

Standard Operating Procedures for Biological
Sample Collection and Storage
(IMAGEN-FU3)

**Standard Operating Procedure
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1. Sample collection

1.1. Sample collection summary

- Blood sample collection will be performed **after** neuroimaging. Blood samples will be processed into plasma, buffy coat (i.e., white blood cells; for DNA isolation) and red blood cells. Whole blood will also be collected in Tempus tubes for direct isolation of RNA.
- Hair sample collection will also be carried out **before blood collection** provided the participant had previously given consent.
- Samples should be labelled with a barcode containing the subject identifier.
- Information about the date of sample collection, visit number and sample type (e.g. plasma, buffy coat etc.) should be recorded. We suggest the use of coloured boxes or coloured tape and clear labelling to aid in distinguishing the sample types.
- Collection tubes and samples to be obtained:
 - 1 x 10ml blood in EDTA tube: for DNA
 - 1 x 10 ml blood in EDTA tube: for collection of plasma and buffy coat and red blood cells
 - 2 x 3 ml blood in Tempus tubes: for RNA isolation
 - 1 x 1.5cm diameter of hair from nape of the neck: for drug analysis

1.2. Materials

Blood collection kit:

- 1 x 23G butterfly needle
- 1 x Vacutainer needle holder
- 1 x Latex gloves
- 1 x Tourniquet
- Alcohol wipes
- Cotton wool
- Small plasters
- 1 x Sharps bin for used needle or needle/holder combination

Sample tubes:

- 2 x 10ml EDTA tubes (BD Vacutainer K2EDTA tube, lavender lid, Catalogue #367525)
- 2 x 3ml Tempus tube (Applied Biosystems Tempus tube, blue lid, Catalogue #4342792)

Hair collection kit:

- 1 x Scissors
- 1 x Alcohol wipe (isopropyl alcohol)
- 1 x Latex/latex-free gloves
- 1 x Collection foil
- 1 x Envelope

1.3. Obtaining samples

1.3.1. Requirements prior to collection:

- Blood and hair should be collected at the end of the last imaging session. The processing of blood samples need to be done right after collection and hence should be done at the end of the institute assessment to allow ample time for processing.
- Sample tubes should be labelled with the barcode prior to collection - **stick the labels long ways on the tube (so the barcode can be read)**.
- Cut off the extra white space of the barcode labels to fit on the cryovials (plasma, buffy coat and red blood cells).
- Hair sample should be taken **prior to blood samples** due to possible faintness following blood collection.
- Envelope should be labelled with the barcode prior to collection.
- **Remind participants not to use hair dyes, bleach or anti-dandruff shampoo on their hair for at least 3 days prior to institute visit.**

1.3.2. Details to be recorded:

- Samples collected (i.e. blood [which tubes, how many tubes and in which order they were filled]).
- Date and time the samples were taken.
- Last time the subject consumed food/drink.
- Any issues (e.g. very short hair, no hair).

Equipment

- The equipment used throughout the study for sample collection and storage should be the same as outlined in this protocol.
- Any significant variations in equipment must be recorded (e.g. blood tube number or type).
- Details of any new purchase of tubes should be recorded (e.g. lot/batch no. and expiry date).

