



**SOP Reference: BCNTB/SOP/004**

Standard Operating Procedure for

Collection and processing of blood samples for circulating cell-free DNA and viable lymphocytes

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Authorised by:

	Name	Designation	Signature	Date approved
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1.0	1 <sup>st</sup> Issue and updated HTA codes of Practice and Standards	Louise Weatherley, Helen Cramp, Prof. Angela Cox, Uma Ekbote	November 2016; April 2017

## **1.0 PURPOSE AND SCOPE**

- 1.1 Blood reflects the many processes that the body undertakes. In diseases, blood can be used to monitor, screen and diagnose.
- 1.2 This SOP covers collection and laboratory processing of blood to generate blood plasma suitable for the isolation of circulating cell-free DNA, serum, buffy coat for DNA extraction and buffy coat containing viable lymphocytes.
- 1.3 This SOP applies to the Institution defined in section 2.2.

## **2.0 DEFINITIONS**

- 2.1 The Breast Cancer Now Tissue Bank shall be referred to as the Tissue Bank or BCNTB.
- 2.2 The Institution is the University of Sheffield.
- 2.3 Sheffield Teaching Hospital Foundation Trust is referred to as STHFT.

## **3.0 REFERENCES**

- 3.1 Human Tissue Act 2004
- 3.2 Human Tissue Authority, Codes of Practice and Standards, April 2017;
  - 3.2.1 Code A: Guiding principles and the fundamental principle of consent
  - 3.2.2 Code E: Research
- 3.3 BCNTB/SOP/009: Approach to Consent
- 3.4 Control of Substances Hazardous to Health (COSHH)
- 3.5 Institute-specific Codes of Practice and Guidance:
  - 3.5.1 STHFT Venepuncture and Intravenous Cannulation Open Learning package (November 2006).
  - 3.5.2 STHFT Hand and Hygiene Policy.
  - 3.5.3 STHFT Infection Control Accreditation Programme (September 2010).
  - 3.5.4 STHFT Aseptic Technique Guidelines (2014).
- 3.6 NMC Code of Conduct (31 March 2015).
- 3.7 Legal Framework:
  - 3.7.1 [http://www.sth.nhs.uk/STHcontDocs/STH\\_Pol/ClinicalGovernance/HandHygienePolicy.doc](http://www.sth.nhs.uk/STHcontDocs/STH_Pol/ClinicalGovernance/HandHygienePolicy.doc)

3.7.2 The Health and Social Care Act 2008: Code of Practice on the prevention and control of infections and related guidance (Department of Health 2010).

#### **4.0 HAZARDS AND PRECAUTIONS**

4.1 Vaccination:

All staff involved in tissue collection should have Hepatitis B vaccination under the guidance of their local Occupational Health Service.

4.2 Blood:

Infection risk, appropriate protective equipment should be worn.

4.3 Consent:

Consent forms should be checked and any inconsistencies noted on the sample report form to ensure swift correction of errors and evidence of due diligence.

4.4 Needles:

Needles must be disposed of in appropriate sharps bin and never re-used or re-capped.

4.5 Liquid Nitrogen:

This poses serious burns and asphyxiation risk. Protective equipment must be used (face mask and cryogenic gloves).

#### **5.0 PROCEDURE**

5.1 Blood collection should be carried out by a suitably trained registered nurse, doctor, phlebotomist or other suitably trained staff.

5.2 The blood collection procedure should be followed as per site-specific protocols, but should take into account if possible the volume of collection and the order of the blood draw as outlined below.

5.3 Bloods drawn for diagnostic purposes should be prioritised.

5.4 Order of draw of BCNTB blood: Blood should be collected into K<sub>2</sub>EDTA (potassium ethylenediaminetetraacetic acid; lavender or purple top tube) first, followed by clotted (red top tube).

5.5 Blood should be collected in the following vacutainer tubes from a registered manufacturer

5.5.1 Four x 6ml K<sub>2</sub>EDTA (lavender or purple top)

5.5.2 Two x 6ml clotted tube with or without clot activator (red top)

- 5.6 Blood tubes should be labelled with patient name, hospital number, or addressograph label or sample barcode, and the time sample taken.
- 5.7 Blood should be processed as soon as possible but **within 2hrs** of blood draw. The time of blood draw is to be noted in the Lab. Record Form (Appendix B).

