Appendix 9.a
Standard Operating Procedure (SOP) for Samples Preparation and Processing: Blood

Version 3.2 – October 2019

1 Introduction

The purpose of this SOP is to describe the instructions for the collection, processing and storage of peripheral blood samples for the HEADSpAcE study.

2 Objective

The purpose of this SOP is to define the procedure and establish the basic quality guidelines with respect to the collection of peripheral blood samples to be processed into fractions and stored at the participant institutions until DNA extraction for further genetic/epigenetic analysis.

3 Required equipment/material

- Centrifuge
- Fridge and cold pack for transport (4°C)
- Pipettes and tips
- Blood (per subject)
- 1x 5 ml Serum-separating tube (SST) for serum
- 1x10 ml vacutainer tubes with EDTA as an anticoagulant (BD Vacutainer® K2E) for plasma and buffy coat
    - ml cryotubes (colour coded with **red, green, white and yellow tops**)
- 4.5 ml cryotubes

Note: Each centre should contact IARC if they experience any problems in obtaining either Vacutainer tubes or cryotubes. These can be provided by IARC.

4 Blood extraction

- Approximately 10ml of blood should be collected in the EDTA tubes. 10 ml of blood will be divided into whole blood, plasma, leucocytes/platelets (buffy coat) and red blood cells (RBC).
- 5ml of blood should be collected in the SST tubes. This will be used to recover serum.

5 Blood processing

5.1 Short-term storage and transport

Blood samples in tubes must be stored and transported at 4°C until processing, for no longer than 3 hours after collection. **Ideally, blood should be processed within 2 hours.**

5.2 Yield

The protocol below describes blood processing for storage of buffy coat, plasma and serum.

From 10ml of blood with EDTA, the following should be obtained:

- 1 tube of whole blood (4.5 ml tube)
- 2 tubes of plasma (in **yellow top** 1.8ml cryotubes)
- 1 tube of buffy coat (in **white top** 1.8ml cryotubes)
- 2 tubes of RBC (in **red top** 1.8ml cryotubes)
- 1 tube with 1.0 ml of serum (in **green top** 1.8ml cryotubes)

5.2.1 Blood

Blood will be processed and frozen under the following protocol:

1. Pipette **4 ml of whole blood** from the vacutainer tube before centrifugation and transfer to the **4.5 ml cryotube**.
2. Centrifuge the remaining blood in the vacutainer for **10 minutes at 3500 x rpm and 4°C.**
3. Three layers will be obtained. The upper layer (plasma) is generally clear and pale yellow in colour. The second layer is a narrow greyish white interface band representing the "buffy coat" fraction. The third or bottom layer is dark red and consists of the RBC.

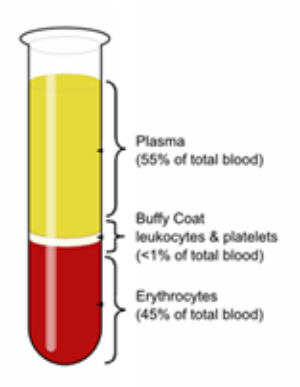

Fig 1. Representation of blood fractions after EDTA tube centrifugation.

5.2.2 Plasma

- Using an appropriate disposable transfer pipette, aspirate off the plasma layer (uppermost yellow layer, about 50-60% of the blood sample) down to approximately 1 mm from the buffy coat layer. Take care not to disturb the buffy coat layer.
- Aliquot recovered **plasma** into 2 cryotubes with **yellow top (~1.8ml each)**.

5.2.3 Buffy coat

- After removing the plasma layer, use a transfer pipette to collect the top 2ml of the remaining specimen making sure that the buffy coat is included. *Note that this layer is sometimes hard to visualize, which is why it is better to include some plasma and RBC.*
- Expel the **buffy coat** into a single cryotube with **white top**.

5.2.4 RBC

- After removing the buffy coat, use a transfer pipette to aspirate the remaining RBC layer.
- Aliquot recovered **RBC layer** into 2 cryotubes with **red top**.

5.2.5 Serum

- Using an appropriate disposable transfer pipette, aspirate off the serum layer above the gel from the serum-separating tube (SST).
- Aliquot recovered serum (~1.0ml) into 1 cryotube with **green top**.

Label each tube with appropriate barcoded label. Labels will be provided by IARC, and include readable subject's full identification number, the type of sample, and a barcode.

5.3 Long-term sample storage

All samples should be stored at -80ºC. Cryotubes should be stored in the white **carton boxes** provided with the tubes. **Record the number and the type of stored sample** in the database provided by IARC (see logsheet). Enter storage location in your local tracking system.

If possible**, store whole blood in a separate freezer** than processed samples; this will avoid the loss of all material in case of any problem with one of the freezers.