BLOOD COLLECTION AND STORAGE

1 Scope

This SOP describes the procedure for performing a peripheral vein blood draw and subsequent storage of samples.

2 Purpose

To describe the processes used for the pre-radiotherapy blood collection within the REQUITE study, including the preparation of the blood tubes, documentation of the blood draw and storage.

3 Process Owner (Responsibility)

The principal investigator of each study site is responsible for ensuring the implementation of this SOP and ensuring specific training is provided to all site staff who will be performing blood collection.

4 Health and Safety

All employees should make themselves aware of any health and safety issues related to the use of blood and bodily fluids, and demonstrate adequate training has been received. Employees are responsible for ensuring the health and safety of themselves and others in the work place.

5 References / Related Documents

- RQ2b Blood Collection Form_2nd Attempt[1]
- RQ6 Sample Kits and Courier Shipping SOP
- RQ7 Radiation Induced Lymphocyte Assay SOP
- RQ11 Database Manual

All related documents will be available at www.REQUITE.eu

6 Materials and Equipment

6.1 General / Inventory / Equipment

Equipment	Recommended Manufacturer / Type	Product Code
-80°C Freezer	New Brunswick	
Laser Scanner	Bar Code Data Limited	Motorola LS1203-7AZU0100ER

6.2 Consumable Supplies

Material	Supplier	Product Code
BD Vacutainer Safety-Lok blood collection set[2]	Becton Dickinson	368654
PAXgene blood RNA tube	Becton Dickinson	762165
Lithium Heparin blood tube	Becton Dickinson	367526
EDTA blood tube (provided by CIGMR)	Becton Dickinson	367525

[1] This form should be completed only if initial blood draw failed

[2] Recommended method of taking blood, but other BD Vacutainer methods for blood draw following local guidelines/ procedures could also be used like e.g. BD Ref. 360213 which fits into a holder BD Ref. 364815.

Material	Supplier	Product Code
Bar-coded labels	CIGMR	various
Gloves, Tourniquet	-	-
Cotton wool, small plasters, alcohol wipes	-	-
Cryobox (122 x 122 x 128 mm W x L x H; order with 5 x 5 divider) or any other repository which is cold-resistant	-	-

6.3 Required Documents

- RQ2 Blood Collection Form (electronically or on paper)
- RQ3 Screening and Recruitment Log
- RQ8 Sample Tracking Log

All related documents are available at www.REQUITE.eu

7 Method

7.1 Preparations

Each centre will be provided with REQUITE sample kits by the Centre for Integrated Genomic Medical Research (**CIGMR**) in the UK. The unique REQUITE identifier (RQ-ID) displayed on each kit will be the patient´s study number for the duration of the study. Issue of the RQ-ID for each patient will be done by allocation of the next available sample kit.

Each sample kit contains:

- One pre-labelled, barcoded EDTA blood tube.
- Additional barcode labels for use on the other REQUITE blood tubes i.e. Lithium Heparin and/or PAXgene (N.B. blood tubes not included).
- 'Blank' barcode labels for use as required on e.g. consent form, patient notes etc. These 'blank' labels only display the RQ-ID, site name and barcode.

For more information on requesting REQUITE sample kits, see RQ6 Sample Kits and Courier Shipping SOP.

The following steps should be performed by a member of the research team in preparation for the blood draw:

7.1.1 Take the next available REQUITE sample kit to allocate a RQ-ID for that patient.

7.1.2 Label a PAXgene or Lithium Heparin tube with the appropriate barcode label provided in the kit. Please ensure that the barcode label is fixed horizontally, as straight as possible on the tube (see Figure 1). Wrap the label once around the tube, to laminate and protect the label. Try to pull it over the tube without causing air bubbles. Incorrect placement of the label will result in the barcode being unreadable by the hand held scanners.

7.1.3 If there is no computer access in the blood draw room, print a copy of the RQ2 Blood Collection Form and affix a barcode label displaying the matching RQ-ID.

