MANCHESTER 1824
The University of Manchester

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

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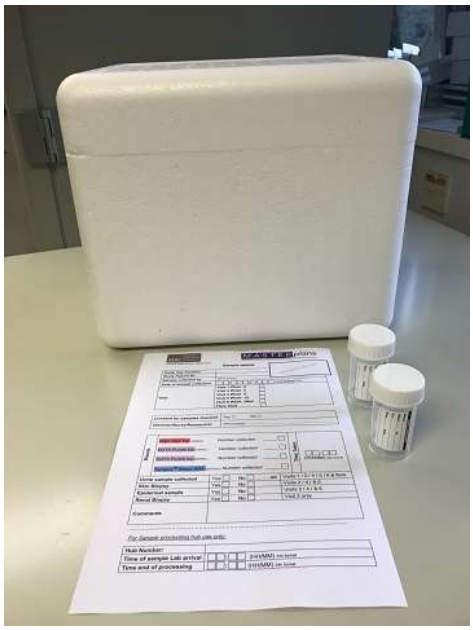
SOP title	**Skin sample collection and photos**



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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 skin procedures in clinic: skin photos, skin biopsies and epidermal sampling. These procedures will be undertaken with patient consent. Skin biopsies and epidermal samples will be sent direct to the University of Leeds for same day delivery.

In some hospitals, the skin procedures will take place externally to the Rheumatology department, e.g. in the Dermatology department.

Epidermal tape stripping

Epidermal sampling using the tape stripping technique is employed to obtain materials from the upper layers of the skin (epidermis) for analysis of diagnostic biomarkers. Tape stripping samples will be collected to assess whether molecular markers for different inflammatory skin responses can improve cutaneous lupus classification with regard to therapeutic outcome.

Videos

Three short videos are available on the MASTERPLANS website to supplement this SOP. These demonstrate:

- How to orientate the skin biopsy in the freezing mould
- How to freeze the skin biopsy in dry ice prior to transport
- How to undertake epidermal tape stripping

The videos can be accessed at www.lupusmasterplans.org/videos.html . The web address should be typed in full as the web page is not accessible from the public part of the MASTERPLANS website.

2. Purpose of this SOP

The purpose of this SOP is to describe the procedure to follow at the clinical site when taking skin photographs and when taking and shipping skin biopsies and epidermal samples for the PLANS study. Separate SOPs are available describing collection and transport of blood and urine samples (SOP-001) and renal biopsies (SOP-004). SOP PLANS-007 describes the set-up of the cameras, which will be supplied pre-configured.

This SOP must be followed to ensure a high standard of quality and storage of biological samples as the process in which a sample is collected, transported, fractionated and stored will affect sample integrity which is vital to ensure valid and robust data following analysis. In particular, the time to freezing the skin biopsies and epidermal samples must be as short as possible, with freezing taking place as soon as the biopsy has been taken.

3. Abbreviations

ePLANS	PLANS web-based Case Report Form (CRF)
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
PSG	MASTERPLANS Project Steering Group: the project's senior operational group. The PSG meets monthly.
SLE	Systemic lupus erythematosus; lupus
SOP	Standard Operating Procedure
SPTOG	PLANS Sample Processing Technical Operations Group

4. Roles and responsibilities

Role	Responsibilities
Clinical / nursing staff	• Book patient visit to coincide with standard care visit • Arrange courier, including packaging and dry ice • Ensure availability of laboratory personnel at University of Leeds • Take the skin biopsy / epidermal samples • Label the tubes containing the skin biopsy / epidermal samples • Freeze the skin biopsy sample(s) in the OCT mould in the dry ice provided. • Complete the Inventory Card, which includes confirmation that the samples have been taken with consent. • Log sample into ePLANS and any local system
Courier	• Deliver packing materials and dry ice • Collect samples in packaging and deliver to University of Leeds research associate (Dr Ade Alase)

Role	Responsibilities
MASTERPLANS study coordinator	• Provide skin kits to clinical sites
Leeds research associate	• Receive skin samples from the courier

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 SO

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 (lab coat and gloves) should be worn whenever handling biological samples.

