Standard Operating Procedure (SOP)

Work Package 8

Sample Collection and Storage

May 2008

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1 Sample collection

1.1 Sample collection -summary

- • Sample collection to be taken at one time point.

Samples to be obtained:

- • 2 x 10ml EDTA tube for DNA
- • 1x 4ml EDTA tube for blood count
- • 1 x 10ml EDTA tube for plasma and buffy coat (to be cryopreserved)
- • 2 x 2.5ml PAXgene tube for RNA

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

- • 3 x 10ml EDTA tube
  (BD Vacutainer EDTA tube, lavender lid, Catalogue #367525)
- 1 x 4ml EDTA tube
  (BD Vacutainer EDTA tube, lavender lid, Catalogue #368860)
- • 2 x 2.5ml PAXgene tube
  (BD, Paxgene blood RNA tube, Catalogue #762165)
- Bar-coded labels *(the IMAGEN labels for the blood, plasma and buffycoats are the smaller label with the long transparent plastic strip attached, stick the labels long ways on the tube (so the barcode can be read) and wrap the plastic strip around the label to ensure the label does not come off in the freezer)*

1.3 Obtaining samples

1.3.1 Requirements prior to collection

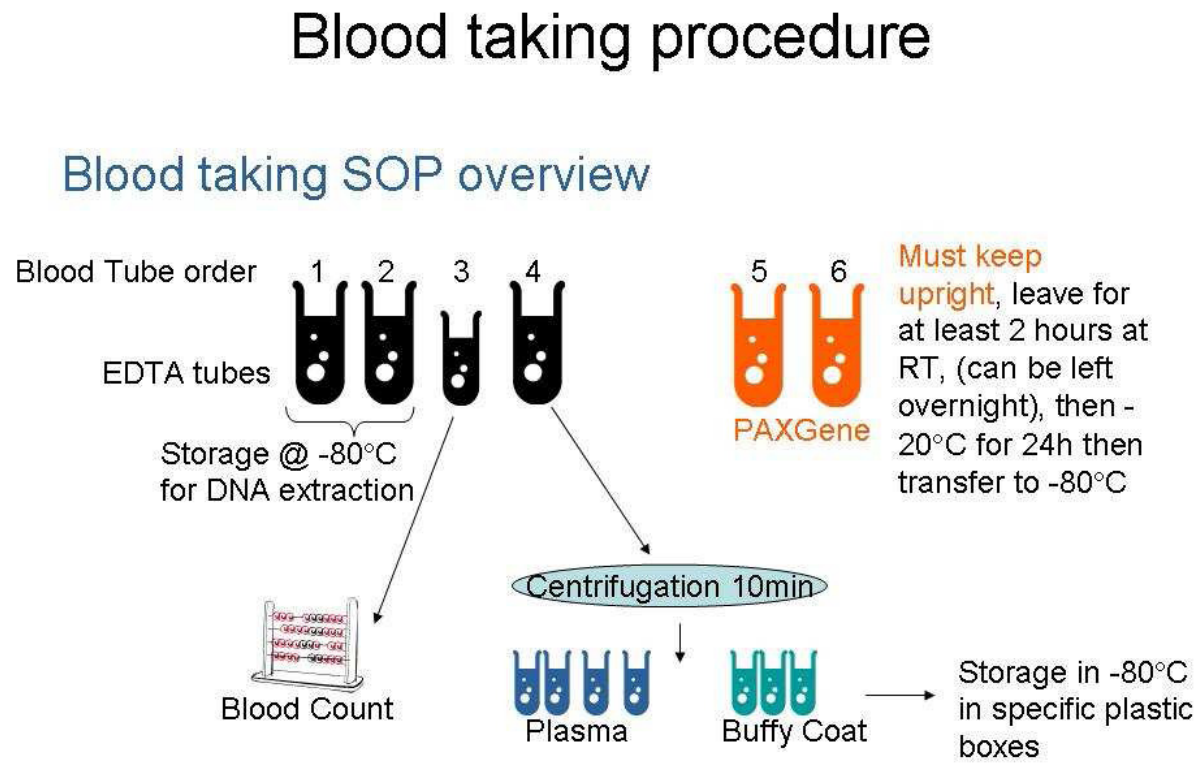
- Bloods should be taken at the end of the last imaging session.
- Sample tubes should be labelled with the bar-code prior to blood collection.

1.3.2 Details to be recorded:

- Samples collected (i.e. blood [which tubes and how many and in which order]).
- Date and time the samples were taken (blood).
- Last time the subject consumed food/drink.

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 blood

1.4.1 Guidelines for venepuncture procedure

Preparation (see complete SOP for a more detailed description of this procedure)

1.4.2 Label tubes with corresponding bar-coded label

1.4.3 Notes on EDTA tube(s)

- 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.
- Since the EDTA 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.