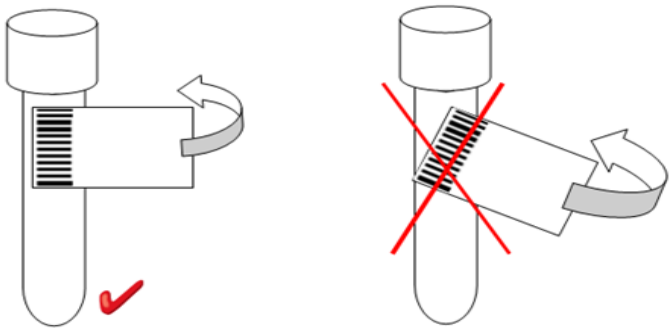


Figure 1: Labelling PAXgene or Lithium Heparin tubes for REQUITE

The following steps should be performed in the blood draw room by the person who will be taking the samples:

7.1.4 Ensure consent has been taken for the patient´s participation in the REQUITE study: confirm verbally with the person who took consent if different from the person responsible for blood draw or check the REQUITE database / RQ3 Screening and Recruitment Log (see RQ11 Database Manual).

7.1.5 Check the EDTA tube, PAXgene or Lithium Heparin tube, consent form and blood collection form (if printed) all display the same RQ-ID.

7.1.6 Complete the RQ2 Blood Collection Form (paper or online, depending on local arrangements). If you are collecting any additional blood samples at your site for sub-studies etc. (i.e. in addition to the required EDTA and either PAXgene or Lithium Heparin) please make a note of this in the RQ2 Blood Collection Form.

7.1.7 **OFF-PROJECT:** When filling out the RQ2 Blood Collection Form, please specify whether the sample is 'Off-Project' or not. Off project patients are those recruited in addition to what was specified in the original REQUITE application (see recruitment table by site below), e.g. MSSM recruiting breast patients.

Target enrolment for REQUITE

Country	Site	Breast	Prostate	Lung	TOTAL
Belgium	UGENT	200	200	100	**500**
Belgium	KULEUVEN	500	150	200	**850**
France	ICM*	500	0	300	**800**
Germany	DKFZ*	400	400	0	**800**
Italy	INT*	100	200	80	**380**
Spain	FPGMX*	100	350	120	**570**
UK	CNFT	0	200	200	**400**
UK	ULEIC	300	200	100	**600**
USA	MSSM	0	400	0	**400**
TOTAL		**2,100**	**2,100**	**1,100**	**5,300**

* Or third parties thereof

'Off-Project' samples should be stored locally and permanently at the recruitment site. They are collected using resources independent of those provided by the REQUITE project. Also, the Subject IDs given on 'Off Project' sample kits are spatially separated from those that are part of the

original REQUITE target. For clarification on off-project samples, please ask your principal investigator or the observational study manager.

REQUITE IDs	Off-Project IDs
RQ50001-6	RQ52001-5
RQ50002-4	RQ52002-7
RQ50003-7	RQ52003-6

7.2 Blood draw

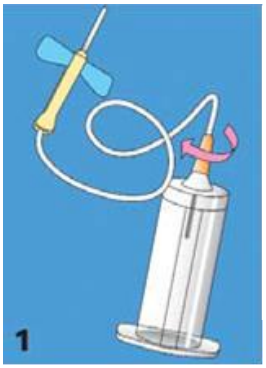
Blood draw should be performed in a seated or lying position depending on local practice or patient preference. The arm should be supported on a pillow or a blood wedge. Gloves should be worn to ensure the rules of asepsis and antisepsis are being followed as well as maintaining sterile conditions for venipuncture. Before the puncture, a tourniquet should be applied, which can be loosed as soon as the blood flows. The tourniquet should not be applied for more than 1 min. For the puncture please select a large vein in the arm of the patient and disinfect the region with an antiseptic.

