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LABORATORY MANUAL

MRC SUPREMO (BIG2-04)

TRANS-SUPREMO & CARDIAC SUB STUDIES

ISRCTN61145589

Version 6, 20/04/2016

Trans-Supremo & Cardiac Sub Studies

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CONTACT INFORMATION

TRANS-SUPREMO

John Bartlett – Principal Investigator, Trans-Supremo sub-study
Tammy Piper – Senior Biomedical Scientist
Monika Sobol – Research Technician

ADDRESS
Biomarkers & Companion
Diagnostics Group,
Edinburgh Cancer Research Centre,
Western General Hospital,
Crewe Road South,
Edinburgh,
EH4 2XR
UK

EMAIL
Tammy.piper@igmm.ed.ac.uk
Monika.sobol@igmm.ed.ac.uk
TELEPHONE
Laboratory Office: +44 (0) 131-651 8605

FAX
+44 (0) 131-651 8711

BNP SAMPLES

Biomarkers & Companion
Diagnostics Group,
Edinburgh Cancer Research Centre,
Western General Hospital,
Crewe Road South,
Edinburgh,
EH4 2XR
UK

When contacting, please include the following information:

- Your name, email address and telephone number
- Your centre details including centre name/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 of 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 1600 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 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 may 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 the 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.

A 25ml blood sample will be taken from all patients who consent, at baseline and at disease recurrence (local and/or distant relapse)

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

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

GENERAL INSTRUCTIONS

Patients can be approached for inclusion to Trans-Supremo and/or the Cardiac Sub-Studies. A separate consent **must** be obtained for participation in these sub studies.

Patient tissue samples will also be used for the pathology audit as per the main trial protocol. A separate consent is **not** required for this as the patient has given consent to take part in the main trial.

For the purposes of this laboratory manual it is assumed that a patient consenting to the Cardiac sub study will also consent to the Trans-Supremo sub study, however, please ensure the correct procedures are followed depending on the consent given:

Consent Given For:	**Relevant pages in document**
Cardiac and Trans-Supremo sub studies	6-11
Cardiac sub study only	6-11 for BNP sample only
Trans-Supremo only	15-20
Trans-Supremo for tissue donation only	22

No additional consent required	**Relevant pages in document**
Pathology Audit (part of the main trial)	24

Trans-Supremo & Cardiac Sub Studies

INSTRUCTIONS

FOR BLOOD SAMPLE

PROCESSING

FOR THE

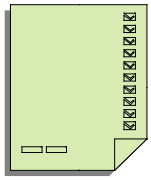
CARDIAC SUB STUDY

(& TRANS-SUPREMO)

SUPPLIES FOR THE CARDIAC SUB STUDY

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) plus labels and sample bag for freezing

Pasteur pipettes (x 2, including 1 spare)

Rigid, protective container, packaging and stamped addressed jiffy envelope for <u>immediate</u> dispatch of BNP sample

Blood sample flow chart for Cardiac sub study– 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 Biomarkers & Companion Diagnostics Group, Edinburgh, UK (+44 (0) 131 651 8711).
5 kits will be despatched immediately. <u>Please do not email requests to the clinical trials office as this may result in a delay in the kits being sent to you.</u>

The following equipment is required but not provided;

- Venepuncture kit
- Plain tube with separator gel; 10 ml (SST Vacutainer or S-Monovette Serum *or equivalent*)
- EDTA tubes; 4 x 5 ml *or equivalent*
- 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 to be applied by the central laboratory 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 7 digit trial number = 3 digit centre number and 4 digit trial number, e.g. B07 9999
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 = whether first visit; second visit; 5 year follow-up, etc. If recording details of local or distant recurrence please include details of the site of recurrence.
5. Date of sampling = DD MM YY e.g. 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**
9. Sign and date the **'Informed Consent Checked'** box to indicate to the laboratory staff that the patient has given informed consent to participate in the biological sub-study – this section is to be completed by the person filling out the form, ***not*** the patient.
   Please do not include copies of the consent form with the sample as the central laboratory must not receive any paperwork with the patient's full name

