

Handling and Processing of Whole Blood Samples.

1.0 Purpose and scope.

Blood samples may be collected from patients to assess various metabolites, for DNA extraction from the white cells found as a normal component of the buffy layer, for isolation of leukocytes either for culture or analysis by flow cytometry. Serum and plasma samples may also be used from a whole blood sample.

2.0 COSHH / Health & Safety

Departmental COSHH/BioCOSHH forms/ Risk assessment should be performed to assess any potential hazards.

3.0 Equipment / reagents

Class II safety cabinet
Pre-cooled centrifuge
EDTA tubes
Heparin tubes
Polypropylene centrifuge tubes
Universal tubes
Lymphoprep
PBS
Cell Lysis buffer
Nuclear Lysis buffer
Rotary Mixer
Disposable Pasteur Pipettes
Water bath
Sodium perchlorate
Chloroform
Ethanol (stored at -20°C)
Eppendorfs
Sterile water
Bleach

3.1 Sample Collection.

Collect blood in labelled sodium EDTA or heparin tubes as appropriate, invert tubes gently to mix. Transport tubes on ice to the laboratory for processing.

3.2 Sample Storage.

Prepared aliquots from whole blood should be stored at -80°C.

4.0 References

4.1 HTA

4.2 University/Faculty Policy

5.0 Procedure

DNA Extraction

1. Collect blood in sodium EDTA tubes, pre-cool centrifuge to 4°C.
2. Switch on class II safety cabinet in designated class II room.
3. In a 50ml polypropylene centrifuge tube, add 5-10ml of blood to 35ml of cell lysis buffer, mix for 2 mins then centrifuge for 10 mins at 3,000rpm at 4°C.
4. Discard the supernatant into bleach to give approximately 1/100 dilution.
5. Resuspend the pellet with 2ml nuclear lysis buffer and transfer the suspension to a new 15ml polypropylene tube.
6. Add 0.5ml 5M sodium perchlorate to each tube.
7. Relocate from the safety cabinet to the bench in the class II lab.
8. Rotary mix the sample at room temperature for 15 mins.
9. Incubate the sample at 65°C for 30 mins, remove from the hot block then add 2.5ml of chloroform.
10. Rotary mix for 10 mins to mix the aqueous and organic phases – it should form a homogenous emulsion. If the sample goes very granular at this stage this means that the sample has not been adequately deproteinised by the sodium perchlorate.
11. Centrifuge the tubes at 1500rpm for 10 mins to break up the emulsion; the DNA containing phase is the **uppermost phase**.
12. Transfer the DNA containing phase to a fresh tube using a disposable Pasteur pipette, discard the chloroform into the waste bottle.
13. Add 2 volumes of pure ethanol (stored at -20°C) to the DNA solution and mix by slow, gentle inversion until the solution is no longer cloudy. The DNA will precipitate out of the solution as a white 'cotton wool' pellet. If left on the bench for a short time, the DNA will float to the surface and can easily be collected onto a disposable microbiology loop.
14. Air dry the DNA by standing the loop DNA upwards in a rack on the bench for 10 mins. Then place the loop into a 1.5ml eppendorf tube and cut off the loop handle.
15. Dissolve the DNA in 200µl of sterile water at 60°C overnight.
16. Once dissolved, DNA may be stored in the freezer in labelled tubes.
17. Amend records where appropriate.

Lymphoprep for leukocytes

1. Collect 10ml blood into heparin tube
2. Switch on Class II safety cabinet in designated class II room
3. Add 10ml PBS to 10ml blood
4. Layer 10ml diluted blood over 5ml lymphoprep in fresh universal tube
5. Centrifuge at 800g for 20 mins (no brake)

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6. Harvest buffy coat band into fresh universal
7. Add PBS to total volume of 25ml, centrifuge 400g for 10 mins (break on)
8. Pool pellets from 2 universals, add PBS to 25ml, centrifuge 200g for 10 mins (break on)
9. Resuspend pellet in PBS or RPMI1640 cell culture media as required for flow cytometry or culture respectively

6.0 Site Specific Details

Personnel: Staff and students within Reproductive and Vascular Biology Group,
Institute of Cellular Medicine

Location: M3066, 3rd Floor Leech Building

Induction: All are provided with a written protocol with safety details. Demonstration is given with continuing supervision as necessary.