Note: Please do not forget to fill out the RQ2 **Blood Collection Form** and record the time of blood sample collection. Patients with known HIV infection / infectious hepatitis are not eligible for the REQUITE study. The blood samples should be taken prior to the start of the radiotherapy.
The labeled sample tubes need to be filled in the following order:

Order	Blood Type	Quantity	Recruitment site*	Remarks
1st	EDTA-Blood	1 x 10 ml	All	-
2nd	Lithium Heparin	1 x 10 ml	UMONT, DKFZ, ULEIC	Please collect **prior to the start of the chemotherapy**.
3rd	PAXgene-RNA	1 x 2.5 ml + stabilizer	CNFT, FPGMX, UGENT, MSSM, INT, KULEUVEN, MAASTRO	Please **do not fill** the PAXgene RNA tube first as as the air from the tubing will fill the blood tube, leaving little to no room for blood volume.

* Or third parties thereof

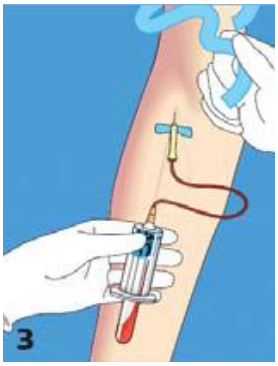
Instructions[3]:



Turn the BD Safety-Lok™ Blood Collection-Set in the BD Vacutainer® holder and remove the protective plastic cover.
Please note that using BD Safety Lok will reduce the risk of additive reflux from the PAXgene tube into the vein. Therefore BD recommends using the blood collection set BD Safety Lok for PAXgene tubes.

[3] All pictures courtesy of © BD-Diagnostics 28.01.2014

Run the venepuncture as usual on the bowed arm. If you have punctured the vein, you will see a small amount of blood flowing in the tube.

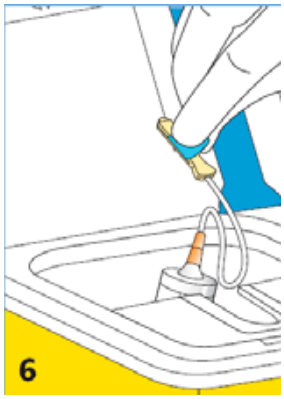


Insert the tube into the holder and release the vacuum while pressing the tube completely into the holder. **Please note for PAXgene:** It is preferable to hold the tube **vertical below the arm** to avoid additive reflux.
If the tube has filled entirely, pull it out of the holder and insert the next tube into the holder if applicable.

Attention:
Invert all blood tubes for **10 times** slowly over head, **especially the PAXgene tubes**.

After the blood collection pull the needle from the vein and cover the puncture site with gentle pressure using a sterile swab.

Push the yellow shield forward until the safety shield is locked in place.



Discard the blood collection set with the holder in an appropriate container following in-house regulations.

After that the blood tubes should be placed in cryoboxes or any other suitable repository for storage.
Lithium Heparin sample is scheduled for live cell apoptosis assays (see RQ7 Radiation Induced Lymphocyte Assay SOP).

7.3 If blood draw fails

7.3.1 Blood draw is considered to have failed if the volume collected is less than 1ml. In the case of a blood draw failure, please record this in the RQ2 Blood Collection Form.

7.3.2 Do not discard the pre-labelled EDTA blood tube provided by CIGMR because it contains the CIGMR internal tube ID (= a small 2D barcode in the right upper corner of the label). If some blood was collected but less than 1ml, freeze as normal at -80^{0}C but still record as a blood draw fail and make a second attempt. The patient should remain on study and questionnaires should be completed at all the usual time points.

7.3.3 Try to repeat the EDTA blood draw at an appropriate time point either on the same date or at least six months following the end of radiotherapy. Note that replacement EDTA tubes are not provided by CIGMR, so it is recommended that a small number of 10ml EDTA blood tubes are available for use in the event of a blood draw failure. Ensure the new EDTA tube is labelled with the correct REQUITE ID barcode (spare labels are provided by CIGMR in the sample kit).