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

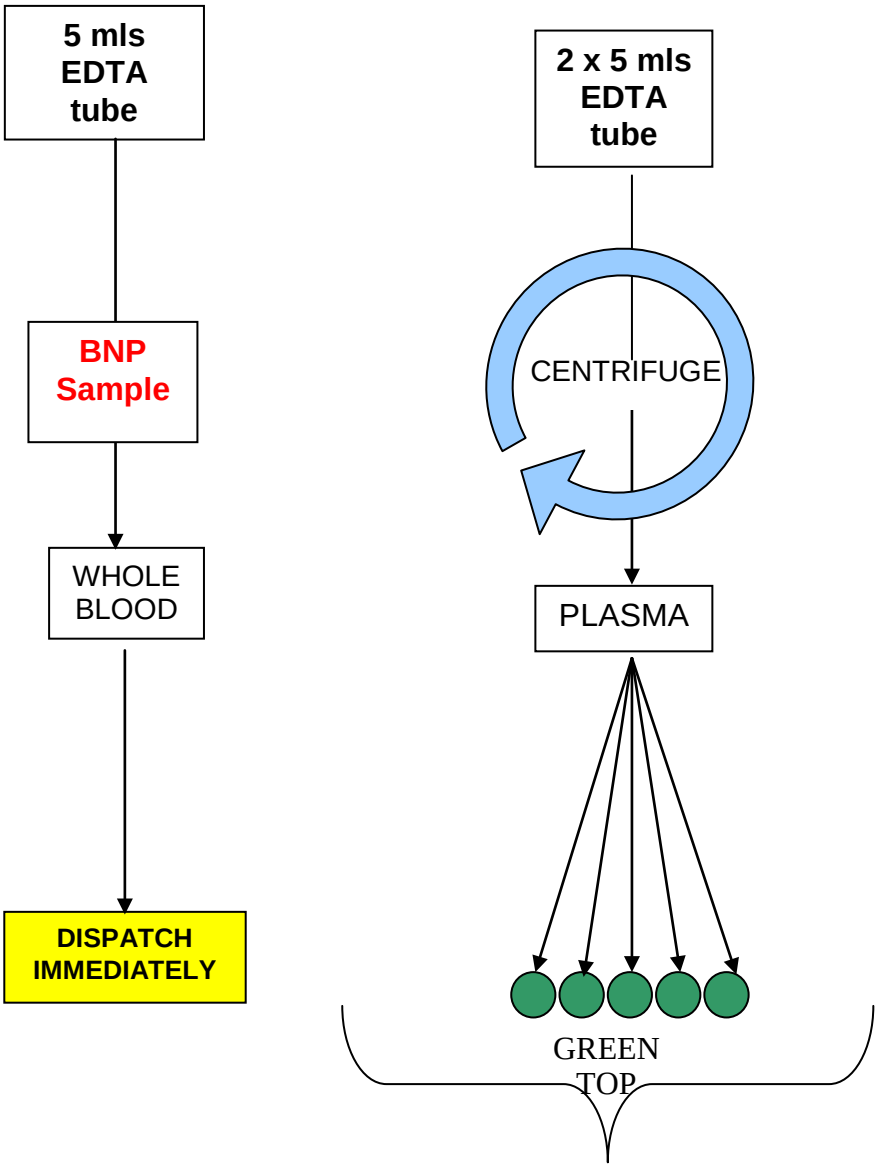
The **top copy (white)** is to be put into the pocket in the **FROZEN sample** bag and placed in the freezer at -80°C (or -20°C, if not available). This is to be despatched on request with the sample to the Biomarkers & Companion Diagnostics Group, Edinburgh.

The second copy (yellow) is to be put into the pocket of the **BNP sample** bag and **DESPATCHED IMMEDIATELY** using the stamped addressed jiffy envelope provided. Samples should be sent to the Biomarkers & Companion Diagnostics Group.

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

BLOOD SAMPLE FLOW CHART: CARDIAC SUB STUDY

A 30 ml blood sample should be taken at the following time points: first visit (baseline), following chemotherapy, following radiotherapy, 1, 5 and 10 years and at disease recurrence, (local and/or distant relapse). The samples should be processed as follows:-



PIPETTE 0.8 - 1ml INTO EACH TUBE
APPLY AN ADHESIVE LABEL (PROVIDED) TO EACH TUBE
PLACE USED *AND* UNUSED TUBES IN BAG PROVIDED
PLACE LAB REQUISITION FORM INTO SLEEVE
FREEZE AT -80°C (OR -20°C, IF NOT AVAILABLE)

Biomarkers & Companion Diagnostics Group: email tammy.piper@igmm.ed.ac.uk or tel +44 (0) 131 651 8605

Cancer Clinical Trials Team: email supremo@isd.csa.scot.nhs.uk or tel 0131 275 6630

BLOOD SAMPLE INSTRUCTIONS: CARDIAC SUB STUDY

The samples need to be separated within 1 hour of sampling and frozen immediately after processing (up to a maximum of 30 minutes).

- A 15ml 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 15 ml of blood in 3 x 5 ml **plastic** EDTA tubes.
- Complete the 5 small labels provided with the patient trial number, patient initials and the date – these will be used to label the small cryovials.
- Complete Laboratory Requisition Form as described on page 7.

BNP SAMPLING:

- Using a ballpoint or marker pen label one of the 5 ml plastic EDTA tubes with the patient trial number, initials and DOB. Put the tube into the rigid container provided. Make sure this container is closed properly and place into the plastic bag with the absorbent pad.
- Place this sample, with the **yellow copy** of the Laboratory Requisition Form, into the stamped, addressed jiffy bag provided and **despatch immediately**. **This sample must arrive within 48 hours**. *(This packaging complies with Royal Mail postal regulations)*.