5. Procedure

5.1 Arrangements to be made prior to the patient visit

5.1.1 Supplies

The PLANS study coordinator will provide packs for collection of skin samples and a pre-configured digital camera for skin photographs. If additional packs are required, these should be requested by contacting the PLANS Study Coordinator using the following details:

Email: <email>
Tel: <phone>

Photos: The following will be provided:

- Sony H300 camera
- 8 GB memory card
- Spare batteries
- Background sheets (for calibrating the photos and recording the site / patient ID on the photos)

Biopsy kits will comprise the following:

- 1 x Rocialle Minor Operations Pack
- 2 x Disinfection wipes

- 2 x Stiefel punch skin biopsy 4.0 mm
- 2x Ethicon coated vicryl 4.0 suture
- 1 x Sterile glove (Medium) for undertaking the surgical procedures
- 2 x Mepore dressing 9 cm x 10cm
- 1 x 5 ml syringe
- 3 x Green needles (one for removing local anaesthetic from the bottle and the other two for orienting the skin biopsies in the mould)
- 1 x Orange needle (for injecting the patient with local anaesthetic)
- 5 x Tissue mould 15mm x 15mm x 5mm for OCT embedding (two to be used and three spare)
- 2x Polypropylene sterile containers, 60 ml with a screw cap

One of the following will be provided per site, as there should be enough for all skin samples:

- 1 x 118 ml bottle of OCT
- 1 x roll of aluminium foil

Kits for epidermal sampling tape stripping will comprise:

- 22 x D-Squame standard adhesive sampling discs of dimensions diameter 22mm, area 3.8 cm^2 (supplier: CuDerm)
- 2 x Disinfection wipes
- 2 x Polypropylene sterile container, 60 ml with a screw cap

The PLANS Study Coordinator will provide:

- Inventory cards
- Freezer labels for identifying the samples:
    - These will have the tube number, site number and patient ID.
    - Identical labels will be provided for the inventory cards.

Clinical sites will provide the following:

- Local anaesthetic, e.g. Xylocaine with adrenalin (owing to transportation difficulties for the study)
- Skin marker pen
- Disposable latex or nitryl gloves

Packing materials:

The courier, having been booked at least a day in advance, will deliver packing materials including a polystyrene box (26 x 26 x 26 cm) and dry ice on the morning of the visit for sending all the skin samples together to the University of Leeds. The courier will collect the box and samples 2 – 3 hours later.

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

5.1.2 Booking patient visits

Skin samples may be collected on a different day and / or in a separate department from the blood and urine samples. Following collection, skin samples are sent immediately by courier directly to the University of Leeds for same day delivery. This may be a different courier from the one for the blood and urine samples.

Skin procedures should be booked early in the morning to allow sufficient time for the samples to reach the University of Leeds on the same day. The timing will depend on the travel distance from the clinical site.

Skin photos, biopsies and epidermal sampling will take place during a routine care visit. Tape stripping should take place in the same visit as any skin biopsy. The patient will not attend visits solely for research activities. The person booking the patient visit should ensure that the following takes place:

Check the Leeds Research Fellow will be available on the date of the planned visit:

<contact name>
<address>
<email>
<phone>

- Please book the courier and dry ice (see below) at the same time as booking the patient visit. The courier must be booked at least a day in advance of the visit.
- Confirm the booking to the Leeds Research Fellow.

5.1.3 Booking a courier collection

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

5.1.4 Visit schedule – skin photos, biopsies and epidermal samples

Week 0 (baseline):

- Skin photos

- 1x skin biopsy (4 mm punch) of lesional skin
- 1x skin biopsy (4 mm punch) of non-lesional skin
- Epidermal sampling of lesional skin (adjacent area to biopsy)
- Epidermal sampling of non-lesional skin (adjacent area to biopsy)

Week 4:

- Skin photos

Week 26:

- Skin photos

5.1.5 Skin photos

In order to validate assessment of skin morphology and response criteria, photographs will be taken of all lesional skin at weeks 0, 4 and 26. Take photos to record all the lesions. Because of the camera distance, you may be able to include several lesions in the same photo.

A background sheet will be included in images to allow calculation of the area of affected skin during image analysis and provide a standard colour and length scale.

All photographs will be close-ups of lesions so the participant will not usually be identifiable. If the lesion is on the face, the photographer will restrict the field of view so that identification of the participant will be unlikely.