1.4.4 Notes on PAXgene tube(s)

- Ensure that the PAXgene RNA tube is at **18-25 ^{0}C prior to use.**
- If the PAXgene tube is the only tube to be drawn, blood should be drawn into a 'discard tube' prior to drawing blood into the PAXgene tube. Otherwise, the PAXgene tube should be the **last** tube drawn in the phlebotomy procedure.
- The PAXgene tube should be kept upright.

Note. Aim to completely fill the PAXgene tube. It contains 6.9 ml of additive to stabilize blood RNA. It yields a ratio of 2.76 ml of additive/ml blood when the evacuated tube is filled correctly to its 2.5 ml draw volume.

1.5 Precautions

- 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.

1.6 Venepuncture

When the first tube is full and blood flow ceases, remove it from the holder and introduce the next vacutainer into the holder. The order of draw should be:

(1) 2 x 10ml EDTA (lavender lid) for DNA
(2) 1 x 4ml blood tube for blood count
(3) 1x 10ml EDTA for plasma and buffy coat
(4) 2 x 2.5ml PAXgene tube
NB: The sample tubes contain additives. It is important to follow the correct order of draw to prevent contamination of samples.

1.7 Immediately following sample collection.

- It is imperative to **gently invert** the EDTA (lavender lid) and PAXgene tubes **at least 10 times** to reach a proper mix of additive and blood. **Do NOT shake**.
- PAXgene tubes should be kept upright after use.
- Safely discard the used needle holder and syringe into the sharps bin.
- Ensure sample tubes are labelled 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.)
- Send all blood samples to the local processing laboratory, 1 x 10ml EDTA sample is required for plasma and buffy coat preparation immediately after blood taking, ideally this process should be performed as soon as possible, see section 2 for details of this procedure.
- Send the small EDTA 4ml tube to the appropriate laboratory for blood count.
- Samples should be moved between laboratories in a sealed container.

2 Sample processing

2.1 Plasma and Buffy Coat preparation

2.1.1 Equipment list

- 1 x EDTA tube for plasma and Buffy coat (lavender lid), not the first tube drawn
- 4 x Blue tube lid cryotube (Sarstedt, Ref. #65.716.001)
- 5-10 x Red tube lids cryotube (Sarstedt, Ref. #65.716.003)
- Filter pipette tips
- General lab equipment
- Dimethyl Sulfoxide (DMSO)
  Fetal calf (bovine) serum (FCS) (Sigma F7524) (Ideally should be heated to 56°C for 30min to ensure inactivation of complement, then can be stored in 50ml aliquots at -20°C for long term storage, you can also buy heat inactivated FCS (Sigma F9665-500ML).

2.1.2 Requirements

- The sample should be processed as soon as possible after blood taking and within 2 hours of the blood draw. This EDTA sample MUST NOT be the first blood sample taken.
- The time elapsed between the taking of the blood and sample processing must be recorded for this sample

2.1.3 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.

2.1.4 Procedure

- Maintain sample at room temperature (18-22 °C) throughout processing.
- The sample needs to be processed in sterile conditions using aseptic technique
- Upon arrival at the lab, centrifuge the sample at 2000g for 10 min, at room temperature. 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.

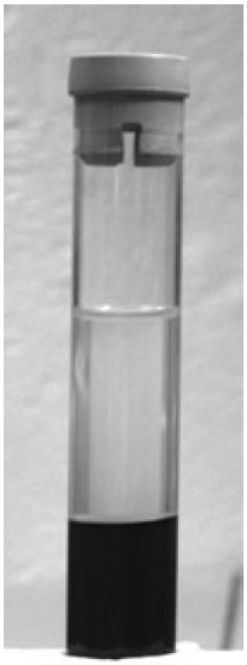


Figure1. This is what the tube should look like after centrifuging

- Label cryotubes with barcode label (ensure barcode is long ways)
- Aliquot the plasma into 2 ml flat-bottom screw-cap microcentrifuge tubes, and cap (1 ml serum/centrifuge tube; up to 5 aliquots/plasma tube). Do not take all the plasma; collection of all plasma may cause contamination with the underlying buffy coat and red blood cell layers.