PLASMA SAMPLING:

- Centrifuge 2 of the 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*. ***Take care not to disturb the cells with the pipette.*** Apply small labels.

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

- Put the filled and any empty, unused cryovials into the small zip topped sample bag provided.
- Fold and place **top copy (white)** into the pocket in the rear of the **sample** bag.
- The **bottom copy (blue)** of the Laboratory Requisition Form Is to be **retained** for your own records
- Place the samples into the freezer at -80°C (-20°C is acceptable for up to 6 months if you do not have access to a -80°C freezer)

- Larger centres will be contacted once per year to arrange collection of frozen samples by courier; however centres that are recruiting faster or those with a -20°C freezer are encouraged to contact us sooner if the available storage space is filling up.

- Smaller centres should contact us directly to arrange a courier collection when they have collected 6 or more patient samples.

- All transport packaging and dry ice will be provided.

- On arrival at the central laboratory the frozen samples will be stored at -80°C until analysis.

SAMPLING PRIORITIES

- We appreciate that it can sometimes be difficult to collect several blood samples from cancer patients. If this is the case please prioritise the blood samples as follows:
    1. BNP whole blood (5 ml EDTA)
    2. Plasma (2 x 5 ml EDTA)

- For each of the individual sample types please put 0.8 ml into each of the cryovials provided. If not enough sample is obtained, it is better to put a larger volume into fewer cryovials than to put a smaller volume in more cryovials i.e. put 0.8 ml into 2 cryovials rather than 0.3 ml into 5 cryovials.

- Complete the form with the number of cryovials filled in the appropriate boxes

- Place all filled and unfilled tubes into the sample bag.

COURIER COLLECTIONS OF FROZEN SAMPLES

Centres will be contacted by the Biomarkers & Companion Diagnostics Group to arrange collection of the frozen samples by courier.
If samples need to be collected before this time then please contact Tammy Piper or Carrie Cunningham (tel: +44 (0) 131 651 8605 or email tammy.piper@igmm.ed.ac.uk) to arrange collection. Please **do not** contact the Clinical Trials Team as this may result in a delay in processing your request.

Please be aware that sample collections will not be arranged for Thursdays or Fridays as samples cannot be delivered to the Biomarkers & Companion Diagnostics Group, Edinburgh over the weekend.

In order to arrange the courier collections please provide the laboratory with the following information:

- How many samples need to be collected
- The name and direct telephone number of the main contact at the centre to liaise with the courier company
- The exact address of where the samples are to be collected from.

The courier company (Davies International) will liaise with the centre directly to arrange uplift of samples.

The following procedure will be used:

- A large polystyrene box will be delivered containing a bag of dry ice and a copy of the instructions to be followed.
- The polystyrene box lid will be taped shut, cut the tape and remove the lid.
- Remove the bag of dry ice from the box.
- Place all the frozen samples into the Bio Bag provided, in their sealed specimen bags, and add sufficient absorbent material (cotton wool or tissue paper, etc) to absorb all potential leakage were the samples to thaw.
- Exclude as much air as possible from the Bio Bag before sealing to make it as compact as possible
- Use some of the dry ice to line the bottom of the polystyrene box, place the Bio Bag on top and then cover with the rest of the dry ice. Replace the lid and seal with tape.
- Close the velcro flaps, swap the address labels in the plastic pocket so the address in Edinburgh is visible and leave the box for collection by the courier from the previously agreed location.
- Contact the courier as soon as the samples are ready for collection Tel: 01869 250350.
- Please note that dry ice should not be handled without gloves.

CHOLESTEROL

Cholesterol should be measured locally at site using a separate sample of blood. This test is required at baseline only.

ALTERNATIVE BNP SAMPLE PROTOCOL (Friday Clinics)

The blood sample taken for BNP analysis must be despatched in the padded envelope provided **immediately** to ensure it arrives at the laboratory within 48 hours. However, please **do not** post samples on a Friday as samples will not be delivered to the laboratory over the weekend.

This protocol has been designed for those centres which routinely hold clinics on Fridays. If your centre holds clinics between Monday and Thursday you should continue to follow the procedures on pages 8-11.

Centres must inform the Biomarkers & Companion Diagnostics Group that they run clinics on a Friday to ensure we provide you the additional supplies required to perform the alternative sampling procedures. Centres should also inform the Clinical Trials Team.

ALTERNATIVE BNP PROCEDURE

- Instead of despatching the BNP sample immediately in the post please complete the following steps:

- Centrifuge the 5ml EDTA tube (whole blood - BNP sample) at 1300g (approximately 2,500 rpm) for 15 minutes. Using the pipette provided, transfer **0.8 ml** of **plasma** into each of the 5 non-coloured cryovials. ***Take care not to disturb the cells with the pipette***. Apply small labels.