1.4. Protocol for taking hair

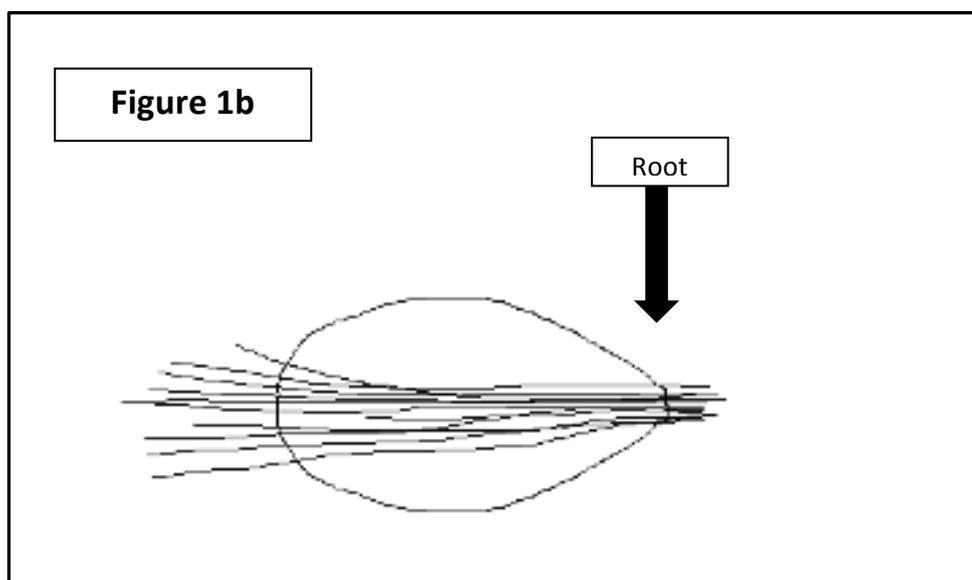
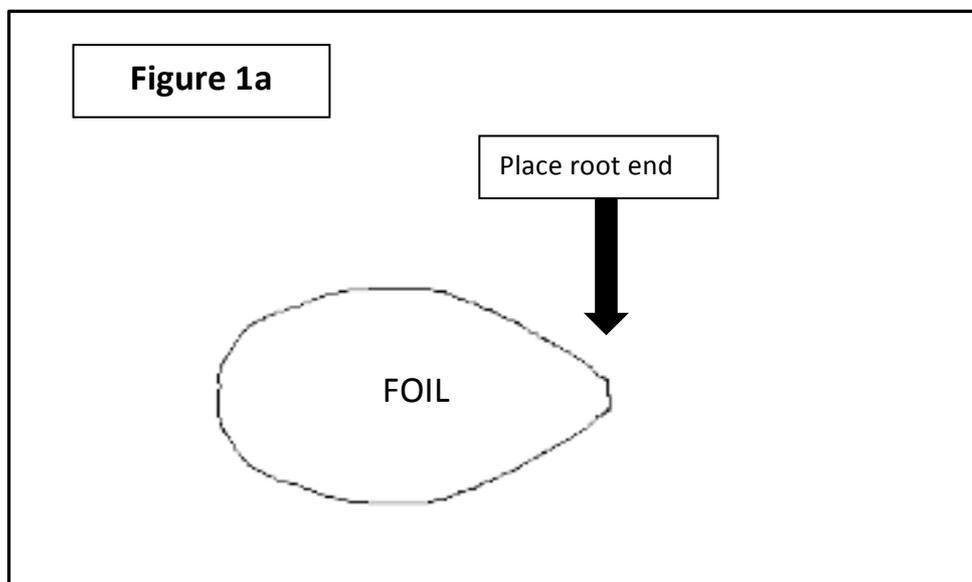
1.4.1 General precautions:

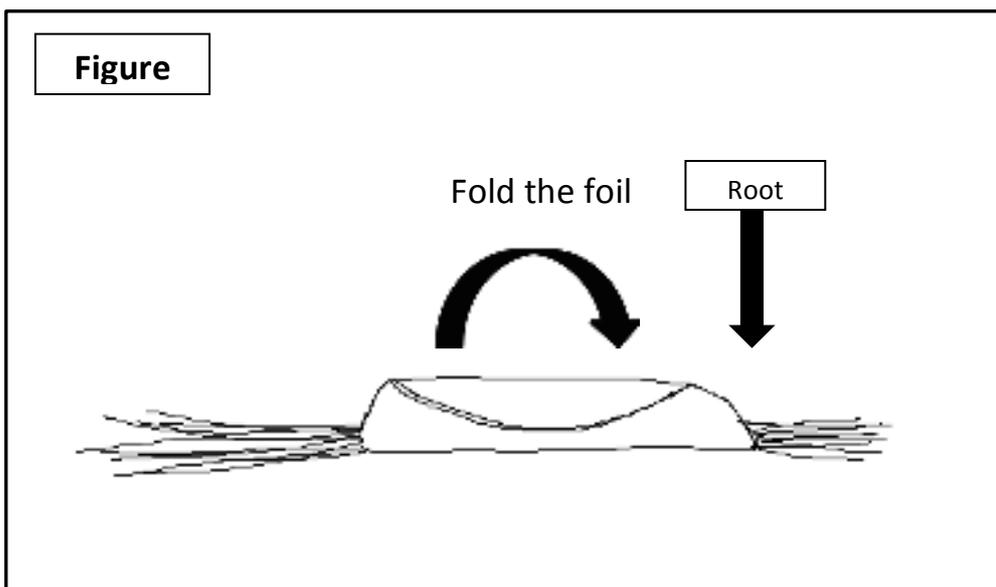
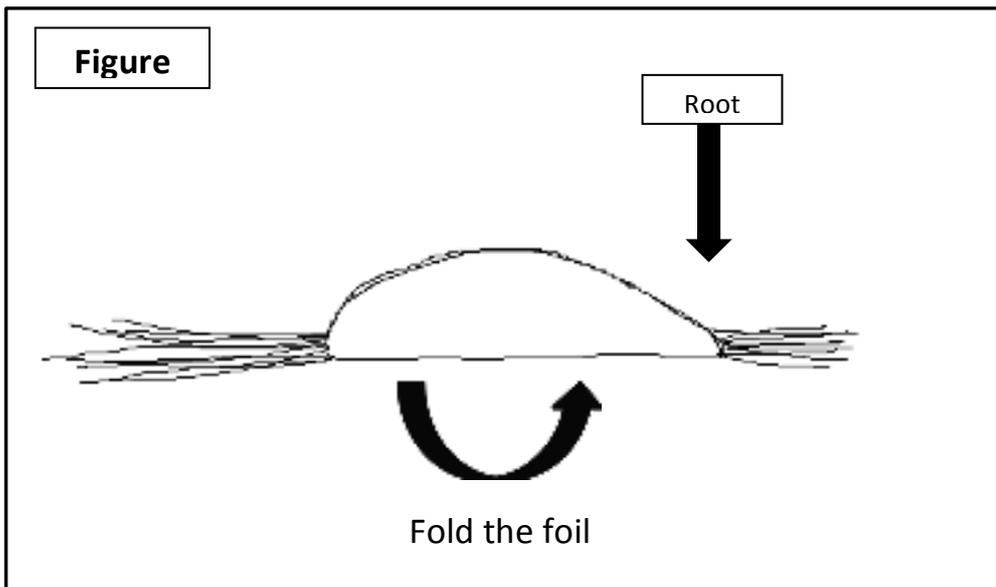
- Take precaution when using the scissors. Ensure that the participant is seated and still, and is aware of the scissors.

1.4.2 Guidelines for hair sampling procedure

- Gloves to be worn if preferred.
- Wipe the scissors with the alcohol wipe.
- Select a bunch of hair (ideally from the nape of the neck).
- Hair bunch thickness to be approximately equal to 1.5cm diameter (approximately 250-350 strands of hair).
- Cut the hair sample as close to the scalp as possible.
- Hair can be taken from multiple sites on the head if the participant prefers for cosmetic reasons, or if the hair is particularly thin.

- If there is no head hair, or very short (shorter than 0.5cm in length), hair can be taken from under the arm (armpit) if the participant is comfortable with this.
- Be careful to keep the root end of the hair strands together.
- Place full hair-length on collection foil, with the root end of all the hair strands pointing in the same direction, positioned at the tapered end of the collection foil (Figure 1a-b).
- Fold the collection foil in half along the centre line (Figure 1c).
- Fold the collection foil lengthwise again (Figure 1d).
- Place the foil in the envelope and seal the envelope.
- Ensure envelope is labelled with the date, time and where on the body the sample was taken from, and ensure the barcoded label is affixed.





1.4.3 Immediately following sample collection

- Hair to be placed on the foil and folded (Figure 1a-1d) and placed into sample envelope.
- Sample envelope to be sealed and labelled with the date, time, and barcode label.

