A PROJECT OF THE BRITISH ASSOCIATION OF DERMATOLOGISTS

A-STAR Bioresource Laboratory Manual

V1.1

Contents

1 OVERVIEW AND CONTACT DETAILS FOR SAMPLE MANAGEMENT AND PROCESSING

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Scope:

This lab manual applies to the Bioresource element of the UK-Irish Atopic eczema Systemic Therapy Register (A-STAR) study to support and enable the collection, processing and retention of biomaterials collected during the Bioresource.

2 SITE REQUIREMENTS

The suitability of each site will be assessed with the A-Star Bioresource site requirements and capability assessment. Each recruiting site must have as a minimum:

- Venepuncture facilities
- Centrifuge capable of spinning serum at 2000g for 10 minutes at room temperature
- Lab space to aliquot samples for storage
- -80°C freezer space sufficient to store up to 1 year worth of collected samples
- Sample collection and storage tubes
- Secure storage facilities for samples and data
- Capacity to post samples on the same day, before the last post

Each Biorepository site must have as a minimum:

- Lab space for additional sample processing
- -80°C freezer space
- When collecting PBMC, liquid nitrogen storage facilities
- Capacity for monitoring sample storage conditions
- Appropriately trained staff to store samples

3 SAMPLE SCHEDULE SUMMARY

Samples are to be collected from participants at;

- V1 - Baseline visit
- V2 - 4 weeks post systemic immuno-modulatory therapy start (+/- 1 week)
- V3 - 16 weeks post systemic immuno-modulatory therapy start (+/- 1 week with +/- 2 weeks in exceptional circumstances)
- V6 – 52 weeks post systemic immune-modulatory therapy start (+/- 4 weeks)

Severity assessments (EASI, POEM and DLQI) need to be performed on the same day as the collection of biomaterial.

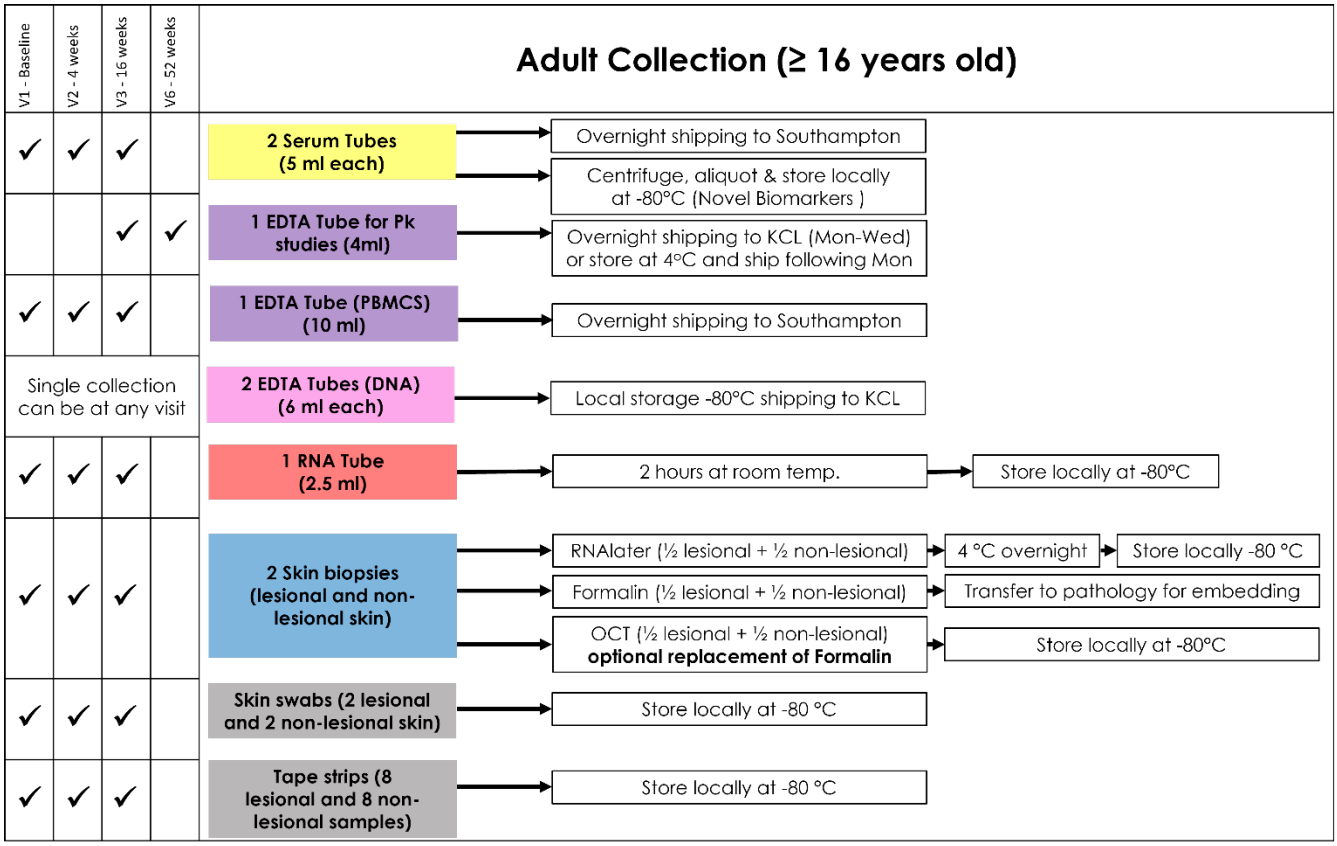
Systemic immune-modulatory therapy start is to be conducted as close to the baseline visit/assessments as possible.

If patient switches main treatment, Baseline 2 assessments will be performed and then further visits will continue as per original schedule (week 4, week 16, week 52).

For treatment-specific sample schedule see pages 4-9.

Patients are to be offered a voucher to the value of £10 per visit to cover reasonable expenses during the course of the sub-study. Contact A-star@gstt.nhs.uk to arrange receipt of the vouchers.

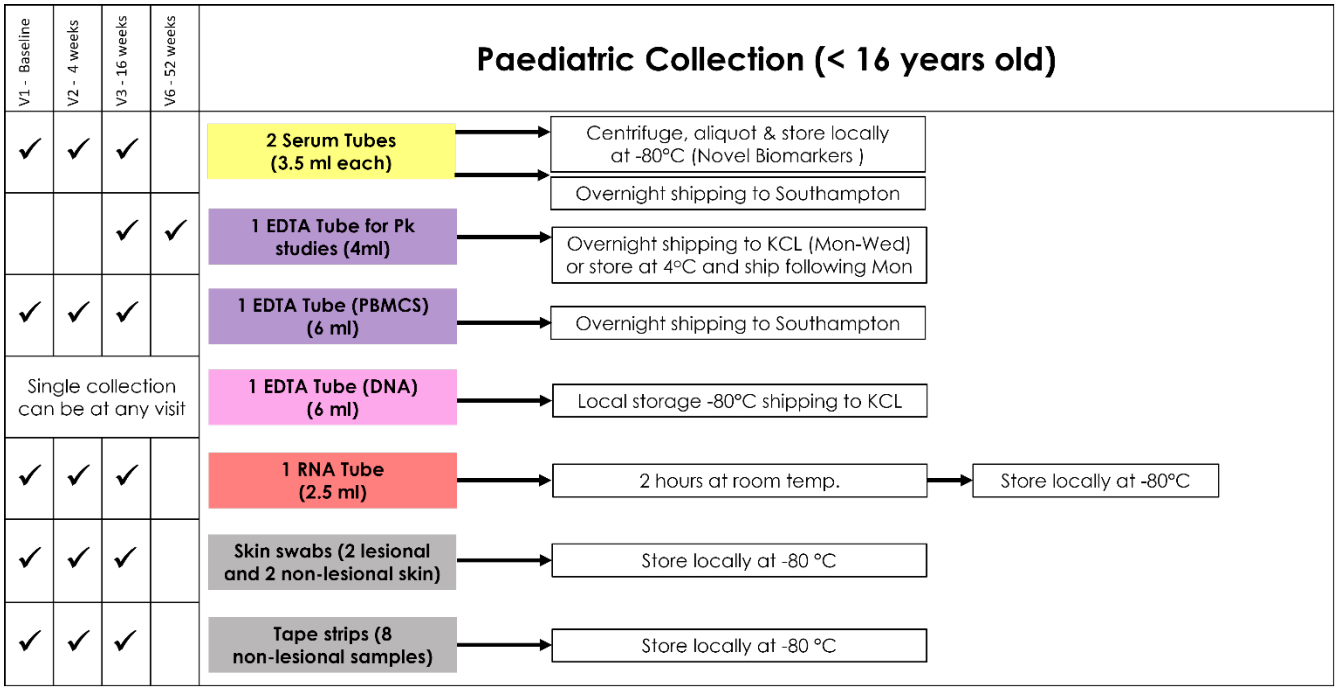
3.1 SAMPLE SCHEDULE – METHOTREXATE (ADULTS)