- Freeze the 5 x non-coloured BNP sample tubes over the weekend (-20°C is suitable for this)

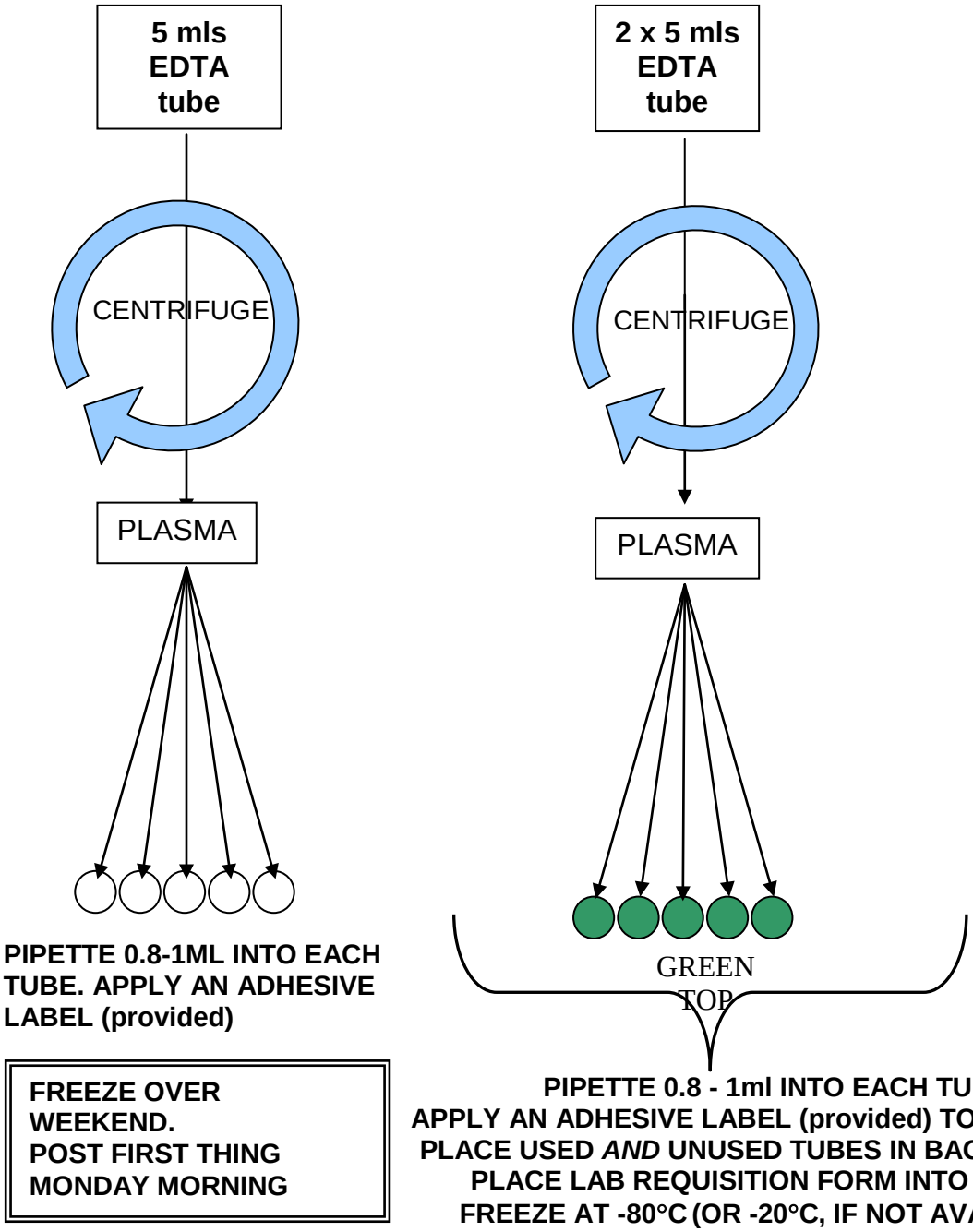
- On the following Monday morning, remove the 5 x non-coloured cryovials from the freezer and place into the rigid container provided. Make sure this container is closed properly and place into the plastic bag with the absorbent pad. Place the samples, with the **yellow copy** of the Laboratory Requisition Form, into the stamped, addressed jiffy bag provided and despatch. *(This packaging complies with Royal Mail postal regulations)*.

- The sample will defrost in the post ready to be processed on arrival at the laboratory. It is **not** necessary to ship these samples on dry-ice.

- If you would like further information or have any questions regarding this procedure please contact the Tammy Piper: +44 (0) 131 651 8605, or email: tammy.piper@igmm.ed.ac.uk).