1.5 Protocol for taking blood

1.5.1 General precautions:

- Gloves must be worn at all times when handling specimens. This includes during removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and clean up of any spills. Tubes, needles, and pipettes must be properly disposed of in biohazard containers, in accordance with institutional requirements.
- Contents of tubes that contain chemical additives may be irritating to eyes, respiratory system and skin.
- For safety information regarding sample tubes and risks associated with venepuncture please refer to the manufacturer's product guidelines and/or your institution's risk assessment.
- Universal precautions and institutional requirements should be followed, including gloves, eye protection or working in a biosafety cabinet for blood processing. All equipment (storage, shipping, and centrifuge) must be labeled as biohazard.
- It is important to take steps to prevent hemolysis in blood samples (see Figure 1). *In vitro* hemolysis can be caused by improper technique during collection of blood specimens (e.g., difficult collections, incorrect needle size, improper tube mixing and incorrectly filled tubes) or by the effects of mechanical processing of blood (e.g., too hot or too cold). This can cause inaccurate laboratory test results by contaminating the surrounding plasma with the contents of hemolyzed red blood cells. For example, the concentration of [potassium](#) inside red blood cells is much higher than in the plasma and so an elevated potassium level is usually found in biochemistry tests of hemolyzed blood.

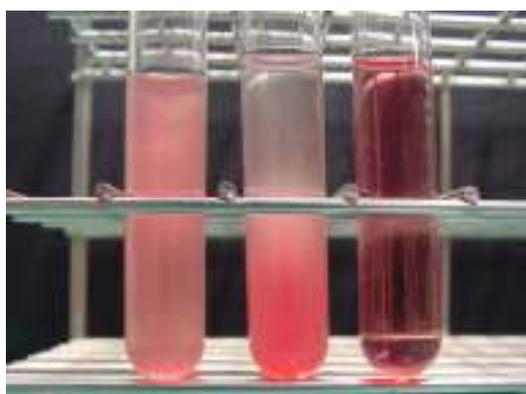


Figure 1: Hemolysis of blood samples: Red blood cells without (left and middle) and with (right) hemolysis. If as little as 0.5% of the red blood cells are hemolyzed, the released hemoglobin will cause the [plasma](#) to appear pale red or cherry red in color. Note that the hemolyzed sample is transparent, because there are no cells to scatter light. [By Y tambe - Y tambe's file, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=989846>].

Techniques to Prevent Hemolysis (which can interfere with many tests):

- Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
- Avoid drawing blood from a hematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venepuncture site is dry before proceeding with draw.
- Avoid a probing, traumatic venepuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute

is optimal).

- Avoid massaging, squeezing, or probing a site.
- Avoid excessive fist clenching.
- If blood flow into tube slows, adjust needle position to remain in the center of the lumen.

Preparation:

- Label the five sample tubes with the provided labels. The labels should be applied along the tube and **NOT around the tube.**
- Ensure subject is sat comfortably in a chair/lying down on a bed.
- Explain the procedure to the subject (to obtain consent and co-operation).
- Select the appropriate blood bottles and place within reach. Ensure sharps container and all necessary equipment are close to hand.
- Wash and thoroughly dry hands. Put on disposable gloves.
- Remove the cover from the valve section of the multi-sample needle.
- Thread the needle into the needle holder and ensure that the needle is firmly seated in the holder.

1.5.2. Guidelines for venepuncture

- Select a vein by observation and palpitation (lightly tapping the vein). Listen to the subject as they will often advise on the best or only site for venepuncture.
 - Place the tourniquet around the subject's upper arm or lower arm 10cm above the venepuncture site. Note: To avoid pinching of the skin, place the tourniquet over the subject's sleeve. Alternatively, place a cotton wool pad under the buckle of the tourniquet.
 - Ask the subject to clench their fist. If the vein is not visible or palpable, ask the subject to "pump hand" or dangle arms over hand rest of chair.
 - If still not visible, lightly tap antecubital fossa or dorsal surface of hand.
 - Disinfect the puncture site well with alcohol skin wipe and allow the skin to dry. Note: It is important that the skin is completely dry to avoid discomfort when inserting the needle.
 - Ask subject to relax hand/arm in a downward position.
 - Remove the cap from the needle, position the needle parallel with the vein with the bevel up and insert it into the vein.
 - Push the first tube into the needle holder and onto the needle valve, puncturing the rubber stopper. Hold the tube vertically, below the donor's arm during blood collection.
 - Release the tourniquet as soon as blood appears in the tube.
- Note: The tourniquet should not be applied for longer than one minute as this can affect blood samples.