- Blood volumes and tissue types collected from adults and paediatric patients are different. This page contains information for **adult patients**.
- Only one venous blood sample for genetic analysis will be required. This can be collected at any visit.
- Samples should not be collected on a Friday or prior to a bank holiday weekend.
- Samples are collected at baseline (V1), 4 weeks (V2), 16 weeks (V3) and 52 weeks (V6) into systemic therapy as specified in the above diagram.
- Samples must be collected in the following order:

1) **Peripheral blood sample collection tubes – adults**
   a) 2 x 5ml Serum tubes (gold top):
      i) One tube to be centrifuged, aliquoted and frozen (see page 10 for details)
      ii) One tube for overnight shipping to Southampton
   b) 4ml EDTA tube (purple top) for overnight shipping to Kings College London
   c) 10ml EDTA tube (purple top) for overnight shipping to Southampton
   d) 2 x 6ml EDTA tube (pink top) for DNA for overnight shipping to Kings College London
   e) 2.5ml into 10ml PAXgene tube (red top) to be stored locally at -80ºC
2) **Skin microbiome samples**
3) **Tape strip samples**
4) **Skin biopsies**

3.2 SAMPLE SCHEDULE – METHOTREXATE (CHILDREN)



- Blood volumes and tissue types collected from adults and paediatric patients are different. This page contains information for **paediatric patients**. Refer to the appendix: blood volume guidelines on page 30 to find the safe volumes allowed for research purposes.
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   c) 6ml EDTA tube (purple top) for overnight shipping to Southampton
   d) 6ml EDTA tube (pink top) for DNA for overnight shipping to Kings College London
   e) 2.5ml into 10ml PAXgene tube (red top) to be stored locally at -80ºC
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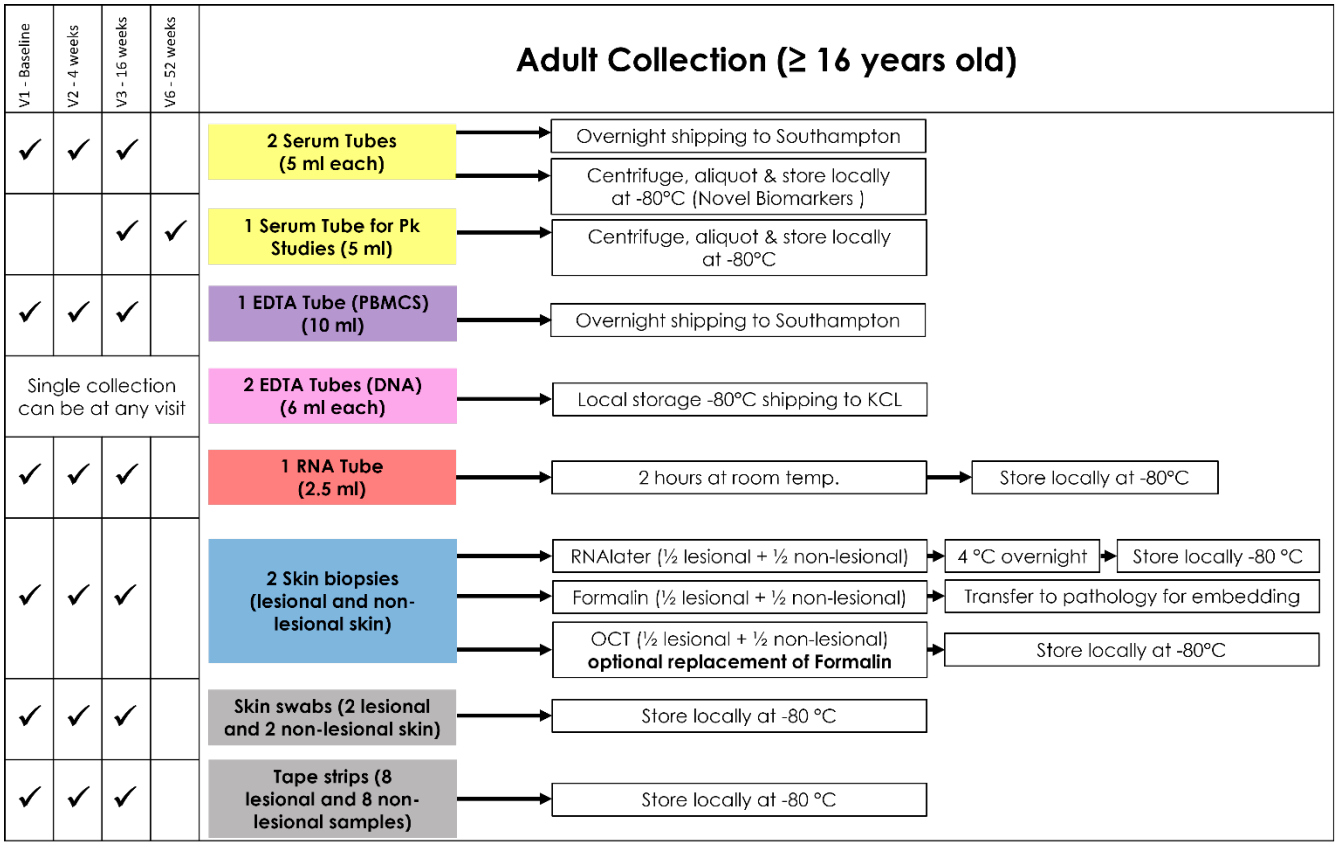
3) **Tape strip samples**

3.3 SAMPLE SCHEDULE – DUPILUMAB (ADULTS)



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- Samples must be collected in the following order:

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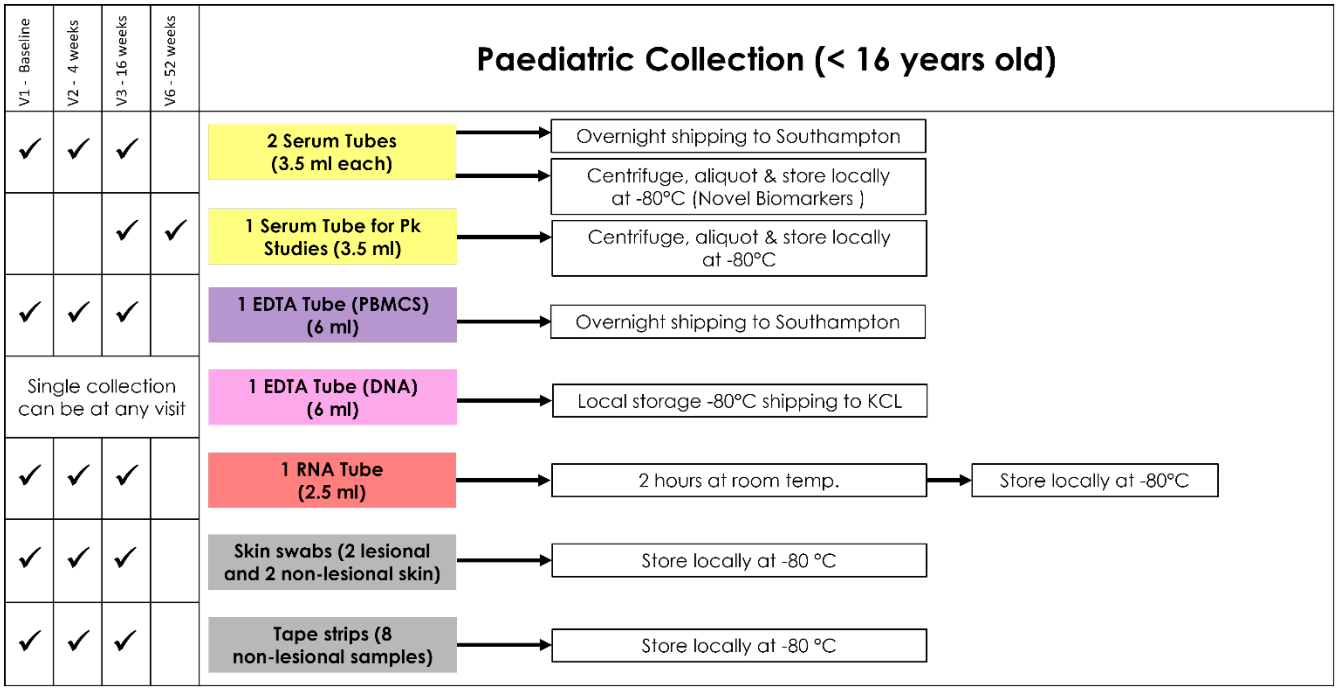
4) **Skin biopsies**

3.4 SAMPLE SCHEDULE – DUPILUMAB (CHILDREN)



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- Samples must be collected in the following order:

1) **Peripheral blood sample collection tubes – paediatric patients**
   a) 3.5ml Serum tube (gold top) to be centrifuged, aliquoted and frozen (see page 10 for details)
   b) 2 x 3.5ml Serum tubes (gold top):
      i) One tube to be centrifuged, aliquoted and frozen (see page 10 for details)
      ii) One tube for overnight shipping to Southampton
   c) 6ml EDTA tube (purple top) for overnight shipping to Southampton
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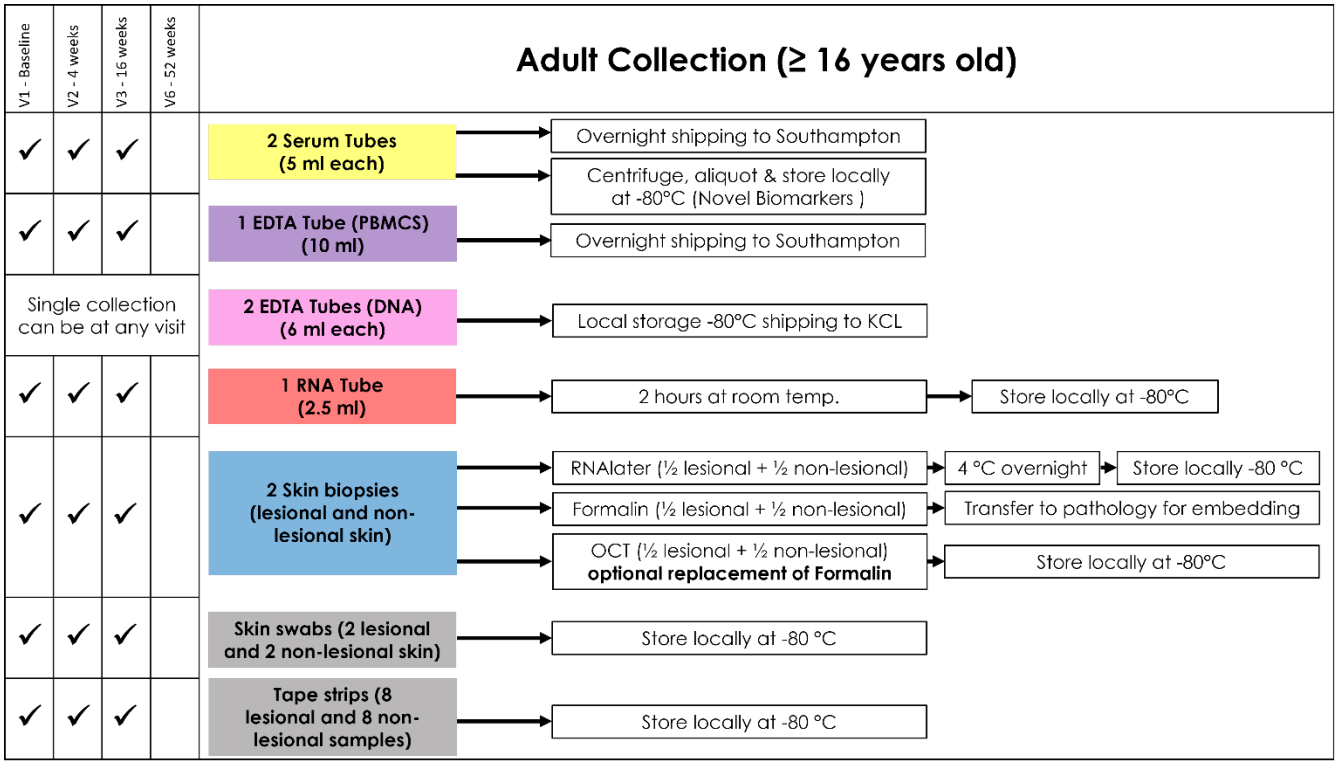
3) **Tape strip samples**

3.5 SAMPLE SCHEDULE – UPADACITINIB & CICLOSPORIN (ADULTS)



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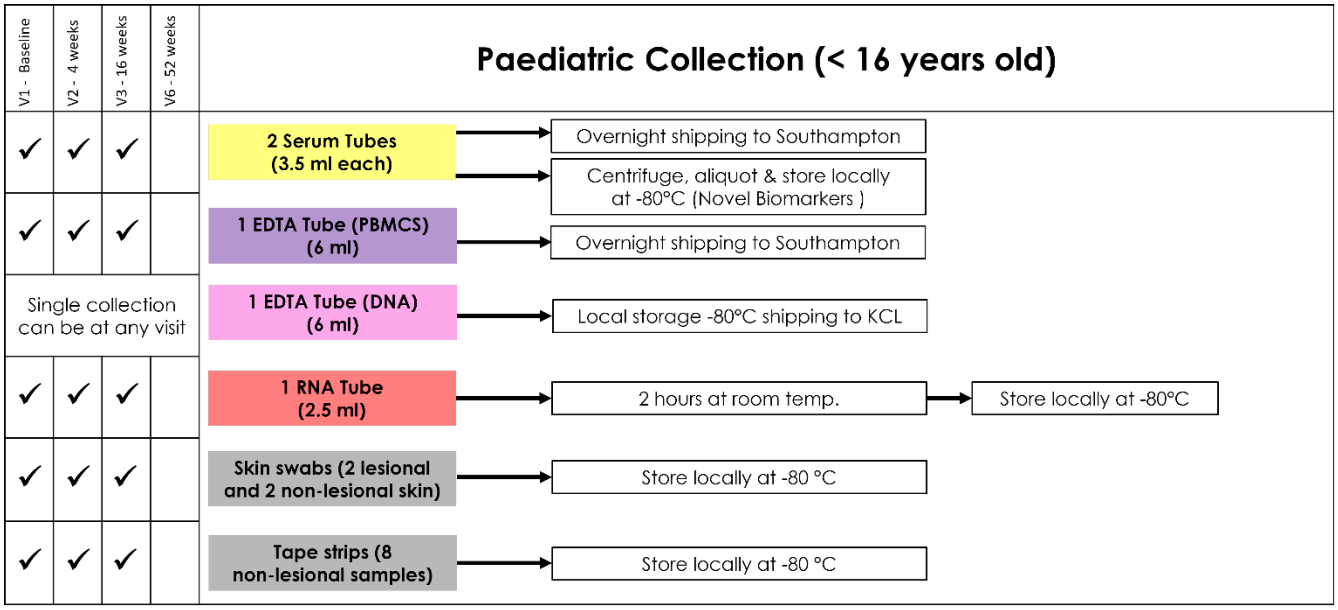
3) **Tape strip samples**
4) **Skin biopsies**

3.6 SAMPLE SCHEDULE – UPADACITINIB & CICLOSPORIN (CHILDREN)



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   d) 2.5ml into 10ml PAXgene tube (red top) to be stored locally at -80ºC
2) **Skin microbiome samples**
3) **Tape strip samples**

4 INSTRUCTIONS FOR COLLECTING AND PROCESSING SAMPLES

4.1 PERIPHERAL BLOOD

Supplies and equipment required

- Tourniquet
- Alcohol wipes
- Non-sterile gloves
- Local anaesthetic cream (optional)
- Sterile needle or winged infusion device of an appropriate size for the vein
- Sterile cotton ball or gauze swab
- Tape or adhesive plaster
- Sharps bin
- Marker for writing on labels

Adult Blood Tubes (≥ 16 years old)

Blood: Serum Sep Clot Activator **5ml** Vacuette tubes, gold top, Greiner bio-one, ref. 456018. Samples for the identification of novel Biomarkers (***all patients***) and Pharmacokinetic analysis (***Dupilumab patients only***)

Blood: EDTA (K2EDTA) **4ml** Vacuette tubes purple top, ***Methotrexate patients only***, Greiner bio-one 454209. Samples for Pharmacokinetic analysis.

Blood: EDTA (K2EDTA) **10ml** Vacutainer tubes, purple top, Fisher Scientific, ref 10331254. Samples for PBMC isolation and analysis

Blood: EDTA (K3EDTA) Vacuette tubes pink top, Greiner bio-one, ref. 456252. Samples for genetic analysis including Filaggrin mutations.

Blood: PAXgene **2.5ml** tubes, red top, Fisher, ref. 12937706. Samples for RNA analysis.

Paediatric Blood Tubes (< 16 years old)

Blood: Serum Sep Clot Activator **3.5ml** Vacuette tubes, gold top, Greiner bio-one, ref. 454214. Samples for the identification of novel Biomarkers (***all patients***) and Pharmacokinetic analysis (***Dupilumab patients only***)

Blood: EDTA (K2EDTA) **4ml** Vacuette tubes, purple top, ***Methotrexate patients only***, Greiner bio-one 454209. Samples for Pharmacokinetic analysis.

Blood: EDTA (K2EDTA) **6ml** Vacuette tubes, purple top, Greiner bio-one 456023. Samples for PBMC isolation and analysis.

Blood: EDTA (K3EDTA) Vacuette tubes pink top, Greiner bio-one, ref. 456252. Samples for genetic analysis including Filaggrin mutations.

Blood: PAXgene **2.5ml** tubes, red top, Fisher, ref. 12937706. Samples for RNA analysis.

BLOOD COLLECTION TUBES AND KIT EXPIRATION DATE

DO NOT use expired kits of tubes. The expiration date should be clearly marked on the collection kits and blood tubes. For all tubes and kits the expiry date is the last day of the month given. If supplies have expired, please request more supplies from the A-STAR Co-ordinating Centre.