ALTERNATIVE BNP SAMPLE FLOW CHART: CARDIAC STUDY

A 15ml blood sample should be taken and handled as follows:-



PIPETTE 0.8-1ML INTO EACH TUBE. APPLY AN ADHESIVE LABEL (provided)

FREEZE OVER WEEKEND.
POST FIRST THING MONDAY MORNING

PIPETTE 0.8 - 1ml INTO EACH TUBE
APPLY AN ADHESIVE LABEL (provided) TO EACH TUBE
PLACE USED *AND* UNUSED TUBES IN BAG PROVIDED
PLACE LAB REQUISITION FORM INTO SLEEVE
FREEZE AT -80°C (OR -20°C, IF NOT AVAILABLE)

or tel +44 (0) 131 651 8605
Cancer Clinical Trials Team: email supremo@isd.csa.scot.nhs.uk or tel 0131 275 6630

NOTES

Trans-Supremo & Cardiac Sub Studies

INSTRUCTIONS

FOR BLOOD SAMPLE

PROCESSING

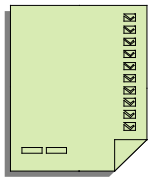
FOR

TRANS-SUPREMO

SUPPLIES FOR TRANS-SUPREMO

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 4, including 1 spare)

Blood sample flow chart for Trans Supremo – see page 19

When you have **2 blood sample kits remaining**, please **photocopy** the Blood Sample Kit Request Form (Appendix 1), **complete** and **fax** to Biomarkers & Companion Diagnostics Group, Edinburgh, UK (+44 (0) 131 651 8711).
5 kits will be despatched immediately. Please do not email requests to the clinical trials office as this may result in a delay in the kits being sent to you.

The following equipment is required but not provided:

- Venepuncture kit
- Plain tube with separator gel; 10 ml (SST Vacutainer or S-Monovette Serum *or equivalent*)
- EDTA tubes; 3 x 5 ml *or equivalent*
- 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 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 to be applied by the laboratory 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 7 digit trial number = 3 digit centre number and 4 digit trial number, e.g. B07 9999
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 = whether first visit; second visit; 5 year follow-up, etc. If recording details of local or distant recurrence please include details of the site of recurrence.
5. Date of sampling = DD MM YY e.g. 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**
9. Sign and date the **'Informed Consent Checked'** box to indicate to the laboratory staff that the patient has given informed consent to participate in the biological sub-study – this section is to be completed by the person filling out the form, ***not*** the patient.
   Please do not include copies of the consent form with the sample as the central laboratory must not receive any paperwork with the patient's full name

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

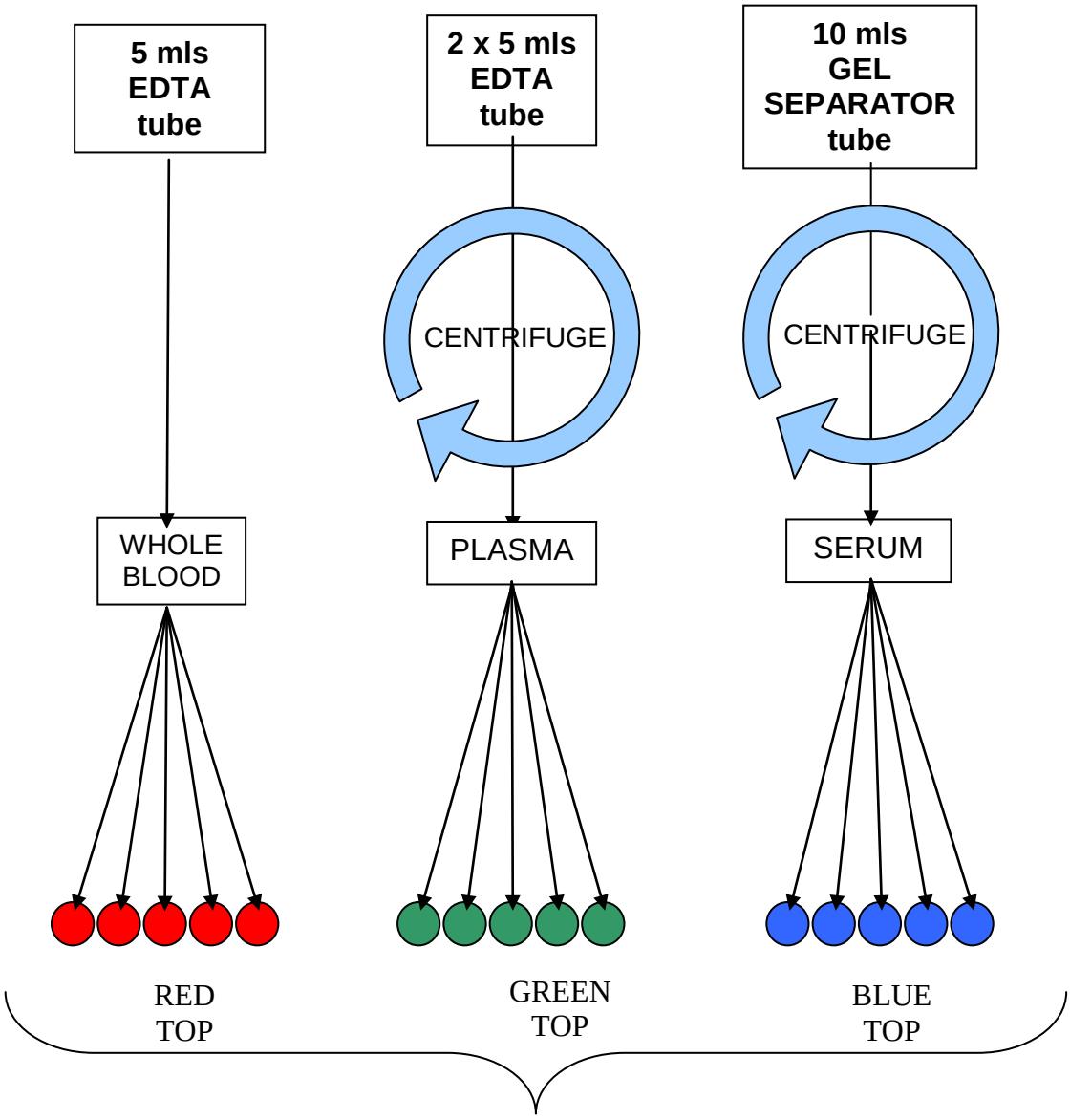
The **top copy (white)** is to be put into the pocket in the **FROZEN sample** bag and placed in the freezer at -80°C (or -20°C, if not available). This is to be despatched on request with the sample to the Biomarkers & Companion Diagnostics Group, Edinburgh.

