

LABORATORY MANUAL

MRC SUPREMO (BIG2-04)

TRANS SUPREMO
&
CARDIAC SUBSTUDIES

ISRCTN61145589



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CONTACT INFORMATION

Fiona Campbell
TRANS SUPREMO SUB STUDY
Endocrine Cancer Group,
University Department of Pathology
Edinburgh Cancer Research Centre,
Western General Hospital
Crewe Road South
Edinburgh EH4 2XR

EMAIL

transsupremo@ed.ac.uk

TELEPHONE

+44 (0) 131-777-3585

FAX

+44 (0) 131-777-3520

When contacting, please include the following information:

- Your name, email address and telephone number
- Your centre details including centre ID number
- Patient trial number (if applicable)

INTRODUCTION

TRIAL SUMMARY

MRC-SUPREMO is a MRC randomised phase III trial assessing the role of chest wall irradiation in women with intermediate risk breast cancer following mastectomy, axillary surgery and appropriate systemic therapy. Patients will be randomly allocated to either receive chest wall irradiation or allocated to a control group. The study will recruit 3700 patients.

CARDIAC SUB STUDY SUMMARY

Recent studies of radiation toxicity in the treatment of breast cancer show that the effects on normal tissues can constitute a significant clinical problem and particularly increased cardiac mortality may offset any potential survival benefit. There are no data on lesser degrees of late cardiac damage, but it seems likely that non fatal ischaemic heart disease must also be induced. An excess of cardiac deaths starts to manifest itself at about 7 years post radiotherapy and increase year on year thereafter. Thus, reported values are dependent on length of follow up. The cardiac sub study will use proteomic analysis of circulating markers to identify patients with cardiac damage. Additional studies relating to detection of markers of relapse or proteomic markers of outcome will also be performed.

A 30 ml blood sample will be taken from all patients who consent, at baseline, following chemotherapy, following radiotherapy, 1, 5 and 10 years and at disease recurrence (local and/or distant relapse).

TRANSLATIONAL SCIENCE SUB STUDY SUMMARY

Within the context of the SUPREMO trial there exists a unique opportunity to expand our knowledge of the molecular mechanisms underlying the relapse of breast cancer and resistance to radiation therapy.

Standard prognostic factors do not define the 60-80% of patients in whom radiotherapy might be safely omitted. The aim of the present study is to identify molecular signatures and validate methods by which such patients can be identified in the clinic using the SUPREMO trial as a test system.

Tissue blocks will be requested, following randomisation, from pathology departments. Blocks will be returned after extraction of small diameter cores of tissue for the production of tissue micro-arrays.

This manual contains general information about sample handling procedures as well as the storage and shipping of specimens and the use of the relevant forms.

****PLEASE READ THIS LABORATORY MANUAL CAREFULLY TO ENSURE PROPER HANDLING OF THE SPECIMENS****

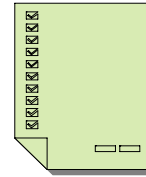
THANK YOU

INSTRUCTIONS FOR BLOOD SAMPLE PROCESSING

SUPPLIES

Each site will be supplied with kits containing the following items;

**Laboratory
Requisition
Form
(1 x NCR)**



**Tubes and coloured caps for
processed specimens
(5 x green; 5 x red; 5 x blue)
plus labels and sample bag for
freezing**



**Pasteur pipettes
(x 5, including 1
spare)**



**Container and stamped
addressed jiffy bag for
immediate dispatch of BNP**



Blood sample flow chart – see page 8

When you have **2 blood sample kits remaining**, please **photocopy** the Blood Sample Kit Request Form (Appendix 1), **complete** and **fax** to Endocrine Cancer Group, Edinburgh, UK (+44 (0) 131 777 3520). *5 kits will be despatched immediately.*

The following equipment is required but not provided;

- Venepuncture kit
- Plain tube with separator gel; 10 ml (SST Vacutainer or S-Monovette Serum)
- EDTA tubes; 4 x 5 ml
- Centrifuge
- Freezer; -80°C (or if not available, -20°C)

USE OF LABORATORY REQUISITION FORMS

Please complete a Laboratory Requisition Form, in **black ball-point pen**, for each blood sample taken, i.e. at first visit and at subsequent follow-up visits, including disease recurrence (local and/or distant relapse).

