Blood tube ID and sample preparation No: 009D

1. Introduction

Accurate identification and preparation of samples is vital to ensure success of the study. It is essential that each sample is individually labelled with the correct subject study details. A sample incorrectly identified is as useless as one that has not been collected at all. In order to minimise poor tube identification and sample preparation, it is necessary that the method only be carried out by the research nurses involved in the study.

2. Responsibilities

Research nurses trained in the method are responsible for ensuring all samples are precisely prepared, suitably labelled and sent to the correct laboratory for analysis.

3. Equipment

- Becton-Dickinson blood tubes (BD)
- 2ml & 5ml Nunc tubes & coloured lids
- Cryogenic marker pens & labels
- Centrifuge
- Pasteur pipettes

4. Method

4.1 Technique of collecting samples

- Ensure all blood samples are collected into the correct tubes, these are listed in table one.

Table 1:

Specimen number	Volume	Type of tube	Make & colour
1	10 & 7mls	EDTA for Glasgow	BD Lavender
2	10mls	lithium/heparin	BD Green
3	10mls	Plain	BD Red
4	2 X 10 & 7mls	EDTA for Wellcome	BD Lavender
5	8.5mls	ACD	BD Yellow
6	2mls	Flouride oxalate	BD Light grey

4.2 Labelling of samples

- All samples should have the following information:-
    - Study code name (The BRIGHT study)
    - Unique subject ID (e.g. LN/00001/001, representing the sample is from the London centre, family number one and fathers blood.
    - Date and time of collection.

- Samples sent to the Wellcome centre will be bar-coded on their arrival, this is for their reference and not the method the study would identify a sample.

- Ensure to label samples with the low temperature marker pen. Standard adhesive labels are not to be used ,as these fall off tubes in storage (particularly when frozen). Be sure to use the correct marker pens and cryogenic labels to label the tubes to avoid identification details being rubbed off, thus invalidating the sample.

4.3 Sample preparation

- In order to ensure that maximum analysis of the samples can be achieved it is vital that all samples are correctly handled. Table 2, 3 and 4 displays how each sample should be handled.

- These samples are to be prepared at each centre. The buffy coats from these tube samples will be stored at each centre and the plasma and serum will be sent to Glasgow in batches. The samples should be prepared as follows:

Table 2:

Sample	Preparation required
1. 17mls EDTA tubes	Store on ice until centrifugation *
2. Lithium/Heparin tubes	Store on ice until centrifugation *
3. Plain tube	Store on ice until centrifugation *

*** Centrifuge these samples at:-**

Speed	**2500rpm**
Temperature	**4°C**
Duration	**15 mins**

- Using a pasteur pipette transfer the serum from the plain (red tube) into 2 "approximately" equal quantities into 2 x 5ml Nunc tubes. Label these tubes with the subject ID details using the cryogenic marker pens & labels. Secure a red cap onto the lid of both Nunc tubes, this enables identification of the samples (e.g serum from the plain BD tube). Store the samples in your centre's designated freezer at -70°C. Complete the record book indicating where the samples have been stored (e.g. which freezer and which shelf number).

- Using a pasteur pipette transfer the plasma from the Lithium/heparin (Green tube) into 2 "approximately" equal quantities into 2 x 5ml Nunc tubes. Label these tubes with the subject ID details using the cryogenic marker pens & labels. Secure a green cap onto the lid of both Nunc tube, this enables identification of the samples (e.g plasma from the green BD tube). Store the samples in your centre's designated freezer at -70°C. Complete the record book indicating where the samples have been stored (e.g. which freezer and which shelf number).

- Using a pasteur pipette transfer the plasma from the EDTA (Lavender tubes) into 2 "approximately" equal quantities into 2 x 5ml Nunc tubes. Label these tubes with the subject ID details using the cryogenic marker pens & labels. Secure a lavender cap onto the lid of both Nunc tube, this enables identification of the samples (e.g plasma from the Lavender BD EDTA tube). Store the samples in your centre's designated freezer at -70oC. Complete the record book indicating where the samples have been stored (e.g. which freezer and which shelf number).