Procedure

1. Once a suitable participant has been identified, approach study participant when appropriate to do so, and ask if you can explain the study and procedure to them. Once they are fully informed and have had the opportunity to ask questions, seek written informed consent to the study.
2. Ask the patient to state their full name and date of birth, check these details against the study identification number.
3. Position the patient comfortably, sitting or lying down, with appropriate arm supported.
4. Discuss previous venepuncture experiences or patient preferences for venepuncture site.
5. Assemble all the equipment, checking that the devices and blood tubes are within their expiry date. Ensure that the blood collection tubes are appropriate to treatment- specific sample schedule.
6. Wash hands and put on non-sterile gloves.
7. Apply the tourniquet, assess, palpate and select an appropriate vein, release the tourniquet.
8. Select a winged infusion device of an appropriate size for the vein.
9. Reapply the tourniquet above the intended venepuncture site. The tourniquet should be tight enough to impede venous return but should not obstruct arterial blood flow, and arterial pulse should still be palpable below the tourniquet.
10. Clean the skin with the alcohol wipe for at least 30 seconds and allow to air dry, do not re-palpate or touch the skin.
11. Carefully remove device from its packaging and inspect for defects.
12. Apply traction to the skin below the intended puncture site.
13. Hold the wings of the device firmly, ensure the level of the needle is upwards and insert through the skin at an angle of 10-30 to the horizontal, observe for flashback of blood into the tubing.
14. Reduce the angle slightly and advance the needle approximately 1-2mm, secure the device with tape if needed.
15. Carefully release the skin traction.
16. Connect vacuum tubes and withdraw the correct amount of blood, in the following order according to treatment specific sample schedule:

Adult Patients (≥ 16 years old)

a. 2 x 5ml serum tubes (gold top, Greiner bio-one, ref. 456018)
b. If required by patient specific sample schedule:
    i. ***Dupilumab patients*** at V3 (week 16) and V6 (week 52) – 1 x 5ml serum tube (gold top, Greiner bio-one, ref. 456018) for Pk dependent studies
    ii. ***Methotrexate patients*** at V3 (week 16) and V6 (week 52) – 1 x 4ml EDTA tube (purple top, Greiner bio-one, ref. 454209)
c. 1 x10ml EDTA tube for PBMC isolation (purple top, Fisher Scientific, ref. 10331254). Gently invert each tube 2 times immediately after drawing blood
d. 2 x 6ml EDTA tubes for DNA extraction (pink top, Greiner bio-one, ref. 456252). Sample can be collected at any visit
e. 1 x 2.5ml RNA tube (red top, Fisher Scientific, ref. 762165). Gently invert the PAXgene tube 10 times immediately after drawing blood

Paediatric Patients (≥ 16 years old)

a. 2 x 3.5ml serum tubes (gold top, Greiner bio-one, ref. 454214)
b. If required by patient specific sample schedule:
    i. ***Dupilumab patients*** at V3 (week 16) and V6 (week 52) – 1 x 3.5ml serum tube (gold top, Greiner bio-one, ref. 454214) for Pk dependent studies
    ii. ***Methotrexate patients*** at V3 (week 16) and V6 (week 52) – 1 x 4ml EDTA tube (purple top, Greiner bio-one, ref. 454209)
c. 1 x 6ml EDTA tube for PBMC isolation (purple top, Greiner bio-one, ref 456023). Gently invert the tube 2 times immediately after drawing blood
d. 1 x 6ml EDTA tube for DNA extraction (pink top, Greiner bio-one, ref. 456252). Sample can be collected at any visit
e. 1 x 2.5ml RNA tube (red top, Fisher Scientific, ref. 762165). Gently invert the PAXgene tube 10 times immediately after drawing blood

17. When the blood withdrawal is nearly finished, release the tourniquet, during the filling of the last tube.
18. When blood collection is complete place a sterile swab over the insertion site, withdraw the needle from the vein and immediately apply pressure to the puncture site until the bleeding stops.
19. Dispose of the needle, winged infusion device and vacuum device in the sharps container.
20. Cover the puncture site with a dressing or an adhesive plaster and advise the patient to leave the dressing in place for at least an hour.
21. Ensure all vacutainers are labelled with the patient's identification number, date and visit number according to the research protocol.
22. Place samples in an appropriate biohazard container and transfer to laboratory for further processing, shipment or storage.

INSTRUTIONS FOR LOCAL STORAGE OF RNA SAMPLES

PAXgene tubes should be stored at room temperature for 2 hours, before transferring to -80ºC for long term storage.

- Ensure sample labelling and tracking in accordance with CAPTURE protocol.

SEPARATION OF SERUM SAMPLES FOR LOCAL STORAGE

Either 1 or 2 serum tubes (dependent on treatment specific sample schedule) require centrifugation and aliquoting prior to storage.

- Blood samples must arrive at the laboratory in sealed vacutainer vials. For protection of the sample during transit, the vacutainer must be placed in a sealed plastic sample bag or into a sealable plastic carry case.
- Blood samples should have been allowed to clot at room temperature (for between 30 minutes to 1 hour) at the collection site.

Separation of Serum from the cellular fraction

- Fractionate the whole blood by centrifuging at 2000xg for 10 minutes at room temperature.
- Care should be taken to avoid the contaminating the serum with red cells. If contamination does occur centrifugation should be repeated and note made on the lab record form.

Aliquoting

- It is expected that approximately 1-2ml of serum will be collected per serum tube.
- Divide the serum into aliquots of approx. 500µl each.
- Store in -80ºC Freezer in 1ml cryovials.
- Ensure sample labelling and tracking in accordance with CAPTURE protocol.

4.2 SKIN MICROBIOME

Purpose:

5 swab samples will be collected for skin microbiome analyses:

- from lesional and non-lesional areas in patients prior to tape stripping and skin biopsy collection (see diagram below for details)
- left antecubital fossa and left volar forearm, and
- a control sample.

Principle:

This Skin Swab aims at recovering the microorganisms on the skin surface. The microbiome is defined as the collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa and viruses) that live in the human body.

Safety procedures and precautions:

This is a sterile procedure

- Sterile gloves must be worn at all times.
- Microbiome sampling should precede skin biopsy sampling as microbiome samples are to be taken from the exact adjacent location as biopsy samples will be taken.
- Skin biopsies will not be taken from paediatric patients, only from patients aged ≥16.

Supplies and equipment required

- Isohelix SK-2S swabs x5
- Sterile wound care pack
- Sterile normal saline pod
- Storage boxes
- Clinell wipes

- Labels

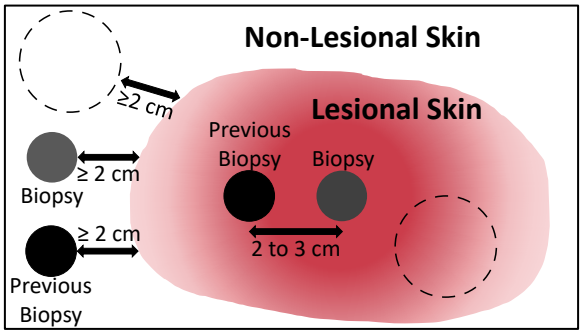
Preparation for the procedure:

- No bathing/showering, no creams or ointments on the areas sampled on the day of the study visit

Considerations for skin Microbiome site

Choose a sun protected area from the patient **prior** to the skin biopsy procedure (see diagram). For adults aged 16 and over, swab samples should be taken from the areas immediately adjacent to biopsy sites. Choose the site according to the following preference:

1: lower back 2: buttock 3: thigh 4: Upper arm



Dashed circles indicate locations for the Skin swab. Filled circles indicate biopsy site

Procedure

1. The skin swabs are taken at Baseline (V1), 4 Weeks (V2) and 16 Weeks (V3).

2. Explain procedure to patient/parents and ask them to expose the skin site to be sampled, WITHOUT touching the sample site.

3. Prepare tube labels with study title, participant ID, sampling site, visit number and date.

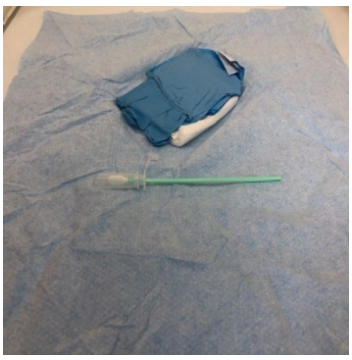
4. Wash your hands thoroughly.

5. Clean the work surface with a Clinell wipe to open up the sterile wound care pack onto it and create a sterile field.

6. Open the packaging of the wound care pack without contaminating the inner package and place the sterile wound care on the clean surface.

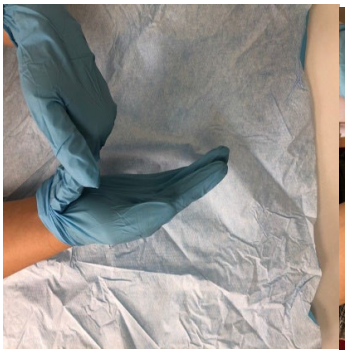

7. Unfold the paper wrapper to create an open sterile field. Pinch up the paper on the first flap and lay it back without touching the inside of the flap. Repeat until all the flaps are open. All the contents inside the paper i.e. gloves, gauze, apron, plastic bag are sterile.