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- Allow at least 10 seconds for a complete blood draw to take place. When the first tube is full and blood flow ceases, remove it from the holder and introduce the next tube into the holder. The order of draw should be:

1 x 10ml EDTA (lavender lid) for DNA

1 x 10ml EDTA (lavender lid) for plasma and buffy coat

2 x 3ml Tempus tube

Note: Always follow this protocol for order of draw

- After the last tube has been drawn and blood flow ceases, remove the tourniquet completely and withdraw the needle from the vein.
- Once the needle has been removed, apply finger pressure over cotton wool ball on puncture site to stop bleeding and to initiate clotting. Ask the subject to press for several minutes until the bleeding stops.

Note: Do not press too hard on the puncture site or bend the subject's arm as this may promote bruising.

- Apply a plaster over puncture site. Dispose of cotton wool ball into clinical waste bag.
- Remove the needle from the syringe and place in sharps bin.

Troubleshooting

Possible cause	Solution
<i>What to do if no blood flows into the tube</i>	
The bevel of the needle tip is sucked against the wall of the vein	Gently rotate the needle within the lumen of the vein
The needle penetrated the vein wall	Gently pull both the tube holder and the needle backwards
The needle is not fully within the vein	Gently push the needle forwards
The tourniquet was too tight or in place too long	Loosen the tourniquet
The tube was already used, or was previously opened	Dispose of and select a new tube
<i>What to do if blood flow ceases midway through collection</i>	
The tube was removed from the holder too soon	Reinsert the tube into the holder until the vacuum is totally depleted
Suction is too strong for the vein (collapsed vein)	Pull the tube out of the holder for a second and then reinsert it
The needle position has altered during the procedure, or the needle is outside the vein	Repeat venepuncture at different site when haematoma occurs

General notes

- If there are difficulties inserting the needle into a vein, try again but ensure the subject is at ease. Do not continue if the subject shows any signs of distress. Anxiety can lead to vasoconstriction of the vein, making it difficult to carry out venepuncture.
- If the subject feels faint, assist them to lie down if there is a couch available, or to lower their head to their knees. Increase ventilation to the room. If the subject seems to be having a panic attack, delay the phlebotomy procedure until they are calm. If there is danger of fitting or swallowing their tongue or vomit, place in recovery position and seek medical advice.
- If the subject shows signs of excessive bruising or haematoma following venepuncture, use a cold compress (cotton wool and cold water) to reduce swelling. If in doubt about cessation of bleeding or the subject becomes distressed, then seek medical advice.

1.5.3. Notes on EDTA tubes (lavender tops)

- Aim to completely fill all tubes. EDTA tubes contain a certain amount of anticoagulant that needs to be mixed in an exact proportion to the blood.
- These tubes contain additives (EDTA or the clot activator silica) that must be mixed for proper anticoagulant (EDTA).
- Since these tubes contain chemical additives, precautions should be taken to prevent possible backflow from the tubes during blood drawing.
- The EDTA tube used for **plasma and buffy coat** preparation **should NOT be the first blood sample** so it is important to label the EDTA tubes with the order in which they were taken.
- Tubes should be stored at 4-25°C (39-77°F).
- Tubes should not be used beyond the designated expiration date.

1.5.4. Notes on Tempus tubes

- With Tempus™ Blood RNA Tube, the blood is drawn directly into a reagent that stabilizes RNA at room temperature for up to five days.
- Do not use the Tempus tubes if they are discolored or contain precipitates
- Do not use the Tempus tubes after their expiration date.
- Filling up the tube to the black mark on the tube label indicates the collection of approximately 3 mL of blood.
- Draw blood directly into Tempus Blood RNA Tubes, shake vigorously or vortex for 10 sec and store it immediately at -80°C.

