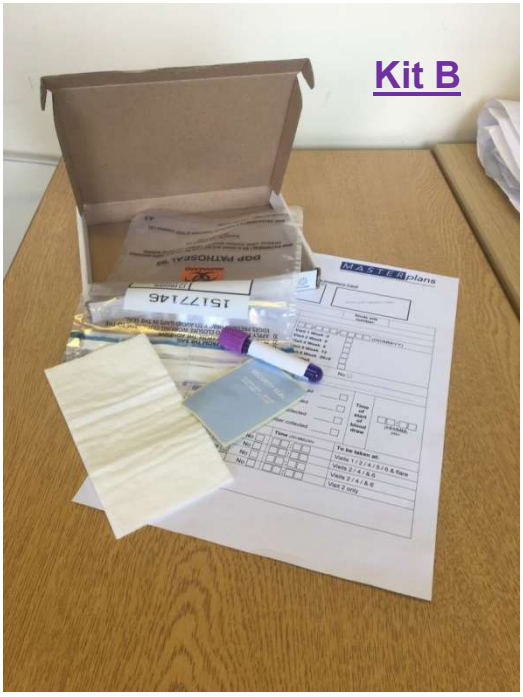
MANCHESTER 1824
The University of Manchester

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



SOP reference number	**PLANS-001 version 4.0**
SOP title	**Blood and urine collection in clinic**

Index

1. Background

The PLANS study is a longitudinal observational study of patients with systemic lupus erythematosus (SLE; lupus). The study forms part of the work of the Medical Research Council-funded MASTERPLANS Consortium. The primary objective of the PLANS study is to answer the following research question:

- Are there differences in the genes and components of blood, urine and skin /kidney tissue of lupus patients who respond well or less well to two drugs (mycophenolate and rituximab), and if so, what are the differences?

This Standard Operating Procedure concerns collection of blood and urine in clinic. Biological samples will be taken with consent from participants and according to their visit schedule. All blood and urine samples except for a 4 ml whole blood tube will be shipped to a local processing hub using a same day courier. Sample processing hubs are located at the Universities of Leeds and Birmingham and University College London. Samples will then be sent from the hub to MASTERPLANS partner organisations for analysis and / or storage pending analysis. The 4 ml whole blood tube will be sent separately to the University of Leeds.

Blood samples taken for the study will be taken at the same time as those for routine blood monitoring as part of NICE guidelines. All samples will be taken at the same time as routine clinical visits.

Acknowledgement: This SOP is based on the SOP RA-MAP001 version 1.0, produced by the RA-MAP project consortium.

2. Purpose of this SOP

The purpose of this SOP is to describe the procedure to follow in clinic when taking and shipping blood and urine samples for the PLANS study. Separate SOPs are available describing collection and transport of skin samples (PLANS-003) and renal biopsies (PLANS-004); however, this SOP covers communications with the dermatology and renal departments concerning these sample collections.

3. Abbreviations

MASTERPLANS	The MRC-funded project 'MAximizing Sle ThERapeutic PotentiaL by Application of Novel and Stratified approaches'
MRC	Medical Research Council
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

4. Roles and responsibilities

Role	Responsibilities
Clinical / nursing staff	• Ensure availability of laboratory personnel at the local sample processing hub • Book patient visit to coincide with standard care visit • Arrange courier • Take the blood and urine samples • Label the tubes containing the blood and urine samples • Complete the Inventory Card, which includes confirmation that the samples have been taken with consent. • Put the 4 ml blood sample in the pack for first class postage to Leeds (must be sent same day). • Make up the box of remaining samples for the hub and hand to courier. • Log samples into ePLANS
Royal Mail	• Deliver 4 ml blood sample to Leeds (next day delivery)
Courier	• Collect samples in packaging and deliver to hub
Study Coordinator	• Provide supplies, e.g. sample tubes, pre-printed labels and Inventory Cards

4.1 Health and safety

Where applicable this SOP must be used in conjunction with Human Tissue Act Codes of Practice and all other relevant University and where appropriate, NHS health and safety policies and SOPs.

All employees should make themselves aware of any health and safety issues related to the use of chemical and biological hazards. Employees are responsible for ensuring the health and safety of themselves and others in the workplace.

Biological samples may represent an infection risk; therefore appropriate personal protective equipment should be worn whenever handling biological samples.

5. Procedure

5.1 Arrangements to be made prior to the patient assessment

5.1.1 Supplies

Biological sample packs for each patient visit will be provided by the PLANS Study Coordinator. These will include:

Labels for sample tubes:

- For each patient, you will receive a supply of labels with the patient ID and tube number. There are two labels for each tube, one for the tube itself and one for the inventory card.