8. Open the Isohelix swabs packaging onto the sterile field WITHOUT touching the swabs.

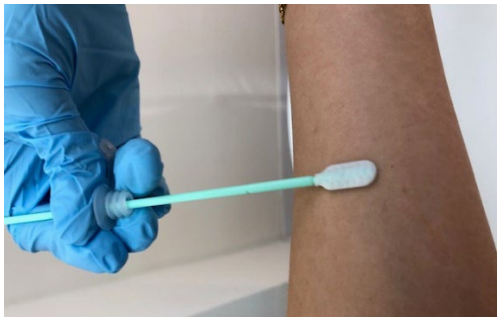
9. Pick up glove for the dominant hand by touching the inside cuff of the glove, without touching the outside. Insert hand into opening and pull the glove completely over.


10. Insert gloved hand into the cuff of the remaining glove. Insert fingers and pull the remaining glove on the non-dominant hand. Keep the gloved hands above the sterile field.



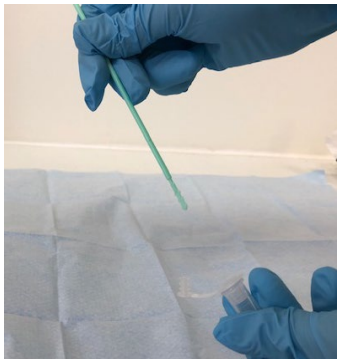
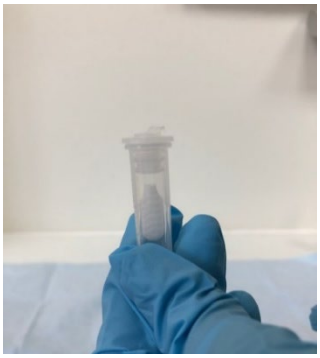

11. Remove the Isohelix swab from the tube, get another person to drip 2 drops of sterile normal saline on the swab WITHOUT touching the swab itself to ensure it remains sterile.


12. Obtain sample over approximately 4cm^2 of skin. Swab the skin with a firm circular motion. Roll over site 10x each for both swab sides, once in clockwise and once in anticlockwise direction.



13. Insert the swab into the clear plastic tube and push the cap into place.

14. Next, hold the cap while pulling the swab handle outwards to release the swab material into the tube. Close the cap. The tube is now completely sealed.

15. Drop the tubes outside of the sterile field and stick on the appropriate label.

16. Repeat steps 11-15 for each sample. Note on the sample label and in CAPTURE whether sample was taken from involved or uninvolved skin for all sites, including antecubital fossa and volar forearm.

17.To obtain the control sample hold the swab in ambient air for a 5 seconds. Repeat steps 13, 14 and 15.

18. Place all tubes in the storage box and transfer to -80ºC freezer within 1 hour.

19. Update CAPTURE with storage information.

4.3 TAPE STRIP

Purpose:

8 tape strips will be collected from **non-lesional skin** areas in patients with atopic eczema to analyse the cytokine profiles. An additional 8 tape strips will also be collected from **lesional skin** in adult patients having skin punch biopsies. One unused tape strip will be stored at each visit as a control.

Principle:

The concentration of filaggrin degradation products is normalized by the protein amount to compensate for variable amounts of stratum corneum (SC) harvested by tape stripping. A tape stripping technique from non-lesional skin of the volar forearm can measure the levels of PCA, trans- and cis-UCA, HIS and TYR, as well as cutaneous cytokine profiles.

Test site

Non-lesional samples will be taken from the Mid left volar forearm on the same site as microbiome samples were taken. **Microbiome sample must take place before tape strip sampling.**

Lesional samples will be taken on the same site as the microbiome samples. For patients having a skin punch biopsy, this should be adjacent to the biopsy site.

Preparation for the procedure

- Tape stripping will not be performed on broken skin.
- No bathing/showering, no creams or ointments on the areas sampled on the day of the visit.

Time point

- Tape stripping is done at baseline (visit 1), 4 weeks (visit 2) and 16 weeks (visit 3).

Reagents and Materials:

- D-100 D-Squame standard sampling discs (22mm)
- Plunger that exerts a standardised pressure (225g/cm2)
- Labelled 2ml Sarstedt microtube
- Gloves
- Marker pen
- Blunt-end tweezers
- Microtube storage box
- Immediate access to -80°C freezer

Procedure:

1. Pre-label the microtubes with study title, participant ID, sampling site, tape number, visit number and date.
2. The mid left volar forearm, without eczema, is exposed. This should ideally be an area free of hair follicles.
3. After washing hands according to the local hand washing policy, don gloves.
4. Using either tweezers or gloved fingers, touching the non-adhesive edge of the strip place an adhesive disc on the selected skin surface. Mark the skin around the disc with four dots using the permanent marker pen so that all discs may be applied to the same area. The dots should not be too close to the tape to avoid contamination with ink.
5. Apply uniform pressure on the sampling disc for 5 seconds using the plunger. Keep your finger on the top of the plunger and press until you feel the inner rod touch your finger. Hold the pressure for 5 seconds.
6. Using either tweezers or gloved fingers, remove the tape from the skin in a fluent movement and Transfer it into the 2ml microtube with attached cap with the adhesive site inside and the white part of the tape uppermost. Do not touch the tape with your fingers.
7. Repeat 7 times (8 in total) from the same skin spot. Use alternating directions for each subsequent strip.
8. Apply the study visit label and write the date and time.
9. Place the microtubes in the storage box and transfer them to a -80°C freezer within 1 hour. Each tape strip should be placed in a separate storage box labelled with the visit number and the tape strip number e.g. V1 TS-NL1, V1 TS-NL2 etc.
10. Ensure sample labelling and tracing in accordance with CAPTURE protocol.

4.4 SKIN BIOPSIES

Purpose:

Two 5mm skin punch biopsy samples will be collected from uninvolved and involved areas in patients with atopic eczema. Half will be used for RNA analyses and half for immunochemical assessment of eczema-related biomarkers.

Safety procedures and precautions:

1. Before taking the skin biopsy, ensure microbiome samples has been collected in the adjacent skin area.
2. Coat and gloves must be worn at all times.
3. Ideally, two persons should be performing this protocol, and while one is taking care of the patient the other should take care of the skin biopsy.
4. All procedures should be performed with sterile, RNAse-free equipment. Any contaminating RNAses will negatively affect the success of subsequent gene expression studies. Avoid touching your skin or hair with your gloved hands while processing the skin biopsies.

Supplies and equipment required

- 20ml vial lidocaine 1% with 1: 200000 adrenaline (Astra Zeneca, Cat No PL 17901/0174) **or** 20ml vial lidocaine 2% with 1:80000 adrenaline (Astra Zeneca, Cat No 17901/0174). **Or** An alternative analgesic can be used under direction from the Principal Investigator. The study management team (a-star@gstt.nhs.uk and Tom.Ewen@Newcastle.ac.uk) must be informed and a file note entered by the PI detailing the alternative product and confirming the PI is authorised to prescribe
- 10ml syringe
- 19 G needle
- 23 G needle
- Suture dressing pack
- Swabs/gauze
- Sterile drape
- Chlorhexidine solution (Sterets Unisept, 0,05% w/v Cutaneous Solution)
- 5mm punch biopsy
- 4/0 prolene
- Fucidin ointment
- Mepore or Tegaderm dressing (Tegaderm, 3M Health Care, Cat No 3586)
- Skin marker
- Sterile Gloves
- Mask and Apron
- RNAlater tubes (Life technologies, 50x1.5 ml, Cat No AM7022)
- Scalpel
- Normal saline (0.9% NaCl – pH is 5.5)
- Gauze
- Pre- -filled formalin Gentafix pot 10% buffered formalin (25 or 60 ml, Genta Medical, LOT 3030517) or equivalent
- Cellpath™ OCT Embedding Matrix (Fisher: 15212776)
- Cork Discs (Electron Microscopy Sciences: 63305)

Procedure:

Preparation before each biopsy:

1. Prepare Petri dishes with normal saline, clearly labelled with “involved or uninvolved skin”
2. Label RNAlater tubes with “lesional or non-lesional”, patient ID, date and time
3. Confirm if ½ the sample should be formalin fixed paraffin embedded (FFPE) or OCT embedded. In the absence of a file note from the PI, all samples should be FFPE.
4. For FFPE samples, label Gentafix pots with “involved or uninvolved skin”, patient ID, date and time
5. For OCT samples, label pot containing normal saline with “involved or uninvolved skin”, patient ID, date and time

Checks prior to excision:

6. Identify correct patient
7. Confirm biopsy sites (Lesional and Non-lesional)
8. Check skin lines to ensure best cosmetic effect, mark biopsy site
9. Check patient’s medication and note of allergies
10. Double check written consent has been given and reiterate all aspects of procedure
11. Position patient comfortably and protect the patient’s clothing

Considerations for biopsy site

Choose a sun protected area following this preference:

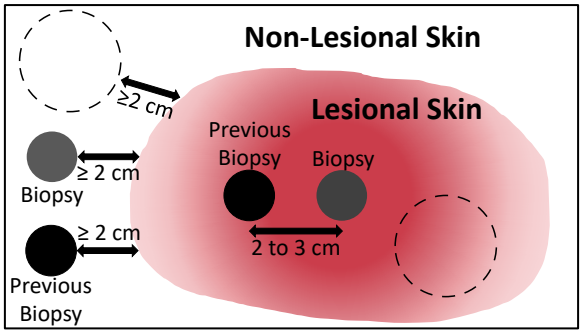
1: lower back 2: buttock 3: thigh 4: Upper arm

Preferably, consecutive biopsies should be taken from active area of eczema 2-3 cm away from the previous biopsy (see diagram). This should be considered when selecting an area at baseline. If the area has cleared as a consequence of the treatment, take the lesional biopsy from the area where the lesion used to be (usually, a light pigmentation or mild redness remains). If it is not possible to identify the area where the skin had been lesional at the earlier visit, refer to an adjacent or symmetrical area where pigmentation/redness is visible, and where there is a similar level of resolution.