7.3.4 Do <u>not</u> use a new blood sample kit because it includes a <u>different</u> REQUITE ID than the one you used before for that patient! Each patient can have only <u>one</u> REQUITE ID during the period of the study.

7.3.5 Complete the 'RQ2b Blood Collection Form_2nd Attempt'.

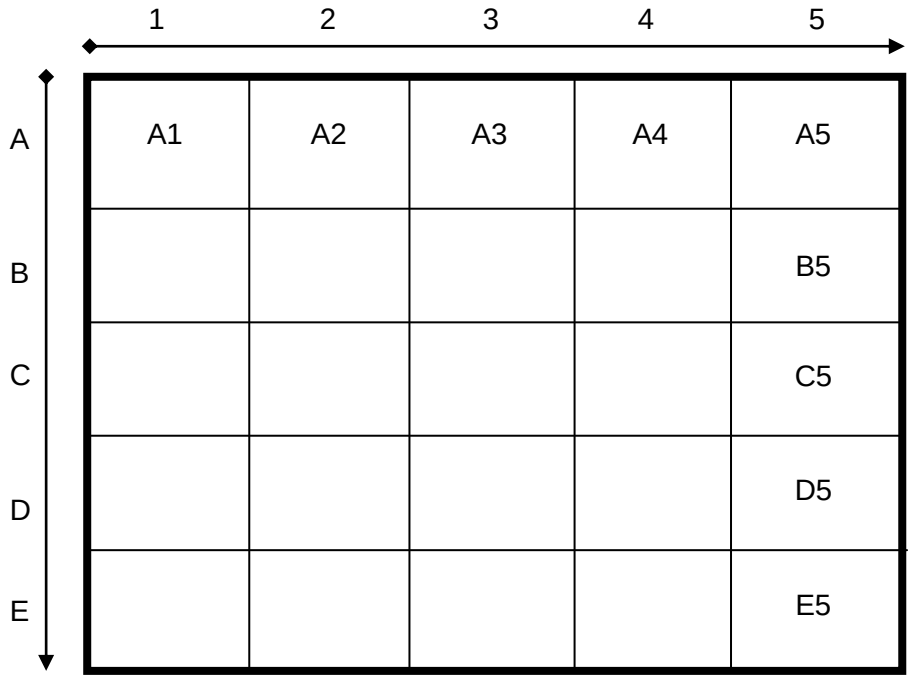
7.3.6 Store the 2nd attempt blood tube together with the failed original tube which contains the 2D code; CIGMR internal tube ID, in one box and return <u>both</u> to the biobank at CIGMR.

7.3.7 Do not try to collect LiH or PAXgene blood samples post-radiotherapy.

7.4 Cryoboxes

The blood samples should be stored in cryoboxes or any other appropriate repository. Please do not store different blood collection tubes in the same cryobox. Use separate cryoboxes for PAXgene, EDTA Blood, Plasma and Buffy Coat samples. Also distinguish between 'Off-project' and REQUITE samples, store them separately from each other. Every box needs a unique label (see Figure 2). The position of each sample should be documented in the excel-sheet RQ8 Sample Tracking Log.The columns of the cryobox are numbered (1,2,3,…) and the rows marked with letters in alphabetical order (A,B,C,...).

Note: The following image shows each position for a **5 x 5** divider cryobox:



7.4.1 In order to ensure all positions are labelled correctly, please orientate the box so that position A1 is in the upper left hand corner (see Figure 2).