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

The **second copy (yellow)** is an additional copy and can be sent with the samples or retained for your records.

BLOOD SAMPLE FLOW CHART: TRANS-SUPREMO

A 25 ml blood sample should be taken at the following time points: first visit (baseline) and at disease recurrence (local and/or distant relapse). The samples should be processed as follows:-



PIPETTE 0.8 - 1ml INTO EACH TUBE
APPLY AN ADHESIVE LABEL (provided) TO EACH TUBE
PLACE USED *AND* UNUSED TUBES IN BAG PROVIDED
PLACE LAB REQUISITION FORM INTO SLEEVE
FREEZE AT -80°C (OR -20°C, IF NOT AVAILABLE)

Biomarkers & Companion Diagnostics Group: email tammy.piper@igmm.ed.ac.uk
or tel +44 (0) 131 651 8605
Cancer Clinical Trials Team: email supremo@isd.csa.scot.nhs.uk or tel 0131 275 6630

BLOOD SAMPLE INSTRUCTIONS: TRANS-SUPREMO

The samples need to be separated within 1 hour and frozen immediately after processing (up to a maximum of 30 minutes).

- A 25 ml blood sample to be taken at baseline (first visit) and at disease recurrence, (local and/or distant relapse) - Collect 10 ml of blood in a plain tube with separator gel and 15 ml of blood in 3 x 5 ml **plastic** EDTA tubes.
- Complete the 15 small labels provided with the patient trial number, patient initials and the date – these will be used to label the small cryovials.
- Complete Laboratory Requisition Form as described on page 7.

WHOLE BLOOD SAMPLING:

- 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 small labels.

PLASMA SAMPLING:

- 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*. ***Take care not to disturb the cells with the pipette.*** Apply small labels.

SERUM SAMPLING:

- 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 small labels.

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

- Put the filled and any empty, unused cryovials into the small zip topped sample bag provided.
- Fold and place **top copy (white)** into the pocket in the rear of the **sample** bag.
- The **bottom copy (blue)** of the Laboratory Requisition Form is to be **retained** for your own records.
- The **second copy (yellow)** is an additional copy and can be sent with the samples or retained for your records.
- Place samples into your freezer at -80°C (-20°C acceptable for up to 6 months if you do not have access to -80°C).

- Larger centres will be contacted once per year to arrange collection of frozen samples by courier; however centres that are recruiting faster or those with a -20°C freezer are encouraged to contact us sooner if the available storage space is filling up.
- Smaller centres should contact us directly to arrange a courier collection when they have collected 6 or more patient samples.
- All transport packaging and dry ice will be provided.
- On arrival at the central laboratory the frozen samples will be stored at -80°C until analysis.

SAMPLING PRIORITIES

- We appreciate that it can sometimes be difficult to collect several blood samples from cancer patients. If this is the case please prioritise the blood samples as follows:

    1. Serum (10 ml gel separator)
    2. Plasma (2 x 5 ml EDTA)
    3. Whole blood (1 x 5 ml EDTA)

- For each of the individual sample types please put 0.8 ml into each of the cryovials provided. If not enough sample is obtained, it is better to put a larger volume into fewer cryovials than to put a smaller volume in more cryovials i.e. put 0.8 ml into 2 cryovials rather than 0.3 ml into 5 cryovials.
- Complete the form with the number of cryovials filled in the appropriate boxes
- Place all filled and unfilled tubes into the sample bag.