If the involved area is too small to sample from repeatedly, use adjacent or symmetrical areas. If taken during treatment, the area should ideally have a similar level of resolution as the active area from which the earlier sample was taken.

Lesional skin biopsy should always be taken away from the edge in an active area of inflammation.

Non-lesional skin biopsy should always be taken 2.0 to 2.5 cm away from the edge of lesional skin accepting this may be ill-defined.



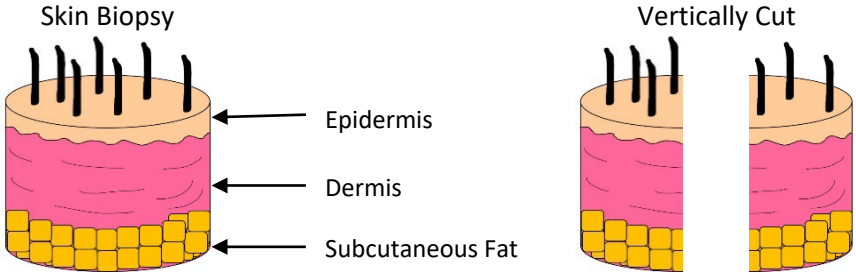
Dashed circles indicate locations for the corresponding Skin swab

Excision:

12. Put on apron/mask/gloves, using sterile technique.
13. Cleanse skin with chlorhexidine solution.
14. Cover the surrounding area with the sterile drape.
15. Check local anaesthetic and anaesthetise desired area.
16. Check effectiveness of anaesthesia, using a needle to prick skin.
17. Stretching the skin perpendicular to the relaxed skin tension lines between thumb and finger either side of the area to be sampled, the punch blade is placed on the skin and rotated under gentle pressure by rolling it between the thumb and finger using a twisting drilling action.
18. Once the biopsy is collected, place it in the normal saline in Petri dish, briefly wash it.
19. Close excision site using appropriate sutures and suturing technique
20. Clean area, apply thin layer of fucidin ointment and cover with appropriate dressing.
21. Check and confirm accuracy of information on pot with assistant.
22. Give appropriate verbal and written advice to patient post biopsy and arrange follow up appointment if required.

5mm punch biopsy:

23. Place the biopsy laying down on side in the Petri dish, epidermis facing sideways, cut the 5 mm punch biopsy vertically into two pieces (2 x 2.5 mm each) with a blade (see diagram. Both halves should contain the same proportion of epidermis and dermis, i.e. cut vertically (in a 90-degree angle) through epidermis and dermis using a single use sterile scalpel).



½ Biopsy in RNAlater:

24. Immediately, place 1/2 biopsy in an RNAlater tube, properly labelled (Lesional or Non-lesional, Patient ID, Visit Code, Date and if possible CAPTURE ID).
25. Invert tube a few times and ensure biopsy is NOT stuck to inner lid or side of tube, and is completely submerged in RNAlater.
26. This sample should be refrigerated as soon as possible and must be held at 4°C within 30 minutes.
27. Biopsy should be kept at 4ºC overnight then transferred to -80ºC the next day, following the processing instructions below.

½ Biopsy in Gentafix pot:

28. Place the other ½ piece (Lesional or Non-lesional-Patient ID-Visit Code- Date) in a marked Gentafix pot and transfer to the local pathology laboratory for embedding in paraffin after a minimum of 6 hours fixation in formalin. The samples should be embedded within the 6 - 72 hours post-collection for medium-term storage prior to shipping to central processing facility.

½ Biopsy in Normal Saline pot (OCT samples only):

29. Place the other ½ piece in marked pots (Lesional or Non-lesional-Patient ID-Visit Code-Date) onto gauze soaked with normal saline, to keep it moist
30. Transport biopsies to nearest research laboratory for further processing within one hour.
31. DO NOT transport on ice

After excision:

32. Document procedure performed in medical notes.
33. Enter samples collected and processed onto Capture database.
34. Process skin biopsy according to instructions below:

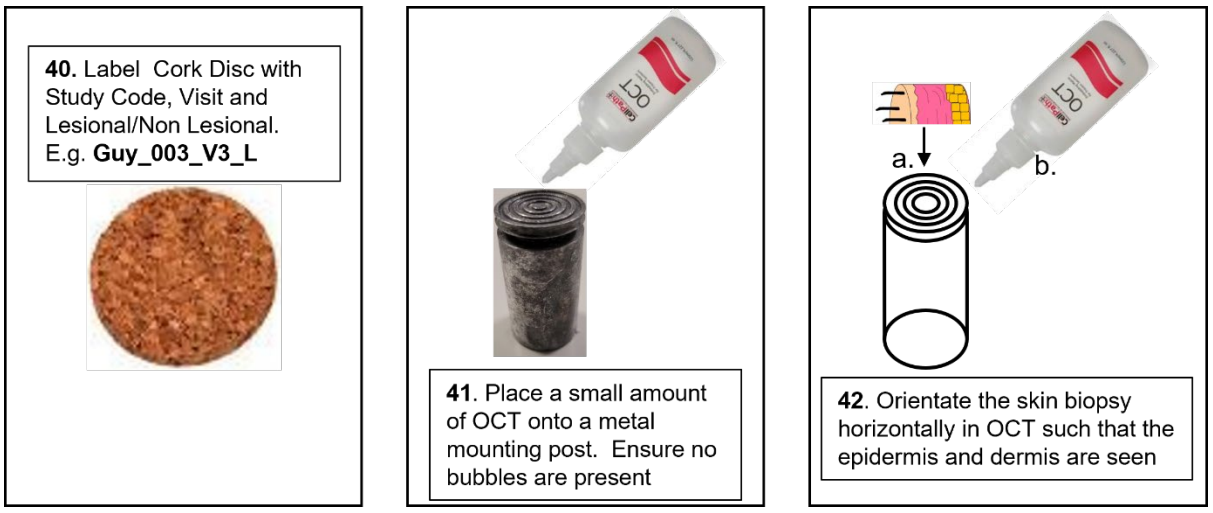
Skin Biopsy for RNAlater biopsy processing:

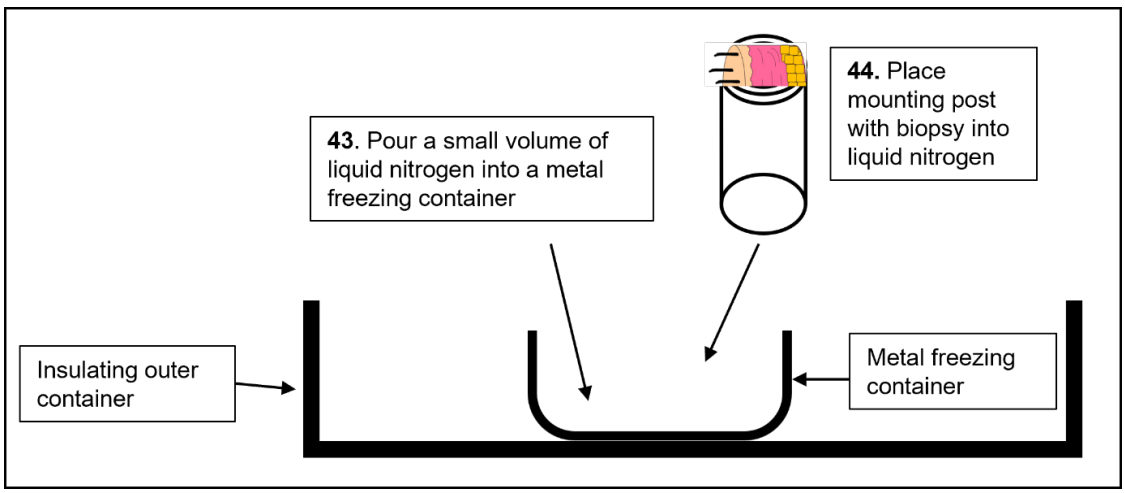
35. Make sure biopsy is NOT stuck to inner lid or side of tube and is fully immersed in RNAlater liquid.
36. Leave biopsy in RNAlater tube to soak overnight in 4ºC fridge (for at least 16 hours).
37. It is recommended to leave tubes upright in a tube rack in fridge.
38. Next day, remove and discard RNAlater liquid from inside the tube using sterile plastic bulb pipettes, thus leaving only the biopsy in the tube.
39. Store tube containing biopsy in -80ºC freezer for medium-term storage, prior to shipping to central processing facility.

Skin Biopsy for OCT processing:

This technique involves the use of liquid nitrogen. You must be trained to handle liquid nitrogen safely prior to OCT embedding.