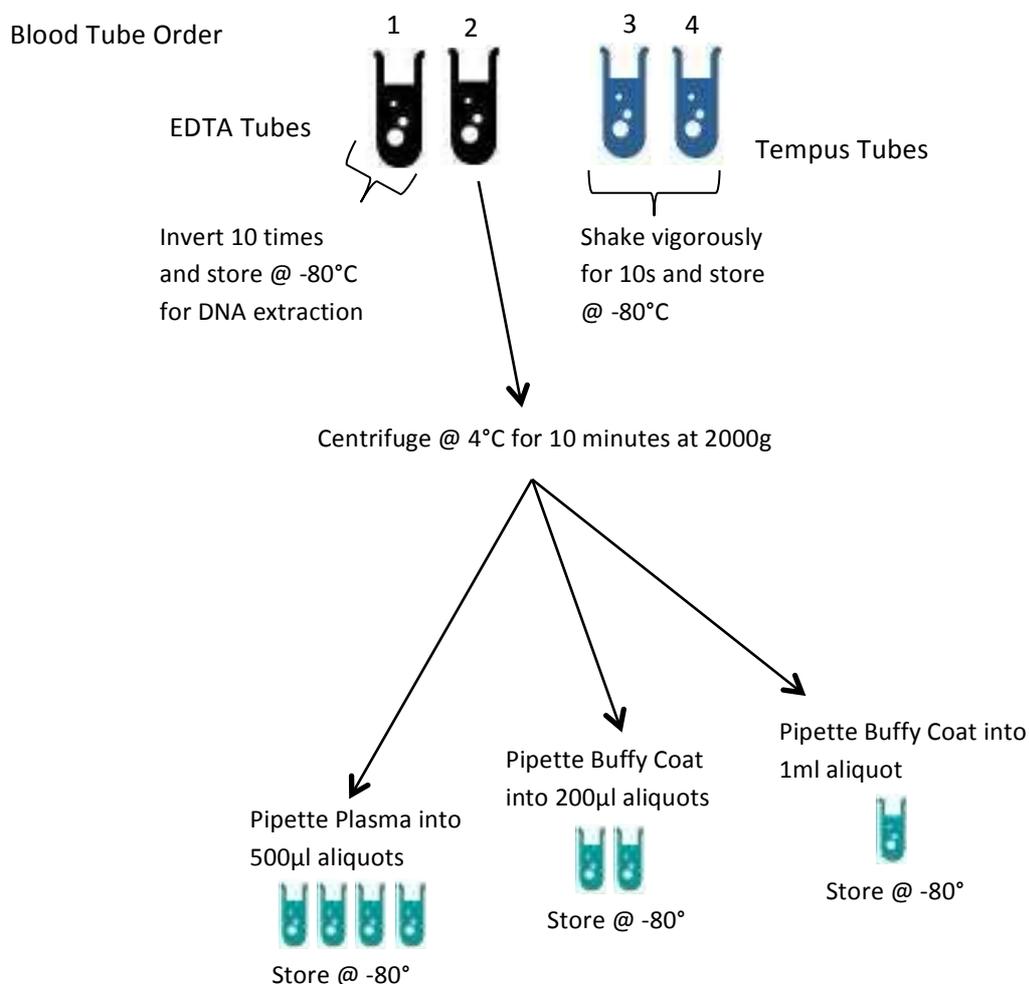
1.5.5 **Immediately following sample collection.**

- **EDTA tubes:** it is imperative to **gently invert** the EDTA (lavender lid) blood tubes **at least 10 times. Do NOT shake.** Afterwards, store the vacutainer tubes upright at 4°C until centrifugation. These **blood samples should be centrifuged within two**

hours of blood collection and process into aliquots immediately.

- **Tempus tubes:** shake vigorously or vortex the Tempus tube for 10s.
- Safely discard the used needle holder and syringe into the sharps bin.
- Ensure all sample tubes are labeled with the date, subject code and the study visit number.
- The time of phlebotomy, processing and final storage should be logged as well as the date and any unusual conditions in the lab (failure of temperature control etc.)
- Wash hands thoroughly
- Samples should be moved between laboratories in a sealed container.

Blood taking SOP overview



2. Sample processing

The time elapsed between the taking of the blood and sample processing must be recorded for all samples

Instructions for removal of BD Hemogard closure:

- Grasp the blood tube with one hand, placing the thumb under the closure. With the other hand, twist the closure while simultaneously pushing up with the thumb of the other hand, only until the tube stopper is loosened.
- Move thumb away before lifting closure. Caution: Do not use thumb to push closure off tube. If the tube contains blood, an exposure hazard exists. Lift closure off tube. In the unlikely event of the plastic shield separating from the rubber stopper, do not reassemble closure. Carefully remove rubber stopper from tube.