***Kit A**: Baseline 1 blood and urine for the hub:*

- 1x 10 ml whole blood tubes (red top) – for serum
- 1x 10 ml EDTA blood tube (purple top)
- 1x 10 ml Tempus tube (blue top)
- 1x 125 ml urine pot
- 1x butterfly needle, if required by the clinical site
- Inventory card
- Pathoshield-3 outer cardboard box labelled KIT A and security seal. The box will be pre-labelled with the hub address and details of the sending hospital.
- Internal Pathoseal bag and absorbent material

***Kit B**: 4 ml whole blood for Leeds:*

- 1x 4 ml EDTA tube (purple top)
- Inventory card
- Pathoshield-8 outer cardboard box.
- The outer cardboard box labelled KIT B and security seal. The box will be pre-labelled with the hub address and details of the sending hospital and will have a first class stamp.
- Internal Pathoseal bag and absorbent material

***Kit C**: Blood and urine samples for the hub:*

- 1x 4 ml EDTA tube (purple top)
- 7x 10 ml EDTA tubes (purple top)
- 2x 10 ml whole blood tubes (red top) – for serum
- 2x 10 ml Tempus tubes (blue top)
- 1x 125 ml urine pot
- 1x butterfly needle, if required by the clinical site
- Inventory card
- Pathoshield-3 outer cardboard box labelled KIT C and security seal. The box will be pre-labelled with the hub address and details of the sending hospital.

- Internal Pathoseal bag and absorbent material

Spare labels will be provided for other hubs, in case the primary hub is not able to accept the sample.

Prior to collection by the courier, the sender will need to add their name and contact number to the outer cardboard box to complete the sender's details.

If additional packs are required, these should be requested by contacting the PLANS Study Coordinator using the following details:

Email: <email>
Tel: <phone>

All other materials will be sourced from the recruitment site's normal supplies.

5.1.2 Booking study visits

Blood and urine samples for hub processing must be taken and processed on the same day, as several of the possible biomarkers are perishable.

- Please check the hub technician (see below) will be available on the day of the planned visit.
- Please ensure assessments are booked first thing in the morning (10.30 AM at the latest) so that samples can reach the processing hub in good time. Samples should leave the hospital as early as possible because four hours' processing are required at the hub on the same day.
- Please book the courier (see below) at the same time as booking the study visit. A half hour delay must be taken into account between bleeding the participant and the sample being collected to enable samples collected for serum to clot whilst being left in an upright position.
- Confirm the booking to the hub technician.

Hub technician availability:

The hub technician can only process a certain number of PLANS blood and urine samples on any given day. It takes around four hours for hub processing to take place, which means that samples must be on their way to the hub before 11.00 AM at the latest. This means patient appointments should be before 10.30 AM at the latest.

It is therefore necessary to confirm with the technician who will process samples from your site **before** confirming an assessment date with the participant. Please telephone or email the technician using the details on the following sticker:

<<Affix hub-specific details here>>

If a technician is not available at your nearest hub, you may have a second hub within 2 – 4 hours of your hospital that you can use. If so, details are entered here:

<<Affix hub-specific details here>>

5.1.3 Booking study visits – skin samples and renal biopsies

Skin and renal samples may be collected on a different day and / or in a separate department from the blood and urine samples and are couriered separately from the blood and urine samples, directly to analysts at the University of Leeds (skin samples) or Imperial College London (renal biopsies). The courier(s) used may not be the same as the one used for blood and urine samples. Accordingly, SOPs for collection of skin samples (PLANS-003) and renal biopsies (PLANS-004) are provided separately.

The research nurse in the rheumatology department is responsible for liaison with the department collecting the skin and renal samples. The nurse will alert the relevant department to the required timing of the research visit. The research nurse may also be responsible for checking the availability of the University analysts and for booking the courier, and possibly other activities according to local arrangements.

5.1.4 Booking a courier collection for blood and urine

Until we have final arrangements in place with a courier company, contact XXX (Study Coordinator), tel. <phone>, email <email>.