Camera set-up:

Details on camera settings are given in SOP PLANS-007. These should be checked at each visit to ensure they have not been accidentally changed. It is easy to check them as settings are visible on the camera screen. SOP PLANS-007 also provides illustrative images demonstrating the importance of correct exposure.

Key settings to be checked:

- Flash set to ON
- Exposure compensation -0.3EV
- ISO 200
- White balance AWB
- Picture effect disabled
- Still image size 20M (maximum)
- Focus Multi AF

For each photograph:

All skin lesions should be photographed.

Remove one background sheet and complete the participant details as follows:

- Participant ID Number
- Date of Photograph
- Visit number / week

Take photographs as follows:

1. The flash must be ON. Turn on the flash as described in SOP-007.
2. The distance of the camera from the skin must be approximately 25 inches (63 cm).
3. The zoom function may be used to ensure the image fills the screen.
4. The scales and participant details on the background sheet must be visible.

Photographs may be checked by pressing the PLAY button, then scrolling through the images using the flash icon (or back by pressing the smiley face icon).

Once all the photographs have been taken, upload the skin photos into the ePLANS system.

1. Connect the camera to your computer using the USB lead supplied with the camera. The port for the lead is on the left side of the camera – you will need to open the small rubber door to expose the port.
2. The other end of the USB lead must be inserted into your computer.
3. Once inserted, the camera should be switched on.
4. The computer will recognise the camera as an additional storage device. If you select Windows Explorer (or your file management system) the camera will likely be shown as a “removable disk”. You will know if it is the correct directory as double clicking on it should reveal the following folder “DCIM”. By double clicking on this folder you should see the following folder “101MSDCF”. The photographs you have taken will be found in this folder.
5. If there will be a delay in uploading photos to ePLANS, you can take a backup copy of the photos at this point and store them on a Trust computer according to local guidance on information security.
6. When ePLANS is available, log in and navigate to the correct patient visit. tab for uploading skin photos. The standard Browse facility will allow you to upload the photos individually in the normal way.

5.1.6 Skin biopsies and epidermal sampling - preparation

The courier will deliver the packaging materials, including an outer box, the polystyrene box and dry ice shortly before the patient visit.

- Stick the label with the University of Leeds return address on to the outer box.

- Complete the sender's details on the box (name and contact number)
- Complete the inventory card as far as possible at this stage. A separate inventory card will be completed for each sample returned in the same box.

Take the following into theatre, minor procedures room or other suitable place:

- Skin biopsy kit
- Kit for epidermal sampling
- Polystyrene box containing dry ice
- Outer box
- Partially completed inventory cards
- Labels

5.1.7 Skin biopsies

Skin biopsies will be taken by fully trained clinical staff according to local procedures. This biopsy is for research purposes only. A histology report will not be provided by the MASTERPLANS investigators. If a diagnostic biopsy is needed at the same time, a second section of skin must be collected and processed as for local routine clinical practice.

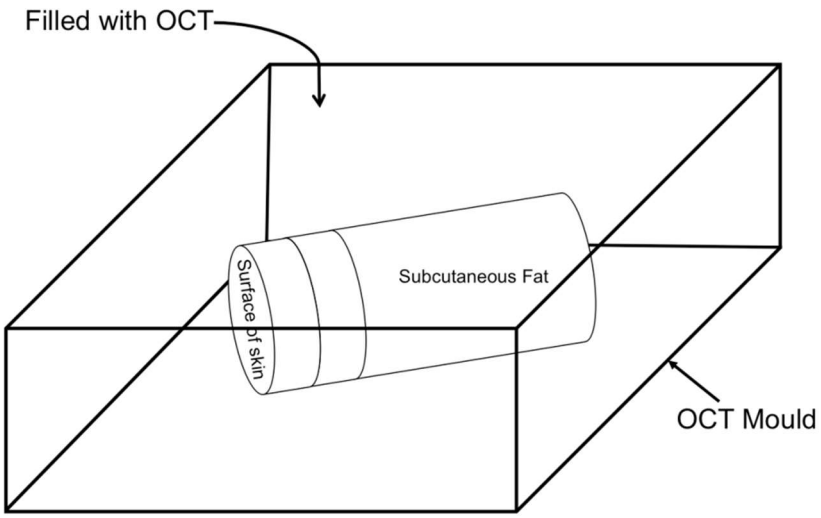
- The area to be biopsied will be discussed and agreed with the patient.
- The selected area to be biopsied is first infiltrated with the local anaesthetic using the syringe and needles provided.
- The selected area is biopsied using the 4 mm punch biopsy. This is rotated down through the epidermis and dermis, and into the subcutaneous fat, yielding a 4 mm cylindrical core of tissue sample.
- A second section will be collected from the nearest area of non-lesional skin amenable to biopsy, using a second 4 mm punch.
- Once obtained, the wound is closed with sutures – usually one or two interrupted nylon sutures using the Ethicon 4.0 suture or as locally supplied. The wound can be bandaged using the Mepore dressing.