COURIER COLLECTIONS OF FROZEN SAMPLES

Centres will be contacted by the Biomarkers & Companion Diagnostics Group to arrange collection of the frozen samples by courier.
If samples need to be collected before this time then please contact Tammy Piper or Carrie Cunningham (tel: +44 (0) 131 651 86 or email tammy.piper@igmm.ed.ac.uk) to arrange collection. Please **do not** contact the Clinical Trials Team as this may result in a delay in processing your request.

Please be aware that sample collections will not be arranged for Thursdays or Fridays as samples cannot be delivered to the Biomarkers & Companion Diagnostics Group, Edinburgh over the weekend.

In order to arrange the courier collections please provide the laboratory with the following information:

- How many samples need to be collected
- The name and direct telephone number of the main contact at the centre to liaise with the courier company
- The exact address of where the samples are to be collected from.

The courier company (Davies International) will liaise with the centre directly to arrange uplift of samples.

The following procedure will be used:

- A large polystyrene box will be delivered containing a bag of dry ice and a copy of the instructions to be followed.
- The polystyrene box lid will be taped shut, cut the tape and remove the lid.
- Remove the bag of dry ice from the box.
- Place all the frozen samples into the Bio Bag provided, in their sealed specimen bags, and add sufficient absorbent material (cotton wool or tissue paper, etc) to absorb all potential leakage were the samples to thaw.
- Exclude as much air as possible from the Bio Bag before sealing to make it as compact as possible
- Use some of the dry ice to line the bottom of the polystyrene box, place the Bio Bag on top and then cover with the rest of the dry ice. Replace the lid and seal with tape.
- Close the velcro flaps, swap the address labels in the plastic pocket so the address in Edinburgh is visible and leave the box for collection by the courier from the previously agreed location.
- Contact the courier as soon as the samples are ready for collection Tel: 01869 250350.
- Please note that dry ice should not be handled without gloves.

NOTES

INSTRUCTIONS

FOR

TISSUE BLOCK

PROCESSING

FOR

TRANS SUPREMO

INSTRUCTIONS FOR TISSUE BLOCK PROCESSING

- Written consent is required from patients who wish to donate tissue for research.
- Tissue blocks will be requested for all patients who consent to the pathology sub-study, at baseline and again at times of local and/or distant recurrence or at first diagnosis of contra-lateral breast cancer, if available.
- Small 0.6 mm cores will be extracted from the blocks for tissue micro-array construction.
- A kit will be supplied for each block requested consisting of;
  - Pathology Request Form (Appendix 3)
  - Small sample bag
  - Addressed, padded envelope
- Complete the Pathology Request Form with all the information indicated.
- **Please note that to ensure unique identification by the data matrix label system to be applied by the laboratory no copying or combined use of the form is allowed!**
- Sign and date, **'Informed Consent Checked'** box to indicate to the laboratory staff that the patient has given informed consent for their tissue to be used for the biological sub-study – this section is to be completed by the person filling out the form, ***not*** the patient.
  Please do not include copies of the consent form with the sample as the central laboratory must not receive any paperwork with the patient's full name
- 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.
- Please send **one** representative tumour block for each patient
- Place the block and form into the addressed, padded envelope and return to: Trans Supremo Sub Study, Biomarkers & Companion Diagnostics, Edinburgh University, Cancer Research Centre, Western General Hospital, Road South, Edinburgh, EH4 2XR, UK
- Blocks will be stored in a secure facility and all data will be held in a confidential manner. Blocks will be returned to the source pathology department upon completion of processing.

If you experience any difficulties or have any questions, please contact the Biomarkers & Companion Diagnostics Group.

NOTES

Trans-Supremo & Cardiac Sub Studies

INSTRUCTIONS

FOR THE

PATHOLOGY AUDIT

INSTRUCTIONS FOR THE PATHOLOGY AUDIT

- All patients who have consented to the main MRC Supremo trial will have a tissue block requested for participation in the quality assurance pathology audit.
- A separate consent **is not** required as the audit is part of the main trial which the patient has already consented to.

***Note 1:* Blocks sent for Trans-Supremo patients are automatically included in the pathology audit so a separate request for these samples will not be made.**

- A kit will be supplied for each block requested consisting of;
  - Pathology Request Form - clearly labelled with 'PATHOLOGY AUDIT'
  - Small sample bag
  - Addressed, padded envelope

- Complete the Pathology Request Form with all the information indicated.
  - The 'Informed Consent Checked' box will be scored through as separate consent is not required for the pathology audit. Please do not write anything in this box – this is used solely to indicate consent given for Trans-Supremo

- 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.

- Please send **one** representative tumour block for each patient

- Place the block and form into the addressed, padded envelope and return to:
  Trans Supremo Sub Study, Biomarkers & Companion Diagnostics Group, Edinburgh University
  Cancer Research Centre, Western General Hospital, Crewe Road South
  Edinburgh, EH4 2XR, UK

- Blocks will be stored in a secure facility and all data will be held in a confidential manner.