5.1.5 Visit schedule

Visit 1 – week -2 to 0 (Baseline 1: could be same day as consent is given; following consent):

- Research blood samples (see 5.2.2)
- Research urine sample (75 ml)
- **Use Kit A**

Visit 2 – week 0 (Baseline 2):

- Research blood samples (see 5.2.2)
- Research urine sample (75 ml)
- **Use Kits B and C**

- Skin biopsy and epidermal sampling (see PLANS-003)
- Renal biopsy (see PLANS-004)

Visit 3 – week 2: No research samples

Visit 4 – week 4: No research samples

Visit 5 – week 12:

- Research blood samples (see 5.2.2)
- Research urine sample (75 ml)
- **Use Kits B and C**

Visit 6 – week 26 +/- 4 weeks:

- Research blood samples (see 5.2.2)
- Research urine sample (75 ml)
- **Use Kits B and C**

Visits 7 & 8: No research samples

Flare visits (after visit 6): No research samples

5.2 Collection of blood and urine samples

Blood and urine samples should be taken first at the study visit, prior to the completion of any questionnaires, to ensure that samples can be collected at the earliest opportunity.

When taking biological samples, please follow your site's standard procedures and observe all appropriate safety practices including those in relation to discarding clinical waste and sharps.

5.2.1 Paperwork

Before collecting blood and urine, select the relevant labels and an Inventory Card for the patient. Tubes are unlabelled and it is essential that the correct labels are used, with a duplicate label being affixed to the Inventory Card as described below.

5.2.2 Order of blood sample draw

Please note this order incorporates samples taken for routine blood monitoring according to local practice, e.g. routine serological tests (ANA, complement, Igs etc) and routine laboratory tests (FBC, U&E, LFT, ESR, CRP).

The maximum blood draw on a single occasion should be no more than:

- Adults and young people from age 16: 130 ml of which no more than 115 ml will be for research purposes
- Young people aged 12 – 15: routine bloods plus 32 ml for research purposes

It is important to draw the blood in the following order. If it becomes apparent that not enough blood will be collected, continue down the list so far as possible and leave out the samples at the end. This will particularly be the case for paediatric patients.

Baseline 1:

1. Routine blood samples

   *Tube(s) for hub (**Kit A**):*
2. ALL PATIENTS: 1x 10 ml plain blood tube (red top) – for serum
3. ADULTS ONLY: 1x EDTA blood tube (purple top)
4. ADULTS ONLY: 1x Tempus tube (blue top)

Baseline 2 and follow-ups:

1. Routine blood samples

   *Tube for Leeds (**Kit B**):*
2. 1x 4 ml EDTA tube (purple top)

   *Tubes to be sent to hub (**Kit C**):*
3. 1x 4 ml EDTA tube (purple top)
4. 1x 10 ml plain blood tube (red top) – for serum
5. 7x 10 ml EDTA tubes (purple top)
6. 1x 10 ml plain blood tube (red top) – for serum
7. 2x 10 ml Tempus tubes (blue top)

Please note: bottle volume may be larger than volume required in sampling schedule. As bottles are only manufactured in certain volumes, bottles of an appropriate volume have been chosen.

5.2.3 Tube-specific requirements

EDTA tubes (purple top):

Immediately after collection, mix gently by inverting tubes 8 – 10 times.

Plain blood tubes (red top):

Immediately after collection, mix gently by inverting tubes 8 – 10 times, then place upright at room temperature for 30 minutes to allow clotting to occur before transportation takes place.

Tempus™ blood RNA tubes:

Prevention of backflow: Tempus tubes contain chemical additives which may cause adverse patient reactions if backflow occurs from the tube into an individual's arm. To prevent this possible backflow, it is important that the following precautions are observed:

- Place the individual's arm in a downwards position
- Hold the tube with the cap up
- Release the tourniquet as soon as the blood starts to flow into the tube.
- Make sure the tube contents do not touch the cap or the end of the needle during venipuncture

Collection of blood: Draw 3 ml blood directly into the tube. Ensuring the tube is filled up to the black mark on the tube will result in 3 ml blood being collected. Immediately after collection, shake vigorously (including tube inversion) or vortex for 10 seconds to make sure the Applied Biosystems Stabilising Reagent makes uniform contact with the sample.

Failure to mix the stabilising reagent with the blood leads to inadequate stabilisation of the gene expression profile and the formation of microclots that can potentially clog the purification filter.

5.2.4 Urine samples

A single 75 ml urine sample will be collected for research in a 125 ml urine pot.

Up to 75 ml urine will be collected for research purposes from both adults and young people in addition to any required for standard care. If it becomes apparent that not enough urine has been will be collected, the priority order is:

1. Urine for standard care
2. Remaining urine for research purposes

Instructions for participants:

1. Guidance should be provided on how to collect a midstream urine specimen in the collection cup, on each occasion a specimen is required:
    - Cleansing of the urethral region immediately prior to urine collection is not required and should be avoided.
    - The subject should not touch the inside of the container.