7.4.2 Each cryobox (lid and box) must be labelled appropriately and either using a sticker or freezer resistant marker pen. It is recommended that different blood types are stored separately to aid sample tracking and avoid the need for cherry picking the EDTA samples destined for CIGMR. The following label information is suggested (please use UPPER-CASE):

- Study name (please add ‚Off-Project' if applicable)
- Recruitment site
- Blood type (EDTA Blood, PAXgene, Plasma, Buffy Coat)
- Box_No. for REQUITE samples

  EDTA Blood – E_xx (e.g. E_01)

  PAXgene – PAX_xx (e.g. PAX_01)

  Plasma – PL_xx (e.g. PL_01)

  Buffy Coat – BC_xx (e.g. BC_01)

- Box_No. for Off-Project samples

  EDTA Blood – E_xx_Off (e.g. E_01_Off)

  PAXgene – PAX_xx_Off(e.g. PAX_01_Off)

  Plasma – PL_xx_Off (e.g. PL_01_Off)

  Buffy Coat – BC_xx_Off (e.g. BC_01_Off)

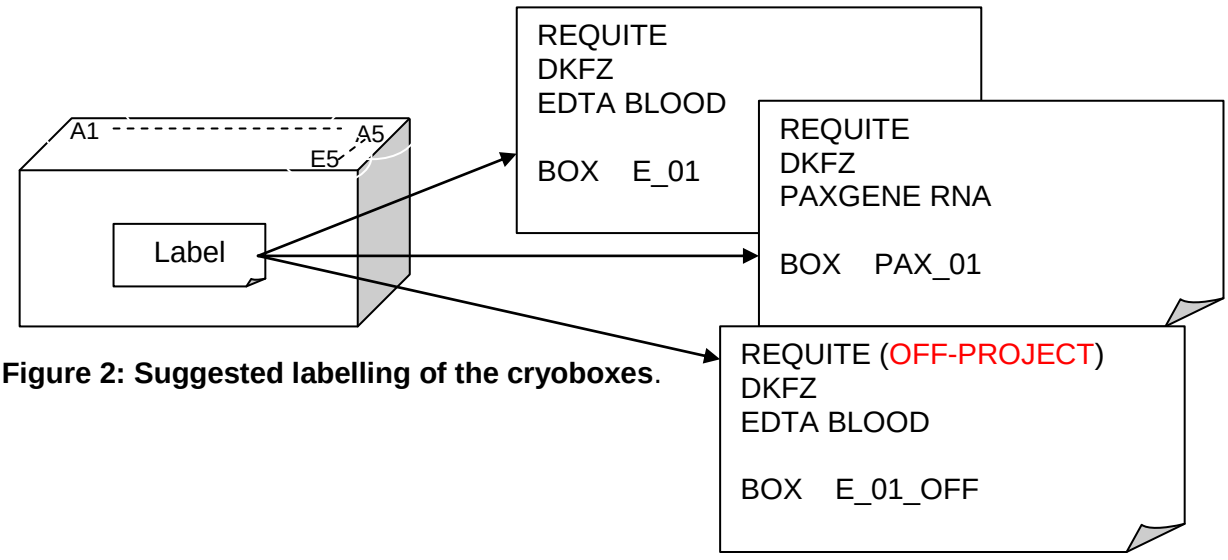


Figure 2: Suggested labelling of the cryoboxes.

Note: If you have a professional LIMS data management system or other administrative software you can add more information such as a box inventory-id or person in charge.

7.5 Storage

7.5.1 EDTA Blood (for DNA)

- **Interim** storage at -80°C after blood collection; shipment to CIGMR at regular intervals as described in RQ6 Sample Kits and Courier Shipping SOP. No processing required.

7.5.2 PAXgene tube (for future analysis)

- **Permanent** local storage at -80°C after blood collection. No processing required.
- Please store the PAXgene tube upright at room temperature for a minimum of 2 hours and a maximum of 72 hours **before transferring to freezer** at -80°C or processing.

7.5.3 Lithium Heparin (for Apoptosis assay)

- **Immediate** Processing within **< 24 h** (see RQ7 Radiation Induced Lymphocyte Assay SOP)
- Plasma and buffy coat separated at the processing stage to be stored appropriately

7.5.4 Off-Project Samples (EDTA and PAXgene)

- **Permanent** local storage at -80°C after blood collection. No processing required.