- Sections will be taken from the tissue block and stained with haematoxylin and eosin at the central laboratory using standardised methods. These slides will be circulated to 3 trial pathologists for blinded review. They will assess the type of tumour, grade and presence of lymphovascular invasion.

***Note 2:* These blocks will be returned to the department of origin as soon as the pathology audit has been performed and the results have been received for the sample.**

ALTERNATIVE INSTRUCTIONS FOR THE PATHOLOGY AUDIT

- If the pathology department refuses to send blocks for the audit we will accept two H&E slides they have prepared locally.

- If you experience any difficulties or need any advice please contact the Biomarkers & Companion Diagnostics Group

NOTES

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: Tammy Piper, Monika Sobol

Fax no: +44 (0) 131 651 8711

(PLEASE PRINT DETAILS)

Contact name _____ Date _____

Email address_____

Centre number_____

Hospital _____

Address _____

_____

_____

_____

For Laboratory Use: - Kits despatched

Comments

Signature Date

If you experience any difficulties or have any questions, please contact the Biomarkers & Companion Diagnostics Group by emailing tammy.piper@igmm.ed.ac.uk; or telephoning +44 (0) 131 651 8605.

Trans-Supremo & Cardiac Sub Studies

Appendix 2

Affix Data matrix label (barcode)

LABORATORY REQUISITION FORM (EXAMPLE)

PATIENT TRIAL NUMBER ☐☐☐☐☐☐☐

Informed Consent (Trans-Supremo) checked:
Name

Sign

Date

PATIENT INITIALS **(First:Middle:Last)** ☐☐☐

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

SAMPLE INFORMATION
(**Please tick** √)

First visit (screening) ☐ Post chemo/preRT ☐ Post RT/other therapy ☐

1 yr visit ☐ 5 yr visit ☐ 10 yr visit ☐ Local recurrence ☐ Distant recurrence ☐

Site _____

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

TIME SAMPLE TAKEN **(HH:MM)** ☐☐ : ☐☐

TIME CENTRIFUGED **(HH:MM)** ☐☐ : ☐☐

TIME SAMPLE FROZEN **(HH:MM)** ☐☐ : ☐☐

N° of 0.8ml samples stored:-

SERUM ☐

PLASMA ☐

WHOLE BLOOD ☐

DO NOT PHOTOCOPY

White copy with frozen samples
Yellow copy with BNP sample
Blue copy to file

NAME OF STUDY NURSE / TECHNICIAN:
(Capital letters)

Sign Date

Affix Data matrix label (barcode)

Appendix 3
PATHOLOGY REQUEST FORM (EXAMPLE)

FAO:
Research nurse (Name) _____

Pathologist (Name) _____

Centre ID _____

Informed Consent (Trans-Supremo) checked:
Name

Sign

Date

Please forward paraffin block(s) relating to the patient below, to the Biomarkers & Companion Diagnostics Group, Edinburgh. Blocks **must** be from the surgical resection and **not** core biopsies.

PATIENT TRIAL NUMBER

PATIENT INITIALS
(First:Middle:Last)

PATIENT DATE OF BIRTH
(DD/MM/YY)

Full Pathology number
(invasive primary tumour)

DO NOT PHOTOCOPY

Block despatched to Biomarkers & Companion Diagnostics Group, Edinburgh UK
Pathology Number:

Type of sample/origin:

Primary ☐ Local relapse ☐ Distant relapse ☐ Contralateral breast cancer ☐

Comments:

Name of person completing the form: (Capital letters)

Signature Date

Block returned to Pathology Dept:-

Signature Date

White copy with block

Blue copy to file

Appendix 4

PATHOLOGY AUDIT

PATHOLOGY REQUEST FORM (EXAMPLE)

FAO:
Research nurse (Name) _____

Pathologist (Name) _____

Centre Name/ ID _____

Informed Consent (Trans-Supremo) checked:
Name

Sign

Date

Please forward one representative paraffin embedded tumour block relating to the patient below, to the Biomarkers & Companion Diagnostics Group, Edinburgh. Blocks MUST be from the surgical resection and NOT core/needle biopsies.

PATIENT TRIAL NUMBER ☐☐☐☐☐☐

PATIENT INITIALS **(First:Middle:Last)** ☐☐☐

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

Full Pathology number (invasive primary tumour) ☐☐☐☐☐☐☐☐☐☐

Block despatched to Biomarkers & Companion Diagnostics Group, Edinburgh UK
Pathology Number:

Type of sample/origin:

Primary ☐ Local relapse ☐ Distant relapse ☐ Contralateral breast cancer ☐

Comments:

Name of person completing the form: (Capital letters)

Signature Date

Block returned to Pathology Dept:-

Signature Date

White copy with block / slides

Blue copy to file

DO NOT PHOTOCOPY