40. Correctly label cork disc and apply a small drop of OCT to the unlabelled side.
41. Place a small amount of OCT onto a metal mounting post. Ensure no bubbles are present.
42. Orientate the skin biopsy horizontally in OCT such that the epidermis and dermis are seen.
43. Pour a small volume of liquid nitrogen into a metal freezing container.
44. Place mounting post with biopsy into liquid nitrogen.
45. As the mounting post freezes, the OCT with the biopsy will also freeze. Ensure that the biopsy is completely covered in OCT. If needed, quickly add a little more OCT on top of the tissue before the block completely freezes.
46. When completely frozen, remove the frozen OCT-tissue block by gently warming the post under warm water.
47. Before the OCT thaws, quickly transfer the block to the correct labelled cork disc pre-treated with OCT on the unlabelled side and allow to freeze in -20°C freezer. When the OCT-tissue block is frozen to the cork, transfer to a labelled plastic bag and keep in a designated Biobank -80°C freezer for long term storage.





4.5 LABELLING AND RECORDING OF SAMPLES

Sample labelling

All blood, microbiome, tape strip and skin biopsy collection tubes should be labelled appropriately so that they identify the patient and the date the sample was taken. The use of multiple identifiers, along with the sample request form, minimises the risk of not being able to identify the sample should details be smudged or rubbed off during processing, storage or transport. This is important as if there is any doubt as to the identity of the sample it will have to be discarded.

Freeze resistant labels will be provided by Jenny.Lumley@newcastle.ac.uk on request. Please copy the Bioresource Project Manager (Tom.Ewen@newcastle.ac.uk) into correspondence. Use a waterproof, permanent marker pen to add additional details not already on the label. Each sample should be labelled with the following information:

1. Study name: **A-STAR Bio**
2. Study Centre 2 letter code:
    - **EU** (NHS Lothian, Edinburgh)
    - **ST** (Guy's and St Thomas' NHS Foundation Trust, Children)
    - **GY (**Guy's and St Thomas' NHS Foundation Trust, Adults)
    - **MU** (Manchester, Salford Royal)
    - **NE** (Newcastle Hospitals NHS Foundation Trust)
    - **OU** (Oxford University Hospitals NHS Foundation Trust)
    - **SH** (Sheffield Teaching Hospital)
    - **SC** (Sheffield Children's Hospital)
    - **SU** (University Hospital Southampton NHS Foundation Trust)
3. Patient ID number: **001, 002, 003…**
4. Visit: **V1** (Baseline), **V2** (Week 4), **V3** (Week 16), or **V6** (Week 52).
5. Sample type:
    - **RNA** (Paxgene blood tube – red top)
    - **Pk** (Serum or EDTA tube – gold top or purple top)
    - **Serum** (Serum tube – gold top)
    - **PBMC** (EDTA tube – purple top)
    - **DNA** (EDTA tube – pink top)
    - **Swab L** (lesional), **Swab NL** (non-lesional) , **Swab AF** (left antecubital fossa), **Swab VF** (volar forearm), **Swab C** (control)
    - **TS-L** (Tape Strip lesional), **TS-NL** (non-lesional). Number tubes **1-8** & **C** (control)
    - **Bx-L** (Biopsy – lesional)**, Bx-NL** (Biopsy – non lesional)
6. Patient initials
7. Date of collection

Register the samples with CAPTURE & include the unique sample code on the label. The unique sample code is comprised of the **Study Name**, **Study Centre**, **Patient ID number**, **Visit** & **Sample Type**. An example of a unique sample code is:

A-STARBio_NE_001_V3_TS-L8

This sample is from Newcastle's first recruited patient. It is the 8th lesional Tape Strip sample from Visit 3.

It is important to note that the participant ID for A-STAR Pharmacovigilance and the Patient ID number for A-STAR Bioresource will be different. Both need to be linked and recorded in your local records.

Refer to the CAPTURE user guide for A-STAR for further instructions. The collection, storage, processing and shipment of the sample must be documented electronically in CAPTURE.

Every sample shipped should be accompanied by a sample manifest form and labels to use after the samples have been processed. See below for further details.

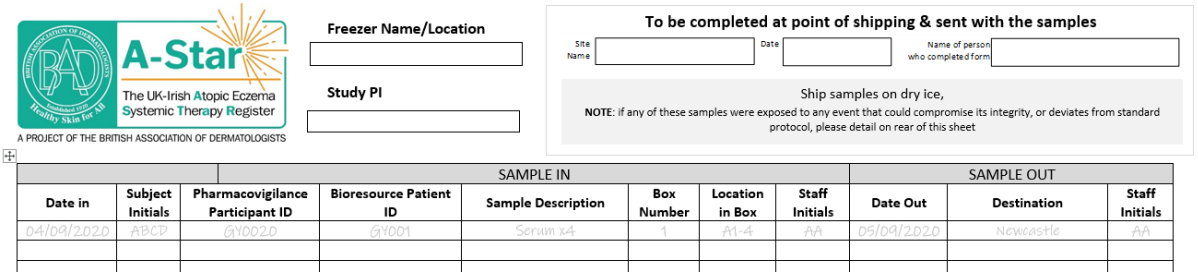
Manifest / sample log

A sample manifest has been created to help you manage and track your samples. This also helps us to process them when they arrive at their destination in dry ice as we'll know exactly what to expect in the shipment box and to flag any missing samples.

- If you would like to, you can use it as sample log for all your A-STAR Bioresource samples and complete it as you take the samples.

Please do include it with the shipment you are sending, and if possible, send it electronically to the lab and the Study Co-ordinating Centre. It is mandatory to update CAPTURE with sample details, including any protocol deviations.

Example of completed sample manifest



A-Star
The UK-Irish Atopic Eczema Systemic Therapy Register
A PROJECT OF THE BRITISH ASSOCIATION OF DERMATOLOGISTS

Freezer Name/Location

Study PI

To be completed at point of shipping & sent with the samples

Site Name | Date | Name of person who completed form

Ship samples on dry ice,

NOTE: if any of these samples were exposed to any event that could compromise its integrity, or deviates from standard protocol, please detail on rear of this sheet

				SAMPLE IN				SAMPLE OUT		
Date in	**Subject Initials**	**Pharmacovigilance Participant ID**	**Bioresource Patient ID**	**Sample Description**	**Box Number**	**Location in Box**	**Staff Initials**	**Date Out**	**Destination**	**Staff Initials**
04/09/2020	ABCD	GY0020	GY001	Serum x4	1	A1-4	AA	05/09/2020	Newcastle	AA

4.6 SHIPMENT AND STORAGE

Local information to be completed for each site:

Site Name	
Location of post room	
Time of last collection	

Laboratory Contact details

Newcastle University

Jenny Lumley (A-STAR Bio)
Dermatological Sciences,
2nd Floor William Leech Building
Medical School, Newcastle University
Framlington Place
Newcastle Upon Tyne
NE2 4HH
Telephone: 0191 208 5644
E-mail: Jenny.Lumley@newcastle.ac.uk

Kings College London

David Baudry (A-STAR Bio)
Skin Therapy Research Unit,
9th Floor - Tower Wing,
Guys Hospital,
Great Maze Pond,
London.
SE1 9RT
Telephone: 0207 188 8203
E-mail: david.baudry@gstt.nhs.uk

Southampton

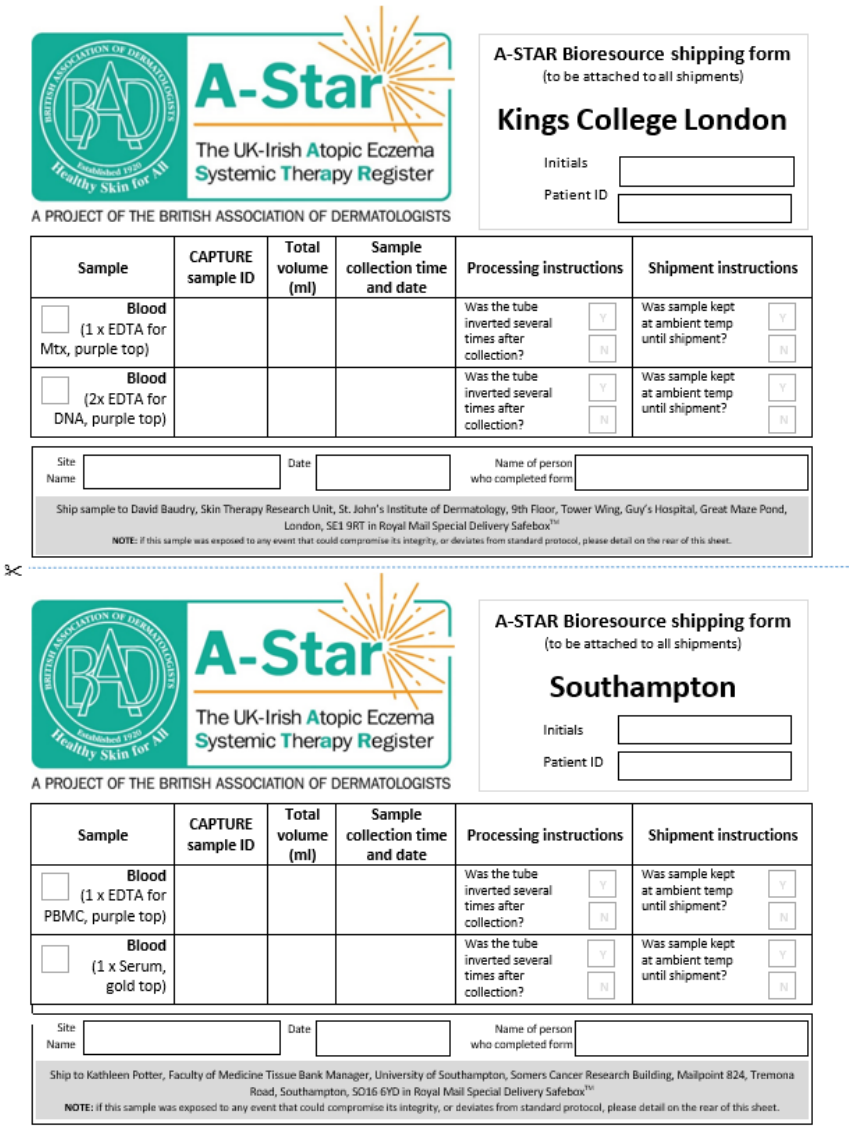
Kathleen Potter Ph.D. (A-STAR Bio)
Faculty of Medicine Tissue Bank Manager
University of Southampton
Somers Cancer Research Building
Mailpoint 824
Tremona Road
Southampton SO16 6YD
Telephone: 02381 205034
Mobile: 07796668925
E-mail: kp1@soton.ac.uk, nh9g15@southamptonalumni.ac.uk, A.Aghajani@soton.ac.uk, A.Woodham-Shulman@soton.ac.uk,