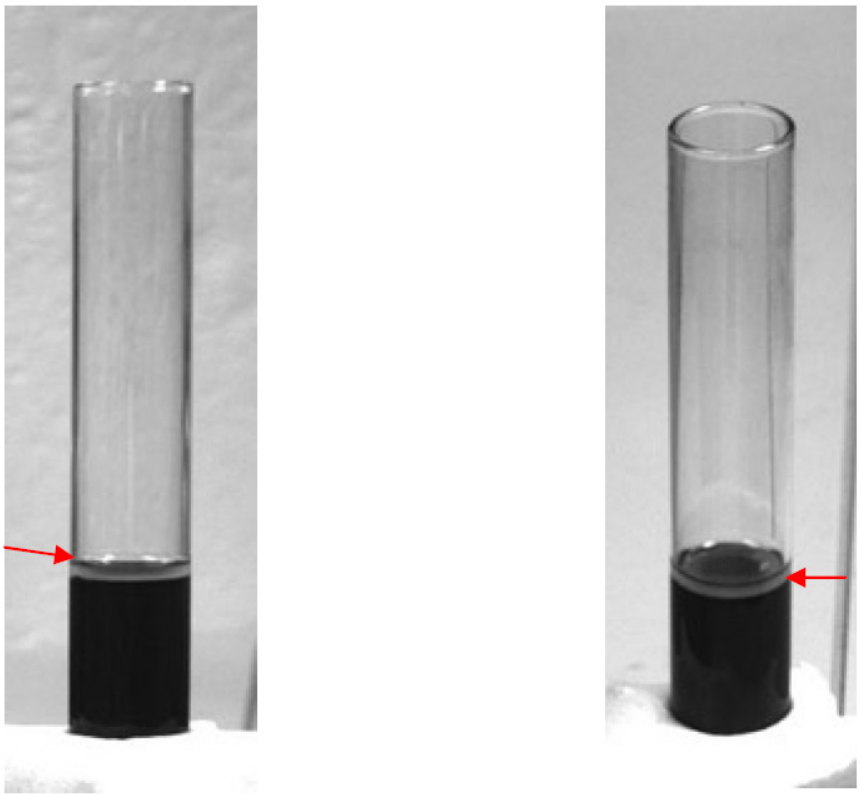


Figure 2. After removal of the plasma the buffy coat layer is left (red arrow)

- Using a cut-off 1ml pipette tip, collect the buffy coat layer into a separate 2 ml 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.
- Split the buffy coat into three separate blue-lid cryo tubes.
- Add ~100µl of 90% FCS and 10% DMSO to the tubes to aid cryopreservation (to save time you can make a master mix for instance for 2 samples make up 540µl of FCS and 60µl of DMSO and then add 100µl to each cryo tube).
- freeze immediately at -20°C for 25 minutes, or alternatively place in a "Mr. Frosty" freezing container filled with isopropanol, (available from VWR, cat no. 55710-200) in the -80°C.
- Transfer to -80 and eventually to liquid nitrogen for long term storage.
- If possible store tubes in 10x10 boxes (VWR 211-9001), a separate one for thebuffycoats and the plasma would be ideal.

2.2 PAXgene RNA tubes

- Upon arrival in the lab, samples should be maintained at room temperature (18-22 °C) for 2 hours before transferring to freezer, in an upright position. (*Note Paxgene tubes can be left for up to 72hours at room temperature prior to freezing, however this is not ideal but if this cannot be avoided (for instance late blood collection on a Friday) please just make a note on the CRF).*
- PAXgene tubes should then be frozen at -20 °C, upright in wire rack.
- Do not freeze in a Styrofoam tray as this may cause the tubes to crack.
- Following 24 h at -20 °C, transfer tubes to -80 °C.
- All times of incubations and freezer transfers should be documented.

2.2.1 General precautions

- For safety information please refer to your institution's risk assessment or to the manufacturer's product guidelines.

3 Sample storage

3.1 General guidelines

- For immediate storage, aliquoted samples and EDTA tubes for DNA need to be labelled with subject identifier, date of sample collection, visit number and sample type (e.g. serum, plasma), and assigned box numbers to aid location in the freezers.

3.2 Protocol for sample storage

3.2.1 Whole blood (for DNA)

- Frozen at -80 ^{0}C.

3.2.2 Plasma

- Frozen at -80 ^{0}C.

3.2.3 Buffy coat

- Frozen at -80 ^{0}C.

3.2.4 PAXgene tube

- PAXgene tubes should be frozen at -20 ^{0}C, upright in wire rack.
- Do not freeze in a Styrofoam tray as this may cause the tubes to crack.
- Following 24 h at -20 ^{0}C, transfer tubes to -80 ^{0}C.

4 Sample transport

- Samples should be sent **every three months** or when you have a sufficient number of samples and packaged appropriately with **sufficient dry ice** to ensure samples do not thaw and packaged in a manner that prevents breakage or leakage.
- Return all samples to the Institute of Psychiatry, London

  Shipment address: Ms. Emma Dempster, MRC-SGDP Centre, PO82, Institute of Psychiatry, 16 De Crespigny Park, London SE5 8AF, UK.