Appendix 2 shows an example of the “Laboratory Requisition Form”.

Please note that to ensure unique identification by the data matrix label system no copying or combined use of the form is allowed!

Please write the numbers and letters clearly and provide complete and accurate information.

Fill in a Laboratory Requisition Form with the following information:

1. Patient trial number (7 digits; 3 digit centre number and 4 digit trial number)
2. Patient`s initials (3 letters; first-middle-last, if only 2 leave middle blank)
3. Patient`s date of birth (DD MM YY e.g. 09 05 65)
4. *Sample information (First visit; second visit, local recurrence; distant recurrence; site if applicable)*
5. Date of sampling (DD MM YY eg 23 02 04)
6. Time of sampling (HH MM eg 14 45) **NB USE 24 HOUR CLOCK**
7. Time sample centrifuged (HH MM eg 15 15) **NB USE 24 HOUR CLOCK**
8. Time of freezing (HH MM eg 16 30) **NB USE 24 HOUR CLOCK**

Please note that the Laboratory Requisition Form is supplied in a “No Carbon Required” (NCR) format.

The **top copy (white)** is to be put into the pocket in the **FROZEN sample** bag and placed in the freezer at -80°C (-20°C). This is to be despatched on request with the sample to the Endocrine Cancer Group, Edinburgh. You will be contacted by the Cancer Clinical Trials Team to arrange collection by courier. If you need to send samples to the Endocrine Cancer Group before this then please contact transsupremo@ed.ac.uk to arrange collection. Please note DO NOT arrange for samples to be collected on a Thursday or Friday as samples should not be delivered to the Endocrine Cancer Group, Edinburgh over the weekend.

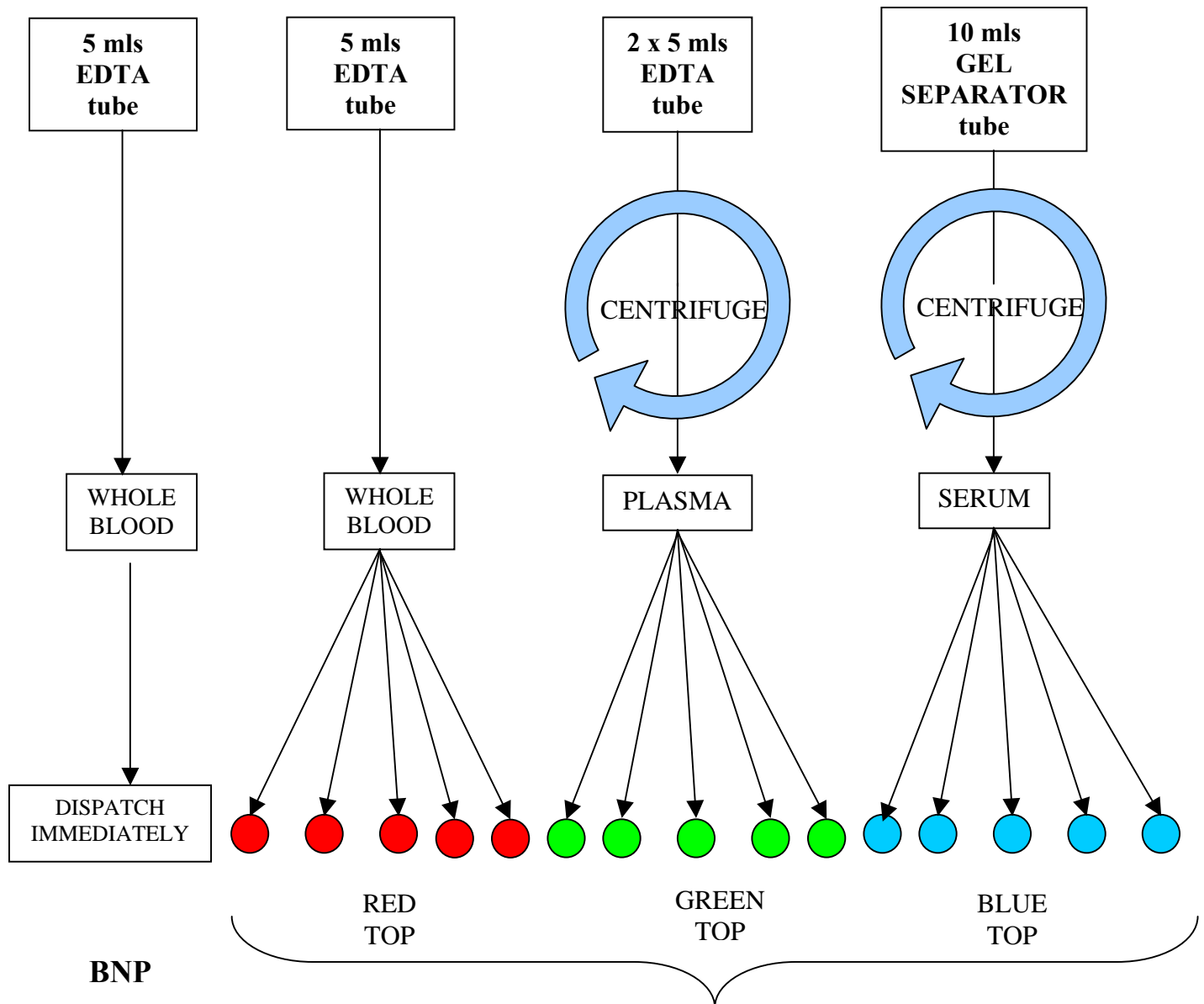
The **second copy (yellow)** is to be put into the **BNP sample** bag and DISPATCHED IMMEDIATELY with the sample to:

Simon Walker/Keith Miller
Trans Supremo Sub Study
Combined Laboratories Reception
2nd Floor
The Royal Infirmary of Edinburgh
Little France Crescent
Edinburgh
EH16 4SU

The **bottom copy (blue)** is to be **retained** for your records.

BLOOD SAMPLE FLOW CHART

A 30 ml blood sample should be taken at baseline (first visit), following chemotherapy, following radiotherapy, 1, 5 and 10 years and at disease recurrence, (local and/or distant relapse), in the following tubes:-



PIPETTE 0.8 - 1ml INTO EACH TUBE
APPLY A STICK ON LABEL TO EACH TUBE
PLACE USED AND UNUSED TUBES IN BAG PROVIDED
WITH FORM INSERTED IN SLEEVE
FREEZE AT -80°C (-20°C, IF NOT AVAILABLE)

Endocrine Cancer Group: email transsupremo@ed.ac.uk or tel +44 (0) 131 777 3585
 Cancer Clinical Trials Team: email supremo@isd.csa.scot.nhs.uk or tel 0131 275 6630

BLOOD SAMPLE INSTRUCTIONS

The samples need to be separated within 1 hour and frozen within 30 minutes.

- A 30 ml blood sample to be taken at baseline (first visit), following chemotherapy, following radiotherapy, 1, 5 and 10 years and at disease recurrence, (local and/or distant relapse). Collect 10 ml of blood in a plain tube with separator gel and 20ml of blood in 4 x 5 ml **plastic** EDTA tubes.
- Complete Laboratory Requisition Form. Fold and place **top copy (white)** into the pocket in the rear of the **sample** bag.
- Place one of the 5 ml plastic EDTA tubes, with the **yellow copy** of the Laboratory requisition form in the addressed jiffy bag provided and **dispatch immediately**. Ensure rigid container is properly closed and place into plastic bag with absorbent pad. **This sample must arrive within 48 hours of dispatch.** (*This packaging complies with Royal Mail postal regulations*).
- Do not centrifuge one of the EDTA tubes (whole blood sample). Using the pipette provided, transfer **0.8 ml** of **whole blood** into each of the **5 red-topped** cryovials* and apply “whole blood” labels.
- Centrifuge the 2 remaining EDTA tubes at 1300g (approximately 2,500 rpm) for 15 minutes. Using the pipette provided, transfer **0.8 ml** of **plasma** into each of the **5 green-topped** cryovials* and apply “plasma” labels. Take care not to disturb the cells with the pipette.
- Allow blood in the plain tube with gel separator to clot for 30 minutes at room temperature. Centrifuge at 1300g (approximately 2,500 rpm) for 15 minutes. Using the pipette provided, transfer **0.8 ml** of **serum** from the gel separator tube into each of the **5 blue-topped** cryovials* and apply “serum” labels.