- After several millilitres have passed (count to five), the midstream portion should be collected in the container without stopping the flow of urine.

5.3 Labelling and logging samples

You are provided with the set of labels for each patient, which should be used in the order printed. Two identical labels are provided for each tube. Put one of the labels on the tube.

Please log samples on the PLANS inventory card, which should be included with the samples. Prepare a separate inventory card for the 4 ml box for Leeds and the box of blood and urine samples destined for the sample processing hub.

Second label: Put the label for the first and last tube on the inventory card. For the single 4 ml blood tube for Leeds, just put the single label on the inventory card and leave the space for the label for the last tube empty.

5.4 Sample shipping

5.4.1 Blood tube (4 ml) for Leeds (Kit B)

The 4 ml blood tube for Leeds will be sent by first class Royal Mail at ambient temperature. It must be sent on the same day as the sample was collected, so that it arrives next day. Please make sure that the sample is not left in an out-tray overnight.

The address to which you will send the 4 ml blood tube is:

<Leeds address>

5.4.2 Blood and urine for the hub (Kits A & C)

The remaining blood tubes and the urine pot will be sent at ambient temperature to your nearest available hub (see Section 5.1.2).

5.4.3 Packaging

Samples from each patient will be returned in individual packs (4 ml blood for Leeds HMDS / everything else for the hub).

Kit B for Leeds HMDS:

- Wrap the 4 ml sample for Leeds in absorbent material (Kit B).
- Continue at ‘All kits’ below.

Kits A & C for hub:

- Seal the urine pot with Parafilm if available and / or place in a regular sealable bag.
- Place all samples for the hubs (Kits A & C) in the absorbent pouches included within the sample packs.
- Continue at ‘All kits’ below.

All kits:

- Place the samples in their absorbent material / pouches in the Pathoseal bag, expel excess air from the bag, peel off the tape to expose the adhesive, and apply pressure to closure working outwards to the edges pressing firmly to avoid gaps in the seal.
- Place the bag in the outer cardboard box with the completed inventory card.
- Close the box and seal it with the security seal.
- The sender's details should be completed on the label attached to the box by adding the name and contact number for the individual sending the samples.

Any unused tubes can be retained by the clinical site as spares.

6. 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: <email>) 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.

7. Other SOPs referenced

SOP	Title of SOP
PLANS-003	Skin photos and sample collection
PLANS-004	Renal biopsy collection

8. References

1. Human Tissue Act 2004

APPENDIX 1: Biological sampling schedule

			V1	V2	V4	V5	V6	V7	V8	Flare visit
Biological samples for research	**Tube type & no.**	**Tube cap colour**	**Wk -2 to 0 Baseline 1**	**Wk 0 Baseline 2**	**Wk4**	**Wk12**	**Wk 26 +/- 4 weeks**	**Wk 40**	**Wk 52**	***If applicable; after V6***
Blood samples in order of collection	Tube volume		Blood volume	Blood volume	Blood volume	Blood volume	Blood volume			Blood volume
1. Routine blood samples	* * * * * AS REQUIRED * * * * *									
2. Whole blood for RNA (to Leeds)	EDTA 1x 4ml	Purple		4 ml		4 ml	4 ml			
3. Whole blood for DNA & epigenetics (to hub)	EDTA 1x 4ml	Purple		4 ml		4 ml	4 ml			
4. Whole blood for serum	Plain blood 1x 10 ml	Red	10ml	10 ml		10 ml	10 ml			
5. Whole blood for PBMCs and plasma	EDTA 7x 10 ml	Purple	10 ml (adults)	70 ml		70 ml	70 ml			
6. Whole blood for serum	Plain blood 1x 10 ml	Red		10 ml		10 ml	10 ml			
7. Whole blood for RNA	Tempus 2x 10 ml	Blue	3 ml (adults)	6 ml		6 ml	6 ml			
Urine samples			**Urine vol.**	**Urine vol.**	**Urine vol.**	**Urine vol.**	**Urine vol.**			**Urine vol.**
1. Routine urine samples	* * * * * AS REQUIRED * * * * *									
2. Research urine sample	1x 125 ml urine pot	N/A	75 ml	75 ml		75 ml	75 ml			
Other samples										
Epidermal sampling	(PLANS-003)	N/A		x						
Skin biopsy	(PLANS-003)	N/A		x						
Renal biopsy	(PLANS-004)	N/A		x						

APPENDIX 2: Hub addresses

Leeds hub:

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

Birmingham hub:

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

UCL hub:

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