Sample SOP 02

Standard Operating Procedure for:

Collection, Processing and Storage of Blood Samples

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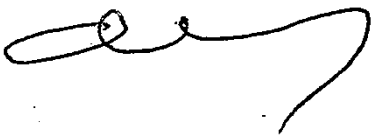
Signature of Author: 

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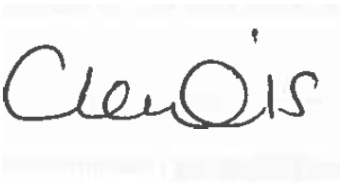
Signature: 

1. Introduction

The Northern Ireland Biobank (NIB) has been established to support translational research programmes locally, nationally and internationally with the aim of establishing new ways of predicting, diagnosing, and treating cancer.

The objective of this Standard Operating Procedure (SOP) is to ensure that there is a standardised method for the collection, processing and storage of blood samples for the NIB, including blood samples for specific clinical research studies/trials. This ensures the quality and integrity of all blood samples archived within the Biobank.

For an overview of the blood samples collected and processed refer to Appendix 1.

Responsibility

It is the responsibility of the Scientific Director of the NIB to ensure that all NIB staff adhere to this SOP.

2. Procedure

2.1. Collection of blood samples

2.1.1. Signed informed consent must be obtained prior to collection of blood samples (See Donor 01 & Donor 03 Informed Consent SOPs).

2.1.2. If required, NIB is ethically approved to collect up to 40 ml of blood. In routine NIB practice, collect 6mls of blood into 1 x 6 ml labelled red–topped serum vacutainer and 12 ml of blood into 3 x 4 ml labelled EDTA vacutainer tubes. Serum is drawn first followed by EDTA. For clinical research studies/trials, please refer to the relevant study protocol for instructions.

2.1.3. Invert the EDTA tubes 5-6 times.

2.1.4. Record the time of blood draw and fasting status on the blood collection form NIB-F-011, NIB-F-012 and NIB-F-013. For clinical research studies/trials, record the relevant data and document as per the study protocol.

2.1.5. EDTA vacutainers can be stored at room temperature until centrifugation; serum vacutainer must be kept at room temperature for 30-45 minutes prior to centrifugation.

2.1.6. Samples must be transported by the Clinical Research Nurse or an appropriately trained staff member directly to the processing laboratory in a PI650 compliant bag for the transport of biological substances. In the Royal Victoria Hospital (RVH) processing will be carried out in the Queen's University Belfast (QUB) Clinical Biochemistry Research Laboratory, Institute of Pathology. In the Belfast City Hospital (BCH) site processing will be carried out in the QUB Molecular Pathology Laboratory (basement CCRCB).

2.2 Preparation of Whole Blood

2.2.1 Remove 3 ml of blood from one EDTA vacutainer and aliquot 1 ml into 3 cryovial tubes pre-labelled with a Unique Sample ID (USID) label (See IT01 Data Entry SOP).

2.2.2 Freeze samples within 1 hour of blood draw, in the designated -80◦C freezers. For BCH, this is located in the basement of CCRCB in the QUB Molecular Pathology Laboratory. For RVH, this is located in freezer store, Institute of Clinical Science B.

2.2.3 Record time of freezing and freezer storage location on whole blood log sheet NIB-F-011.

2.2.4 Enter details into the NIB Information Management System (See IT01 Data Entry SOP).

2.3 Preparation of Plasma

2.3.1 Centrifuge two of the EDTA vacutainers at 2000 g for 10 minutes at 4^0C. Where possible, the centrifuge must be programmed for a controlled gentle deceleration.

2.3.2 Record the time samples went into centrifuge on the plasma and buffy coat layer log sheet NIB-F-012.

2.3.3 When centrifuging is complete, transfer the plasma into a 15ml falcon tube, taking care not to disturb or remove buffy coat white cell layer.

2.3.4 Centrifuge for a second time at 2000g for 10minutes.

2.3.5 When centrifuging is complete, aliquot the plasma into 3 x 1 ml cryovials pre-labelled with a USID.

2.3.6 Retain the 2 x EDTA tubes for preparation of buffy coat layer (see below). Freeze samples within 1 hour of blood draw, in the designated -80^0C temporary freezer. For BCH, this is located in the basement of CCRCB in the QUB Molecular Pathology Laboratory. For RVH, this is located in freezer store, Institute of Clinical Science B.

2.3.7 Record time of freezing and freezer storage location on plasma and buffy coat log sheet NIB-F-012.

2.3.8 Enter details into the NIB Information Management System (See IT01 Data Entry SOP).

2.4 Preparation of Buffy Coat layer

2.4.1 Using the 2 previously centrifuged EDTA vacutainer tubes, carefully remove the buffy coat layer from each vacutainer, and transfer into a cryovial pre-labelled with a USID.

2.4.2 Record the time of preparation on form NIB-F-012.

2.4.3 Freeze sample within 1 hour of blood draw, in the designated -80◦C freezers. 2.4.4 For BCH, this is located in the basement of CCRCB in the QUB Molecular Pathology Laboratory. For RVH, this is located in freezer store, Institute of Clinical Science B.

