

STANDARD OPERATING PROCEDURE 5**TITLE: FRACTIONATING BLOOD INTO SERA, PLASMA & WHOLE BLOOD CELL PELLETS FROM HIV-INFECTED INDIVIDUALS**

Purpose	The purpose of this SOP is to describe the procedure to be followed to fractionate blood from HIV-infected individuals and healthy controls into plasma and blood cell fractions.
Scope	BioBank staff
Responsibilities	HSE, HTA and local rules (e.g. CL3)
Equipment	Centrifuge, Automated pipette, Gilson pipettes
Consumables	1.5ml and 50ml centrifuge tubes, 5ml sterile pipettes, sterile filter P1000 tips

General Procedures Before Processing Blood

1. Biobottles holding EDTA & SST tubes will be removed from the cardboard boxes & taken into the Class II safety cabinets in room 3.27 of the CL3 facility. Samples will be inspected for spillage by removal of the Biobottle top & by inspection of the clear plastic bags. In the event of leakage, the spill procedure will be enacted (SOP4).
3. 'MSC' refers to the Class II microbiological safety cabinet. All processing of blood will be carried out in an MSC within the CL3 unit and the working surface of the MSC wiped down with 70% IMS before use.
4. A 'waste pot' containing 50% (V/V) P3 Sterile diluted with tap water (one-third to half-full) should be made up for use in the MSC and an autoclave bag used as a liner for the dry waste bucket within the MSC.
5. The Biobank booking in form should be filled out and samples logged on datasheet. Details include: patient number/date sample collected/date and time of processing/time of storage/initials of person who processed the sample.

Minimising Cross-Contamination

- Spray gloved hands with 70% IMS between samples, wipe down the Gilson pipette with 70% IMS between samples and wipe down all surfaces of the MSC with 70% IMS between samples.
- Do not process blood from control volunteers with blood from infected donors at the same time.

Procedure: Fractionating Blood Into Sera, Plasma & Whole Blood Cell Pellets

1. Inside the MSC, place each unopened vacutainer containing blood in a re-usable 50ml conical tube. Cap the tubes and balance *in situ* in the centrifuge buckets. Seal the centrifuge buckets and transfer to the rotor and centrifuge the samples at 3000 rpm, 10mins at room temperature.

2. Remove the sealed centrifuge buckets to the MSC and inspect for spillage. If leakage is observed, enact spillage procedure (SOP 4), if not remove the 50ml centrifuge tubes, extract the vacutainers with a spatula and place in a rack inside the MSC.
3. Open the vacutainer & remove the plasma fraction (top layer) from the EDTA-vacutainers with a sterile 5ml pipette (leaving the buffy coat layer intact. Pool into a sterile 15/50ml conical tube & gently mix.
4. Aliquot the plasma fraction in 1ml volumes into labelled 1.5ml Eppendorf tubes for storage.
5. Finally, gently mixing the remaining fraction using a P1000 and sterile tip. From 2x EDTA-vacutainers, aliquot 1ml volumes of the blood into labelled 1.5ml Eppendorf tubes for storage.
6. Place all aliquots into the -80c freezer.

General Procedures After Processing Blood

1. Seal the autoclave bag containing dry waste with autoclave tape, remove from the MSC and double bag. Add an indicator strip to the bag and leave for autoclaving.
2. Mix the contents of the waste pot by inversion and remove the waste pot to the sink area. Leave the waste pot for a minimum of 24hours before opening. Tip the waste into the strainer provided, allow to drain and then place in an autoclave bag. Leave the bag for autoclaving after sealing, double bagging and adding an indicator strip as above.
3. Wipe down pipettes with 70% IMS. Remove all equipment & consumables from the MSC and wipe down the working surface with 70% IMS when finished.

Health & Safety

1. All processing of infected blood will take place in the CL3 unit within the Department of Infectious Diseases. Staff involved with the BioBank will strictly adhere to the in-house CL3 unit Codes of Safe Working Practice. Staff will wear protective gowns/jumpsuits (including covering of the shoes), eye-protection, mask and be double gloved in the CL3 ante- room (Room 3.25). Upon leaving the CL3 lab, the external glove layer will be discarded into autoclave bags immediately next to the exit door and the inner gloves wiped with 70% IMS.
2. All BioBank staff working with infectious material will be trained by senior personnel in CL3 processes and will be immunised against hepatitis B by KCL Occupational Health. All staff will read the CL3 Codes of Safe Working Practice, this Standard Operating Procedure and the risk assessment, and sign to confirm that they have done so.

Cross Referenced SOPS

SOP2: Tracking samples, SOP4: Spillage Procedure, SOP7: Isolation of Viable Lymphocytes, CL3 codes of practice