(* If insufficient sample is obtained, please indicate on the Laboratory Requisition Form the number of cryovials stored.)

- Put the filled and empty cryovials into the small zip topped sample bag provided.
- The **bottom copy (blue)** to be **retained** for your own records.
- Place samples into your freezer at -80°C (-20°C acceptable for two - four months if you do not have access to -80°C).
- You will be contacted to arrange collection of samples by courier. All transport packaging and dry ice will be provided.
- On arrival at the central laboratory, the samples will be stored at -80°C until analysis.

CHOLESTEROL

Cholesterol should be measured locally at site using a separate sample of blood.

NOTES

INSTRUCTIONS FOR TISSUE BLOCK PROCESSING

INSTRUCTIONS FOR TISSUE BLOCK PROCESSING

- Tissue blocks will be requested for all patients who consent, at baseline *and again at times of local and/or distant recurrence or at first diagnosis of contralateral breast cancer, if available*. The patient's specific written Informed Consent has been obtained for this process.
- A kit will be supplied for each block requested consisting of;
 - Pathology Request Form (Appendix 3) with patient study details
 - Small sample bag
- After completion of the Pathology Request form, please fold and place **top copy (white)** into the pocket in the rear of the **sample bag**. The **bottom copy (blue)** is to be **retained** for your records.
- Place the blocks into appropriate packaging (labels are provided) and return to
Fiona Campbell
TRANS SUPREMO SUB STUDY
Endocrine Cancer Group,
University Department of Pathology
Edinburgh Cancer Research Centre,
Western General Hospital
Crewe Road South
Edinburgh EH4 2XR
- Blocks will be stored in a secure facility and all data will be held in a confidential manner.
- Small diameter cores of tissue will be extracted from the block for the production of tissue micro-arrays etc.
- All blocks will be returned to the pathology department of origin. Please document receipt on the blue copy of the Pathology Request Form held in your files.

If you experience any difficulties or have any questions, please email Fiona Campbell (transsupremo@ed.ac.uk); or telephone +44 (0) 131 777 3585 (Endocrine Cancer Group)

APPENDIX 1

BLOOD SAMPLE KIT REQUEST FAX

TO ORDER 5 KITS

When you have 2 **blood sample kits remaining**, please **photocopy** this form, **complete** and **fax** to:-

**FAO: Fiona Campbell,
John Bartlett
Fax no:
+44 (0) 131 777 3520**

(PLEASE PRINT DETAILS)

Contact name _____ Date _____

Email address _____

Centre number _____

Hospital _____

Address _____

For office use:- Kits despatched
Comments

Signature

Date

If you experience any difficulties or have any questions, please contact your local Trials Unit or email transsupremo@ed.ac.uk; or telephone +44 (0) 131 777 3585 (Endocrine Cancer Group).

Affix Data matrix label
(barcode)

LABORATORY REQUISITION FORM

PATIENT TRIAL NUMBER

PATIENT INITIALS (First:Middle:Last)

PATIENT DATE OF BIRTH (DD/MM/YY) / /

Informed Consent (Trans-Supremo) checked:
Name
Sign
Date

SAMPLE INFORMATION
(Please tick)

First visit(screening) Post chemo/preRT Post Radiotherapy
 1 year visit 5 year visit 10 year visit Local recurrence Distant recurrence

DATE OF SAMPLING (DD/MM/YY) / /

TIME SAMPLE TAKEN (HH:MM) :

TIME CENTRIFUGED (HH:MM) :

TIME SAMPLE FROZEN (HH:MM) :

N^o of 0.8ml samples stored:-
SERUM
PLASMA
WHOLE BLOOD

**White copy with frozen samples
yellow copy with BNP sample
Blue copy to file**

NAME OF STUDY NURSE / TECHNICIAN:
(Capital letters)

Sign _____ Date _____