2.1 Equipment list

- 1 x EDTA tube for plasma and buffy coat (lavender lid), not the first tube drawn
- Centrifuge with swinging bucket rotor
- Sterile screw-cap cryotubes (1,5 or 2 ml; e.g., VWR, Ref. 720-0516)
- Filter pipette tips
- Small ice bucket
- General lab equipment

2.2 Requirements

The samples should be processed as soon as possible after blood taking and within 2 hours of blood collection. Label the cryotubes with the barcode labels provided. Ensure the barcode is **long ways**. If the barcode label is too long, cut off the white space on the barcode label until it fits on the cryotube.

2.3 Plasma and Buffy Coat preparation from the EDTA tube

This protocol describes the isolation of plasma, white blood cells and red blood cells from whole blood.

Procedure:

- After collection, gently mix the blood by inverting the tube 8 to 10 times. Store the vacutainer tubes upright at 4°C until centrifugation. Maintain sample at 4°C throughout processing.
- Centrifuge the sample in a horizontal rotor (swing-out head) at 2000g for 10 min, at 4°C. This causes separation of the sample into 3 distinct phases: the upper layer is the plasma (contains clotting factors), the narrow middle layer is the 'buffy coat'

(white blood cells), and the bottom layer is the red blood cells.

Warning: Excessive centrifuge speed (over 2000 g) may cause tube breakage and exposure to blood and possible injury. If needed, RCF for a centrifuge can be calculated. For an on-line calculator tool, please refer to:

<http://www.sciencegateway.org/tools/rotor.htm>

- After centrifugation, the plasma layer will be at the top of the tube. Mononuclear cells and platelets will be in a whitish layer, called the “buffy coat”, just under the plasma and above the red blood cells (Figure 2).

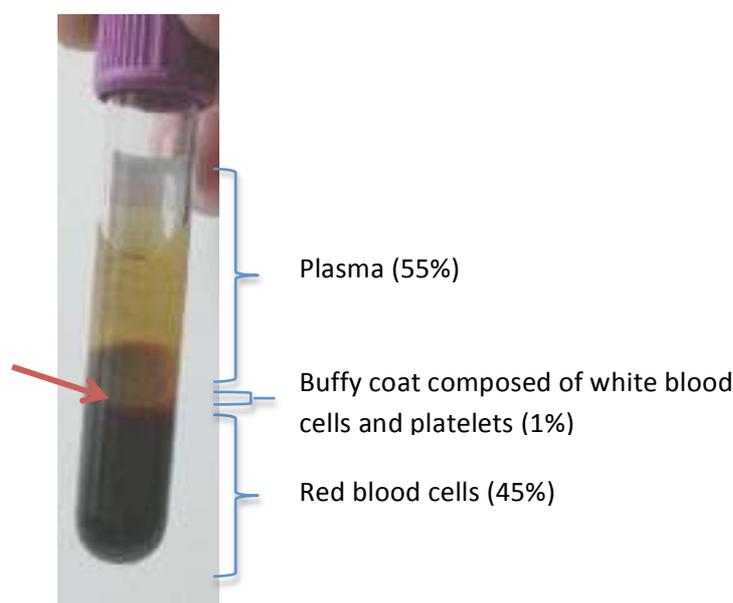


Figure 2: This is what the tube should look like after centrifugation

- **Collecting the plasma:** Carefully collect the plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer. Do not take all the plasma; collection of all plasma may cause contamination with the underlying buffy coat and red blood cell layers. If more than one tube is collected, pool the plasma samples into a 15 ml conical tube and mix. Pipette the plasma into 500 μ l aliquots in labeled cryovials. Fill up to four cryovials and dispose the rest into the appropriate waste bin. Close the caps tightly and place on ice.
- **Collecting the buffy coat:** Using a cut-off 1ml pipette tip, collect the buffy coat layer (Figure 1, red arrow) into a separate cryotube and mix by pipetting up and down a number of times. The resulting sample will be enriched for white blood cells, but will also contain some of the overlying plasma and underlying red blood cells. Aliquot this into 2 appropriately labeled cryovials, approximately 200 μ l in each (i.e., 2 aliquots/subject). Close the caps tightly and place the cryovials on ice.

