐ (DD/MM/YYYY)	Time of freezing	☐☐:☐☐ (HH:MM)24hr	
Total cell count _____	Total No. of vials. _____	Av. No. of cells per vial _____	No. of vials for UCL _____	No. of vials for UCB _____
*NEUTROPHIL step 6 (second) centrifugation 400g	☐☐:☐☐ (HH:MM) 24hr	Time of freezing	☐☐:☐☐ (HH:MM) 24hr	Temp _____ °C
SERUM	Number of tubes _____ Total volume: _____ml	Processed same day? YES ☐ NO ☐ If no, record date: ☐☐/☐☐/☐☐☐☐ (DD/MM/YYYY)		
Step 2 (centrifugation)	☐☐:☐☐ (HH:MM) 24hr	Time of freezing	☐☐:☐☐ (HH:MM) 24hr	Temp _____ °C
TEMPUS	Number of tubes _____	TUBE 1: FULL / PARTIALLY FULL (please circle.) TUBE 2: FULL / PARTIALLY FULL (please circle.)		
Date ☐☐/☐☐/☐☐☐☐ (DD/MM/YYYY)		Time of freezing	☐☐:☐☐ (HH:MM)24hr	Temp _____ °C
DNA 4ml Tube	Number of tubes _____	Total volume: _____ml		
Record date: ☐☐/☐☐/☐☐☐ (DD/MM/YYYY)		Time of freezing	☐☐:☐☐ (HH:MM) 24hr	Temp _____ °C

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APPENDIX E: Order of processing

Key:

RNA Tempus tubes	**Blue**
Serum	**Red**
Plasma	**Light blue**
PBMC's	**Green**
Neutrophils.	**Purple**
Urine	**Orange**
C1	**Centrifuge 1 – stays at Room Temp**
C2	**Centrifuge 2. – will need to be cooled to 4°C after first Leucosep spin.**

The following example has been suggested per sample.

Two Centrifuges:

You should be aware that a sample will be arriving.

Before they arrive, take 2 Leucosep tubes (per patient) out of the fridge and leave on the bench to come to room temperature.

Protocol: Two Centrifuges.

On arrival:

Vortex the tempus tubes and freeze at -80°C

1. **C1. Pour the urine into 2 x 50ml tubes (equal measure for balanced spin).**
    a. **Place the urine and 2 red top tubes in to spin for 15 minutes.**
2. **C2. Transfer blood from purple top tubes into 2 Leucosep tube (note amount) 15mins + no brake = (approx 25mins total to stop)**
3. **While waiting for the spins to end, prepare tubes and items required for processing.**
4. **When C1 ends filter urine and aliquot.**
    a. **Freeze at -80°C.**
5. **When C2 ends (Remove tubes and set centrifuge to cool to 4 °C) – remove plasma from Leucosep tube into 2 x 15ml tubes.**
6. **C1 – spin plasma 15 minutes.**
7. **Collect PBMC layer, into 15ml tubes – 1 per Leucosep tube. Add PBS.**

8. C2 (at 4 °C) – Spin PBMC's 10 minutes.
9. Aliquot serum and freeze at -80°C
10. Remove Leucosep filter – add 20mls RBC lysis buffer & invert.
    a. Transfer contents to 4x 50ml tubes.
    b. Top up with RBC lysis buffer & put in 37°C for 5 minutes.
11. Begin to aliquot the plasma.
    a. When incubation ends – C1 centrifuge Neutrophils (time??)
    b. Finish aliquoting Plasma
12. Resuspend PBMC's, combine the two 15ml tubes, top up with PBS and count cells.
13. C2: Spin PBMC's 10 minutes at 4 °C
14. Discard supernatant, resuspend in PBS, combine into one 50ml falcon. Top up to 50ml PBS.
    a. Count cells
15. C1: Spin neutrophils. 5 minutes.
16. Prepare freezing media for PBMC's.
    a. Resuspend cells in 0.5ml room temp FCS.
    b. Add freezing media dropwise in a 1:1 ration.
    c. Aliquot
17. Discard supernatant, resuspend neutrophils in 1ml TRizol.
18. Put Neutrophils and PBMC aliquots into a coolcell and freeze at -80°C for 24hrs.
    a. After 24hrs, transfer PBMC's to Liquid Nitrogen storage and neutrophils into a box for further -80°C storage.

Protocol: One Centrifuge.

Vortex the tempus tubes and freeze at -80°C

1. Pour the urine into 2 x 50ml tubes (equal measure for balanced spin).
    a. Place the urine and 2 red top tubes in to spin for 15 minutes.
2. Transfer blood from purple top tubes into 2 leucosep tube (note amount)
3. When centrifuge ends, replace with leucosep tubes and spin. 15mins + no brake = (approx 25mins total to stop)
4. Filter urine and aliquot.
    a. Freeze at -80°C.
5. Aliquot serum and freeze at -80°C
6. When centrifuge ends remove plasma from Leucosep tubes into 2 x 15ml tubes.
7. Spin plasma 15 minutes.
8. Collect PBMC layer, into 15ml tubes – 1 per leucosep tube. Add PBS.
9. Remove Leucosep filter – add 20mls RBC lysis buffer & invert.
    a. Transfer contents to 4x 50ml tubes.
    b. Top up with RBC lysis buffer & put in 37°C for 5 minutes.

10. When centrifuge ends spin Neutophils.
11. Aliquot plasma
12. When centrifuge ends discard supernatant, resuspend in PBS, combine into one 50ml falcon. Top up to 50ml PBS.
    a. Count cells
13. Spin neutrophils. 5 minutes.
14. When centrifuge ends cool to 4 °C
15. Once cooled, spin PBMC's 10 minutes.
16. Discard supernatant, resuspend neutrophils in 1ml TRizol.
17. When spin ends, resuspend PBMC's, combine the two 15ml tubes, top up with PBS and count cells.
18. Spin PBMC's 10 minutes at 4 °C
19. Prepare freezing media for PBMC's.
    a. Resuspend cells in 0.5ml room temp FCS.
    b. Add freezing media dropwise in a 1:1 ration.
    c. Aliquot
20. Put Neutrophils and PBMC aliquots into a coolcell and freeze at -80°C for 24hrs.
    a. After 24hrs, transfer PBMC's to Liquid Nitrogen storage and neutrophils into a box for further -80°C storage.