Following each biopsy:

- On completion of each biopsy procedure, put on the disposable latex / nitryl gloves.
- Fill the tissue mould with OCT.
- Place the biopsy into the OCT mould on its side to ensure correct orientation for later processing (see figure 1 below).
- If necessary, remove bubbles with the second green needle. Some people prefer to use a pastette, if locally available.
- Using the scalpel provided, push the biopsy into the OCT so that all surfaces are well covered.

- Place the OCT mould on to dry ice as soon as possible until the OCT turns white and solid. This takes around 5 minutes.
- While waiting for the OCT sample to turn solid, put one label with the appropriate sample reference number on a piece of foil. Put the duplicate label on to the corresponding inventory card.
- Remove the biopsy from the OCT mould, wrap it in foil and put it back in dry ice.
- Place the frozen OCT block in foil into the sterile container. Place the container containing the frozen sample into dry ice.
- Complete the inventory card(s) and place them in the outer box.

Figure 1: Biopsy must be placed on its side within the OCT mould so that tissue layers are in the correct orientation for later processing.



5.1.8 Epidermal samples

5.1.8.1 Choice of site

- Tape stripping samples should be obtained from the same region of skin as the skin biopsy. This may be exactly the same area of skin or a nearby area of skin with similar appearance.
- This will include a lesional and a non-lesional site. Areas that are not suitable include anywhere there is a break in the skin surface (rhadgades, oozing lesions, erosions).

5.1.8.2 Sample acquisition

- Each clear disc has a white non-sticky section. Please use this for handling and manoeuvring the tape discs, either using fingers or tweezers. Place the tape on the

chosen area of skin for 5 seconds with gentle pressure. While the tape is in place on the skin, mark the position of the tape by 2 – 3 dots using a skin marker pen.

- Remove the tape disc by pulling rapidly away from the skin, holding the disc at the end in the marked white area using fingers. You can use tweezers if you prefer and these are available locally.
- Discard the first tape disc obtained.
- Repeat the process with a new disc on the same skin site, using the dots marked on the skin to ensure that the new disc is placed directly over the area that the first disc was placed. Place the disc in an empty polypropylene sterile 60 ml container using either fingers or tweezers, depending on which you used previously.
    - Repeat this process with an additional nine more discs, so that there are ten discs in total. Place each disc in the same polypropylene container.
- In the event of bleeding or erosions occurring on the area of the skin sampled during tape stripping, do not proceed with more tapes at this site, but continue to collect the remaining required number of samples from an adjacent area if possible.
- On completion of the epidermal sampling procedure, put one label on the sterile container and the other on the inventory card.
- Complete the inventory card and put it in the outer box. Take care to specify location of samples and whether they are lesional / non-lesional.
- Put on the disposable latex / nitryl gloves and put the sterile container in the dry ice.

5.1.9 Packing skin biopsies and epidermal samples:

- Ensure the inventory cards are in the outer package (not in the dry ice).
- Close the polystyrene container securely and place it in the outer package.
- The sender's details should be completed on the outer box by adding the name and contact number for the individual sending the samples.
- Hand the package to the courier.

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: 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.

7. Other SOPs referenced

SOP	Title of SOP
PLANS-001	PLANS-001 Blood and urine collection
PLANS-004	PLANS-004 Renal sample collection
PLANS-007	PLANS-007 Photography guide for Sony H300

8. References

1. Human Tissue Act 2004
2. Human Tissue Authority, Codes of Practice 2009; Codes of Practice