Protocol Summary Form

BLOOD COLLECTION SOP

EQUIPMENT USED

3 × 4.0 ml Purple top (EDTA) blood tubes

21 G Vacutainer system needles

Cotton Swab/Gauze

Alcohol Swab

Tourniquet

Plastic gloves

3 × 4.0 ml EDTA purple blood tubes must be filled. Samples should be slowly inverted 8 to 10 times to ensure the mixing of the sample and the anti-coagulant liquid inside the tube.

METHOD FOR BLOOD EXTRACTION

1. Obtain informed consent from participant prior to blood taking.
2. Explain the procedure clearly to participant giving time for any questions, ensuring the patient is comfortable about the procedure.
3. Ensure all equipment is ready to hand in a tray next to the participant.
4. Identify a good-sized vein, usually in the antecubital fossae or on the dorsum (back) of the hand.
5. Apply a tourniquet proximal to the site of venepuncture to ensure engorgement of vein with blood.
6. Prepare the Vacutainer system.
7. Clean the site of venepunture with an alcohol swab.
8. Insert needle into vein looking for blood flashback in the special window of the Vacutainer system.
9. Place purple EDTA tubes into the Vacutainer system to allow self-filling of blood.
10. Once enough blood has been withdrawn, undo the tourniquet with the needle still in place.
11. Take cotton swab and place over site of needle insertion and gently remove the needle.
12. Apply direct pressure with the cotton swab over the puncture site to stem any bleeding. This should be carried out for 2 mins, after which the swab should be removed to ensure bleeding has stopped. If not affix the swab with gauze tape.
13. Carefully label the tubes with participant study number and date and time blood sample was taken.

DNA ISOLATION PROTOCOL (WHOLE BLOOD)

REAGENTS USED

TE buffer (RBS Lysis Buffer)- 10mM Tris, 1mM EDTA (pH=8.0)

WBC Lysis Buffer- 75mM NaCl, 2mM EDTA, 1% SDS

6M NaCl

96% Ethanol

Proteinase K

METHOD FOR DNA EXTRACTION

1. In a 15ml Falcon tube add 3-4ml of blood sample. Add TE buffer up to 14-15 ml. Centrifuge at 3,000rpm for 10 min.
2. Discard the supernatant. Add 14ml TE buffer. Disturb the pellet (by shaking the tubes by hand). Centrifuge at 3000rpm for 10 min.
3. Repeat the second step. The pellet must be white. If not, repeat once more.
4. Add 25µl of Proteinase K (20mkg/ml) and 5ml of WBC lysis buffer. Disturb the pellet by gently pipetting (with 1000µl filter tip).
5. Incubate the tubes at 56ºC for 2.5-3 hours (or overnight).
6. Put the tubes on ice to decrease the temperature up to 20-25ºC.
7. Add 2ml 6M NaCl. Shake the tubes. Leave for 20 minutes shaking periodically (3-4 times). Centrifuge at 3000rpm for 25-30 min.
8. The supernatant should be clear. The pellet should be rigid, not viscous. If not shake again and repeat the centrifugation step.
9. In 50ml transparent clear tubes add 35-40ml 96% ethanol. Add the aqueonus phase into the tube with ethanol to precipitate DNA. Shake very gently. The DNA medusa should be generated.
10. Take DNA medusa carefully with pipette (take as little as possible ethanol with medusa). Wash the medusa in 1ml 70% ethanol (in 1.5ml sterile eppendorf tubes). Aspirate ethanol.
11. Dry the DNA for a while. Add 350µl TE buffer (pH=8.0). Put the tubes with DNAs at 37ºC overnight to solve DNAs.
12. Keep DNA samples at -20ºC.