- **Collecting red blood cells:** Take 1 ml of red cells and pipette into a labeled cryotube, close the cap tightly and place the cryovial on ice.
- **All processing should be completed within 1 hour of centrifugation.**
- Check that all aliquot vial caps are secure and that all vials are labeled. Place all aliquots upright in a specimen boxes (i.e., 10x10 boxes (VWR 211-9001), using different boxes for the plasma and the buffycoats and freeze immediately at -80°C. All specimens should remain at -80°C prior to shipping. The samples should not be thawed prior to shipping.
- Samples will be shipped on dry ice. Refer to “Shipping” instructions, below.

2.5 Processing of Tempus RNA tubes

- Samples should be vigorously mixed for 10 secs and stored immediately in a -80°C freezer.

3. Storage following sample collection

- Hair samples in the sealed envelopes should be stored together in a dry container, away from direct sunlight.
- Hair samples should be stored at room temperature. **Do not freeze.**
- Hair samples should be stored in a safe-place – ideally a locked cabinet or container.
- Blood samples should be sorted and labeled with a barcode containing the subject identifier, information about the date of sample collection, visit number and sample type (e.g. plasma, buffy coat, etc.), and assigned boxes, coloured and numbered to aid location in the freezers, should be recorded. We suggest the use of coloured boxes or coloured stickers with clear labels to aid in distinguishing the sample types.
- Blood samples should be stored at -80 °C in:
 - 10 x 10 specimen boxes for cryovials (aliquots buffy coat, plasma and red blood cells)
 - Or kept upright in racks for collection tubes (blood sample in EDTA tube and blood samples in Tempus tubes).

4. Data to be recorded:

For each type of biological sample to be collected, the following needs to be recorded (in an excel file):

1. Subject ID
2. Type of samples collected (plasma, buffy coat, red blood cells, blood clot,

Tempus blood, or hair sample).

3. Date and time of sample collection.
4. Last time the subject consumed food/drink.
5. Number and volume of aliquots prepared.
6. Date and time into -80°C.
7. Date and time of shipping.
8. Any freeze-thaw that occurs with a sample for any reason.
9. Any variations or deviations from the SOP, problems, or issues (e.g. very short hair, no hair).

5. Shipping instructions

- Return all hair samples to Clinical Educational & Health Psychology, UCL, London every three months or when there are a sufficient number of samples.
- Hair samples should be posted in the packaging provided by UCL. Samples to be sent via recorded/tracked delivery (no specific requirements of temperature control but **do not freeze**).
- Return all blood samples to the Institute of Psychiatry, London every three months.
- Blood samples should be packaged in a manner that prevents breakage or leakage (e.g., in cardboard boxes). Samples in collection tubes may be wrapped individually, or a rack of tubes may be wrapped together (note: test tube racks will not be sent back). A few tubes may be wrapped together in alternate ways (e.g. in a bubble-wrap envelope), provided that they will not come loose and break during shipment. **Important: do not freeze collection tubes in a Styrofoam tray** as this may cause the tubes to crack, particularly when shipping the samples to London.
- Blood samples should be shipped with sufficient dry ice to ensure samples do not thaw during transport. **Important: avoid direct contact of samples with dry ice.**
- Please ensure that the delivery company used has the capacity to ship dry ice.
- Shipment address **for hair**:
 Claire Mokrysz
 Clinical Educational & Health Psychology,
 University College London,
 1-19 Torrington Place,
 London WC1E 7HB, UK
- Shipment address **for blood**:
 Nicole Tay
 MRC-SGDP Centre,
 Institute of Psychiatry,
 16 De Crespigny Park,
 London SE5 8AF, UK.

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- **Please notify London when you intend to make a shipment and provide information regarding delivery date. It is important for the London site to know this so that they may arrange for staff to process the samples.** Please also provide information regarding which kind of samples the site will be receiving

6. Additional Information

The equipment listed below will be provided by London:

- 2 x EDTA Tubes
- 2 x Tempus Tubes
- 7 x Cryovials (for aliquots)
- Butterfly needle with pre-attached holder (BD Vacutainer® Safety-Lok™ Blood Collection Set)
- Labels
- Hair collection Kit (provided by UCL)

Please notify London if you require any additional equipment (i.e., more tubes or more labels). Please provide your shipping address to Nicole (nicole.tay@kcl.ac.uk) and the equipment required before the start of your Institute Assessment.