- After centrifugation and the plasma/serum has been transferred into the appropriate Nunc tubes, transfer the buffy coats from the green and purple cap samples as follows. Using a fresh pasteur pipette for each type of sample, take the buffy coat (approximately 0.75mls) into 1 X 2ml Nunc tubes. For the buffy coat from the green lithium/heparin BD tube, a green cap should be inserted into the lid. This allows the origin of the buffy coat sample to be identified. There will be 2 buffy coats from the 2 Lavender EDTA samples, these should be placed into 2 X 2ml Nunc tubes and labelled with lavender caps in the lids. Label all these tubes with the subject ID details using the cryogenic marker pens &

labels. Store the sample in your centres designated freezer at -70°C. Complete the record book indicating where the samples have been stored (e.g. which freezer and which shelf number).

- If possible, store all the samples for a subject/family member on the same shelf in the designated freezer.

- Every two months batches of the plasma and serum samples will be sent to Glasgow. These will be sent on DRY ICE and securely sealed. Check with the nurse co-ordinator every two months how the samples are to be transported. DO NOT post any samples without consulting the nurse co-ordinator first

Table 3:

Sample	Preparation required
4. 3 X 27ml EDTA	Store at room temperature (DO NOT put in cool box)
5. 1 X ACD	Store at room temperature (DO NOT put in cool box)

- These samples will be packaged & posted at the GP surgery you are working in. Follow the EXACT packaging guidelines given in table 5.

It is vital that these samples DO NOT get refrigerated or frozen, as this will invalidate the DNA extraction process. Care must also be taken when posting these samples during very cold weather.

Table 4:

Sample	Preparation required
6. 2ml X Flouride oxalate	Store at room temperature

- Arrangements should have been made by the Doctor in each of the 6 centres for glucose to be measured in their local laboratory. Check with the co-ordinating Doctor at your centre that this has been arranged prior to collecting any blood samples. Should there be any problems with this local measurement consult with the nurse co-ordinator.

4.4 Dispatch of samples

- It is vital that samples are sent to the appropriate laboratories using the standard procedures, this ensures that the results obtained from them will not be invalidated by poor handling.

- Place the desired tubes into a plastic forming device. Seal the device and wrap it with two sheets of blue laboratory towel. Place it in a plastic bag and ensure it is sealed correctly. Put it in a jiffy bag and ensure the seal is securely fastened. Label the jiffy bag with the recommended tape -PATHOLOGICAL SPECIMEN, FRAGILE WITH CARE - Post it to the desired laboratory (Wellcome Centre, Oxford).

- When posting samples it is vital that the necessary temperature conditions are maintained.

- Broken vials should be avoided by packaging the samples in the correct containers. On no account should postal staff be subjected to a package which is likely to spill, care should be taken to ensure safe handling for all people who may come into contact with the package, apart from the designated centre or nurse posting it.

- The package to be posted should be labelled clearly with the following details:-

    - The source of the sample
    - The addressee
    - The subject(s) unique identification number
    - Temperature for storage of sample(s)

- Prior to posting any samples, notify the addressee. For samples to The Wellcome Centre for Human Genetics, Fax or E-mail them to ensure they are aware of how many samples they are expecting and when. Samples for this addressee however, should not be posted on a Friday unless special arrangements have been made. Samples for Glasgow should be posted in bulk loads and the centre notified by telephone as to when they will be expected.

4.5 Urine collections

- The volume of urine should be recorded in the phenotypic book. 1 X 50ml aliquots should be decanted from the collection and 2 X 5ml Nunc tubes of urine should be prepared, correctly labelled (Using cryogenic markers & labels)and stored in your centre designated -20oC freezer. Ensure they are labelled by unique subject ID number only, using the low temperature marker pens.

5. Additional Information

- Each centre will need to liase with their laboratory when it is convenient to centrifuge the samples for the study and how to use the equipment provided by that laboratory. Each centre may use different centrifuges, BUT the centrifugation conditions must remain the same.

- Postal arrangements for samples should be discussed with the Nurse Co-ordinator. Some samples will be sent via the Royal Mail, some by courier and some by Datapost. Ensure you are aware of how each samples are to

be sent, PRIOR to sending them.

6. Reference Documents

none.