Samples for same day shipping

For samples that require same day shipping, as outlined in the sample collection schedule, use the immediate shipping form.

- All sections of the form must be completed and be included with the sample shipment, an example is included below
- Samples to be shipped in Royal Mail Safebox™ by Special Delivery. Instructions for packaging are contained within the Safebox. The tracking information must be recorded prior to postage.
- Samples should not be shipped on a Friday or prior to a bank holiday weekend.
- Samples must arrive at the local post room at least 30 minutes prior to the time of the last collection to ensure overnight delivery.

Immediate shipping forms



A-Star
The UK-Irish Atopic Eczema Systemic Therapy Register
A PROJECT OF THE BRITISH ASSOCIATION OF DERMATOLOGISTS

A-STAR Bioresource shipping form
(to be attached to all shipments)

Kings College London

Initials
Patient ID

Sample	CAPTURE sample ID	Total volume (ml)	Sample collection time and date	Processing instructions	Shipment instructions
☐ Blood (1 x EDTA for Mtx, purple top)				Was the tube inverted several times after collection? Y / N	Was sample kept at ambient temp until shipment? Y / N
☐ Blood (2x EDTA for DNA, purple top)				Was the tube inverted several times after collection? Y / N	Was sample kept at ambient temp until shipment? Y / N

Site Name | Date | Name of person who completed form

Ship sample to David Baudry, Skin Therapy Research Unit, St. John's Institute of Dermatology, 9th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London, SE1 9RT in Royal Mail Special Delivery Safebox™

NOTE: If this sample was exposed to any event that could compromise its integrity, or deviates from standard protocol, please detail on the rear of this sheet.

A-Star
The UK-Irish Atopic Eczema Systemic Therapy Register
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A-STAR Bioresource shipping form
(to be attached to all shipments)

Southampton

Initials
Patient ID

Sample	CAPTURE sample ID	Total volume (ml)	Sample collection time and date	Processing instructions	Shipment instructions
☐ Blood (1 x EDTA for PBMC, purple top)				Was the tube inverted several times after collection? Y / N	Was sample kept at ambient temp until shipment? Y / N
☐ Blood (1 x Serum, gold top)				Was the tube inverted several times after collection? Y / N	Was sample kept at ambient temp until shipment? Y / N

Site Name | Date | Name of person who completed form

Ship to Kathleen Potter, Faculty of Medicine Tissue Bank Manager, University of Southampton, Somers Cancer Research Building, Mailpoint 824, Tremona Road, Southampton, SO16 6YD in Royal Mail Special Delivery Safebox™

NOTE: If this sample was exposed to any event that could compromise its integrity, or deviates from standard protocol, please detail on the rear of this sheet.

Samples for batch shipments

- Contact the Study co-ordinating Centre to arrange collection of batch shipments (A-star@gstt.nhs.uk)
- For samples that require batch shipping, as outlined in the sample collection schedule, use the destination specific shipping form.
- Samples to be shipped in dry ice or at ambient temperature according to instructions on shipping form.
- Samples should not be shipped on a Thursday or Friday or prior to a bank holiday weekend.
- All sections of the form must be completed and be included with the sample shipment, an example is included below

Batch shipping forms

A-STAR Bioresource sample shipping form
(to be attached to all shipments)

Batch shipment to Kings College London

Site Name | Date | Name of person who completed form

Ship to David Baudry, Skin Therapy Research Unit St. John's Institute of Dermatology, 9th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT on dry ice

NOTE: if any of these samples were exposed to any event that could compromise its integrity, or deviates from standard protocol, detail on rear of this sheet

			Blood (Serum aliquots)		**Skin swabs** (Non-lesional)		**Skin swabs** (Lesional)		**Tape Strips** (Non-lesional)	
A-STAR Participant ID	Patient Initials	Date sample taken	CAPTURE ***sample ID***	Shipped	CAPTURE ***sample ID***	Shipped	CAPTURE ***sample ID***	Shipped	CAPTURE ***sample ID***	Shipped

A-STAR Bioresource sample shipping form
(to be attached to all shipments)

Batch shipment to Newcastle University

Site Name | Date | Name of person who completed form

Ship to Jenny Lumley, Dermatological Sciences Room M2.217, Second Floor William Leech Building, Newcastle University, Framlington Place, Newcastle Upon Tyne, NE2 4HH.

NOTE: if any of these samples were exposed to any event that could compromise its integrity, or deviates from standard protocol, detail on rear of this sheet

			Dry Ice						**Ambient**			
			Blood (RNA, PAXgene)		**Skin Biopsy** (Non-lesional RNA later)		**Skin Biopsy** (Lesional RNA later)		**Skin Biopsy** (Non-lesional FFPE)		**Skin Biopsy** (Lesional FFPE)	
A-STAR Participant ID	Patient Initials	Date sample taken	CAPTURE ***sample ID***	Shipped	CAPTURE ***sample ID***	Shipped	CAPTURE ***sample ID***	Shipped	CAPTURE ***sample ID***	Shipped	CAPTURE ***sample ID***	Shipped

5 APPENDIX: BLOOD VOLUME GUIDELINES

Blood Volumes Allowed for Research Purposes

BASED ON BLOOD VOLUME OF:	**kg**	**mL/kg**
Pre-Term Infant	1 - 2	100
Term Infant - Adult	>2	80

Body Weight (KG)	**Body Weight (LBS)**	**Total Blood Volume (mL)**	**MAXIMUM ALLOWABLE BLOOD VOLUME (ML) IN ONE BLOOD DRAW (2.5% of total blood volume)**	**TOTAL VOLUME (CLINICAL + RESEARCH) MAXIMUM VOLUME (ML) DRAWN IN A 30-DAY PERIOD**
1	2.2	100	2.5	5
2	4.4	200	5	10
3	6.3	240	6	12
4	8.8	320	8	16
5	11	400	10	20
6	13.2	480	12	24
7	15.4	560	14	28
8	17.6	640	16	32
9	19.8	720	18	36
10	22	800	20	40
11 - 15	24 - 33	880 - 1200	22 - 30	44 - 60
16 - 20	35 - 44	1280 - 1600	32 - 40	64 - 80
21 - 25	46 - 55	1680 - 2000	42 - 50	64 - 100
26 - 30	57 - 66	2080 - 2400	52 - 60	104 - 120
31 - 35	67 - 77	2480 - 2800	62 - 70	124 - 140
36 - 40	78 - 88	2880 - 3200	72 - 80	144 - 160
41 - 45	89 - 99	3280 - 3600	82 - 90	164 - 180
46 - 50	100 - 110	3680 - 4000	92 - 100	184 - 200
51 - 55	112 - 121	4080 - 4400	102 - 110	204 - 220
56 - 60	123 - 132	4480 - 4800	112 - 120	224 - 240
61 - 65	134 - 143	4880 - 5200	122 - 130	244 - 260
68 - 70	145 - 154	5280 - 5600	132 - 140	264 - 280
71 - 75	156 - 185	5680 - 6000	142 - 150	284 - 300
76 - 80	167 - 176	6080 - 6400	152 - 160	304 - 360
81 - 85	178 - 187	6480 - 6800	162 - 170	324 - 340
86 - 90	189 - 198	6880 - 7200	172 - 180	344 - 360
91 - 95	200 - 209	7280 - 7600	182 - 190	364 - 380
96 - 100	211 - 220	7680 - 80000	192 - 200	384 - 400