## **6.0 PROCESSING**

### **6.1 Clotted Tubes (red top)**

- 6.1.1 Sample must be mixed gently and left for minimum of 30 minutes to allow the blood to clot at room temperature.
- 6.1.2 The vacutainers should be centrifuged at RCF 850g for 10 minutes at 8-20 degrees C.
- 6.1.3 The serum should then be split into aliquots of approximately 500 µl, in 1.2ml screw top cryovials using a sterile Pasteur pipette or Gilson pipette (or similar) with sterile filter tips.
- 6.1.4 Each vial should be labelled with sample type (serum) and a unique BCNTB barcode, before placing the tubes for storage in a -80°C freezer. The number of serum cryovials and the time of storage should be noted in the Lab. Record Form (Appendix B).

### **6.2 K<sub>2</sub>EDTA (purple top)**

- 6.2.1 The vacutainers should be centrifuged at RCF 850 g for 10 minutes at 8-20 degrees C. The time of centrifugation should be noted in the Lab Record Form (Appendix B: centrifugation 1).
- 6.2.2 Plasma should be drawn off into four labelled 15ml centrifuge tubes (one for each vacutainer) using Pasteur pipettes or Gilson pipette (or similar) with sterile filter tips, taking care NOT to disturb the buffy coat layer.
- 6.2.3 The 15ml centrifuge tubes should be centrifuged at RCF 1600g for 10 minutes at 8-20 degrees C. The time of centrifugation should be noted in the Lab Record Form (Appendix B: centrifugation 2).
- 6.2.4 Meanwhile, draw off the buffy coat layer from the four original vacutainers into four 1.2ml screw top cryovials.
- 6.2.5 To two of these cryovials, add 1ml of DMSO (dimethylsulphoxide) freeze mix (see Appendix A) and mix gently. The two cryovials containing DMSO freeze mix will be frozen slowly and ultimately placed in liquid nitrogen for long-term storage to maintain cell viability.
- 6.2.6 All four cryovials should be labelled with a unique BCNTB barcode, and sample type (either “buffy N2” or “buffy” for those with and without DMSO freeze mix respectively). Transfer the four buffy coat cryovials to the -80 freezer. The two with DMSO freeze mix (“buffy N2”) are to be put in the slow-freeze pot, ensuring that it has polystyrene holder and tissue to wrap them in for slow freezing). The number of buffy

coat cryovials of each type, and the time of storage should be noted in the Lab. Record Form.

- 6.2.7 Following the second centrifugation, the plasma should be carefully removed from the 15ml centrifuge tubes without disturbing the pellet, and split into aliquots of 500 µl in 1.2 ml screw top cryovials using a sterile Pasteur pipette or Gilson pipette (or similar) with sterile filter tips.
- 6.2.8 Each individual cryovial should be labelled with sample type (plasma) and a unique BCNTB barcode, before placing the tubes for storage in the -80 °C freezer. The number of plasma cryovials of each type and the time of storage should be noted in the Lab. Record Form (Appendix B).

### 6.3 **Booking & Logging of blood samples**

- 6.3.1 Blood samples are booked and logged in accordance with local guidelines: All sites use barcodes for blood samples.
- 6.3.2 Once the samples are logged in the System, only unique number or barcode should be used to identify the sample.

### 6.4 **Temporary Storage & Transfer**

- 6.4.1 Where transfer of samples is required for long-term storage, samples must be moved in batches, on dry ice, using an appropriate container.
- 6.4.2 The sample location must be updated in the sample database.
- 6.4.3 Buffy coat samples in DMSO (buffy N2) should be transferred to liquid nitrogen for long-term storage (i.e. storage beyond 3 months).

## **APPENDIX- A**

DMSO Freeze Mix:

- 70% Tissue Culture Media (RPMI)
- 20% Foetal Calf Serum
- 10% Dimethyl sulfoxide

Mixed and pre aliquoted into 1ml cryovials and stored at -20 degrees C until required.

**APPENDIX- B**

**Laboratory Record Form**

Bar code label

**Patient Information**

Initials  Sample No.   
Date of Birth

**Blood sample information**

Date samples taken   
Time samples taken   
No. of tubes K<sub>2</sub>EDTA (Purple)  Clotted (Red)   
Time samples centrifuged 1  2

**Number of samples stored and time**

Serum	<input type="text"/>	Time	<input type="text"/>		
Plasma	<input type="text"/>	Time	<input type="text"/>		
Buffy	<input type="text"/>	Time	<input type="text"/>		
Buffy N2	<input type="text"/>	Time	<input type="text"/>		
Whole blood	<input type="text"/>	Time	<input type="text"/>	TR56	<input type="text"/>

**Name of person collecting samples**

**Name of person(s) processing samples**