7.6 Documentation

In order to ensure the traceability of the EDTA blood samples that are sent to CIGMR, a sample tracking record is required. For recruiting sites that do not have a professional LIMS or other administrative software, the provided excel-sheet, **RQ8 Sample Tracking Log** should be used. This document must be updated each time a blood sample is stored on site to ensure that all samples are accounted for. The excel-sheet **RQ8 Sample Tracking Log** contains the following variables:

REQUITE_COHORT	PROJECT_TYPE	BLOOD_TYPE	FREEZER_ID	REQUITE_ID (*please scan*)	BOX	POSITION	VOLUME_ML	COMMENTS	SHIPPING_DATE_DD_MM_YYYY	CIGMR_CONFIRMATION_OF_RECEIPT
DKFZ	REQUITE	EDTA	1123	RQ12344-1	E_01	A01	10			
DKFZ	REQUITE	PAXgene	1145	RQ12344-1	PAX_01	A01	5			
DKFZ	REQUITE	EDTA	1123	RQ12345-6	E_01	A02	10			
DKFZ	REQUITE	PAXgene	1145	RQ12345-6	PAX_01	A02	10			
DKFZ	REQUITE	Plasma	1145	RQ12345-6	PL_01	A01	3			
DKFZ	REQUITE	Buffy Coat	1145	RQ12345-6	BC_01	A01	2			
DKFZ	Off-Project	EDTA	1123	RQ12501-2	E_01_Off	A01	10			
DKFZ	Off-Project	PAXgene	1145	RQ12501-2	PAX_01_Off	A01	10			
DKFZ	Off-Project	PAXgene	1145	RQ12509-6	PAX_01_Off	A02	10			

- **REQUITE_COHORT**
  Please enter the name of your cohort (UGENT, KULEUVEN, UMONT, DKFZ, INT, FPGMX, CNFT, ULEIC, MSSN, MAASTRO)

- **PROJECT_TYPE**
  Please differ between ‘REQUITE’ samples and ‘Off-Project’ samples.

- **BLOOD_TYPE**
  Either EDTA, PAXgene, Plasma or Buffy Coat.
  Only EDTA-Blood is shipped to CIGMR.

- **REQUITE_ID**
  Please scan the RQ-ID with your Barcode-Scanner to avoid any typographical errors. Place the cursor on the appropriate ID-field.

- **FREEZER_ID**
  Please give the freezer_ID or name of the freezer where the box is stored at -80°C.

- **BOX**
  Please give the box number for every sample.

- **POSITION**
  Please give the position at which the sample is located in the box (see 7.3 Cryoboxes)

- **VOLUME_ML**
  Please enter the estimated volume of the tube.

- **COMMENTS**
  Optional field. Please report all damaged samples (i.e. tubes that have been dropped, labels have ripped, etc), missing samples or any other information regarding the sample.

- **SHIPPING_DATE_DD_MM_YYYY (only necessary for EDTA shipment to CIGMR)**
  Please enter the shipping date dd.mm.yyyy / e.g. 28.01.2014, when you have sent the EDTA samples to CIGMR.

- **CIGMR_CONFIRMATION_OF_RECEIPT (only necessary for EDTA shipment to CIGMR)**
  Obtain confirmation from CIGMR that the samples have arrived in a good condition in Manchester, for example 'received in good condition 30.01.2014'. Please keep the confirmation email for your security.

7.7 Shipment to CIGMR

The shipment of the EDTA blood tubes to CIGMR takes place at regular intervals. We recommend you to arrange transportation when 50 or 100 blood samples are collected. UK bank holidays must also be considered when booking to avoid shipments sitting in the loading bay for prolonged periods of time. Additional details with regards to booking shipments, completing sample tracking records and any other courier related queries can be found in document RQ6 Sample Kits and Courier Shipping SOP.

8 Document History

8.1 This is the version 1.6 of the SOP.