2.4.4 Record time of freezing and freezer storage location on plasma and buffy coat log sheet NIB-F-012.

2.4.5 Enter details into the NIB Information Management System (See IT01 Data Entry SOP).

2.5 Preparation of Serum

2.5.1 Allow the blood sample to clot at room temperature for 30-45 minutes.

2.5.2 Centrifuge the sample at 2000g for 15 min at 4^0C.

2.5.3 Aliquot the separated serum into 2 x 1 ml cryovials pre-labelled with a USID.

2.5.4 Freeze samples within 1 hour of blood draw, in the designated -80◦C freezers. For BCH, this is located in the basement of CCRCB in the QUB

2.5.5 Molecular Pathology Laboratory. For RVH, this is located in freezer store, Institute of Clinical Science B.

2.5.6 Record time of freezing and freezer storage location on the serum log sheet NIB-F-013.

2.5.7 Enter details onto the NIB Information Management System (See IT01 Data Entry SOP).

2.6 Circulating tumour cells

For full details please refer to Laboratory Manual for Clinical Trials for the Processing, Storage and Shipping of Circulating Tumour Cells (CTC) for specific study / clinical trial protocol / lab manual.

2.6.1. Collect whole blood aseptically by venepuncture into appropriate tubes supplied by Principal Investigator for specific study / clinical trial.

2.6.2 Label with appropriate project identifier/code

Date of sample collection

Time of sample collection (24 hour notation HH:MM)

2.6.3 Transport to appropriate principal investigator

2.6.4 Complete NIB-F-021

2.7 Extraction of DNA from Blood

Detailed instructions can be found in the technical manual for the Maxwell® 16 LEV Blood DNA Kit and Maxwell® 16 Buccal Swab LEV DNA Purification Kit.

2.7.1 Mix all blood samples for at least 5 minutes at room temperature.

2.7.2 Prepare and label incubation tubes compatible with heating block.

2.7.3 Add 30µl of Proteinase K (PK) Solution to each incubation tube.

2.7.4 Add liquid blood (up to 300µl) to each incubation tube.

2.7.5 Add 300µl of Lysis Buffer to each incubation tube.

2.7.6 Vortex each tube for 10 seconds.

2.7.7 Incubate each tube in the heating block (set to 56°C) for 20 minutes. During this incubation, prepare cartridges.

2.7.8 Change gloves before handling cartridges, LEV Plungers and Elution Tubes. Place the cartridges to be used in the Maxwell®16 LEV Cartridge Rack. Place each cartridge in the rack with the label side facing away from the Elution Tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.

2.7.9 Place one plunger into well #8 of each cartridge.

2.7.10 Place an empty Elution Tube into the Elution Tube position for each cartridge in the Maxwell®16 LEV Cartridge Rack. Add 50µl of Elution Buffer to the bottom of each Elution Tube.

2.7.11 Transfer each blood lysate sample from the incubation tube to well #1 of each cartridge. (Well #1 is the well closest to the cartridge label and furthest from the user.)

2.7.12 Turn on the Maxwell® 16 Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.

2.7.13 Verify that the instrument settings indicate an “LEV” hardware configuration and “Rsch” operational mode setting.

2.7.14 Select “Run” on the Menu screen, and press the Run/Stop button to start the method.

2.7.15 Select “DNA” on the menu screen, then select “OK” at the Verification screen.

2.7.16 Select “Blood” on the Menu screen, then select “OK” at the Verification screen.

2.7.17 Open the door when prompted to do so on the screen. Press the Run/Stop button to extend the platform

2.7.18 When run has finished remove tubes and store at -20^{0}C.

2.8 Extraction of DNA from Plasma

Detailed instructions can be found in the technical manual for the Maxwell® RSC ccfDNA Plasma Kit.

2.8.1 Change gloves before handling Maxwell® RSC Cartridges, CSC/RSC Plungers and Elution Tubes. Place each cartridge in the rack with the label side facing away from the Elution Tubes. Press down on the cartridge to

snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.

2.8.2 Place one plunger into well #8 of each cartridge.

2.8.3 Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 60µl of Elution Buffer to the bottom of each elution tube. This will give a final elution volume after processing of approximately 50µl.

2.8.4 Add 0.2–1.0ml of plasma to the Binding Buffer in well #1. Mixing is not required.

2.8.5 Turn on the Maxwell® 16 Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.

2.8.6 Verify that the instrument settings indicate an “LEV” hardware configuration and “Rsch” operational mode setting.

2.8.7 Select “Run” on the Menu screen, and press the Run/Stop button to start the method.

2.8.8 Select “User” on the menu screen, then select “OK” at the Verification screen.

2.8.9 Select “CF DNA” on the Menu screen, then select “OK” at the Verification screen.

2.8.10 Open the door when prompted to do so on the screen. Press the Run/Stop button to extend the platform

2.8.11 When run has finished remove tubes and store at -20^{0}C.

3. Quality

Time from blood collection to freezing should be no more than one hour. Variances from this must be documented on the blood collection forms as required (NIB-F-011, NIB-F-012, NIB-F-013, NIB-F-021, NIB-F-022) and in the NIB IMS.

4. Safety

All local health and safety regulations must be adhered to:

4.1. Royal Victoria Hospital laboratory

All local health and safety regulations must be adhered to.

4.2. NICTC laboratory

All local health and safety regulations must be adhered to.

Appendix 1

Summary of blood samples and derivatives

Vacutainer tube	Volume (mL)	Fraction	No. of aliquots
EDTA (4ml) x 3	12	Blood Plasma Buffy coat	3 (1ml) 3 (1ml) 1
Z Serum clot activator (6ml) x 1	6	Serum	2 (1ml)
Total	**18**		**9**