Australian Breast Cancer Tissue Bank

STANDARD OPERATING PROCEDURE

Title: Blood Sample Processing and Storage

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Related SOPs

SOP 4.1.007 Blood Collection Process
SOP 5.1.006 Data Entry: Blood and Tissue Samples
SOP 5.1.012 Instructions for Label Printing for Cryovials
SOP 5.1.016 Data Entry: Management of Storage Containers
SOP 8.1.004 Operation and Maintenance Schedule Centrifuge 5702R
SOP 10.1.011 Transfer of Material between Collection Sites

Revision History

Version	Issue Date	Reason for amendment	Sections amended	Replaces Version	Revised by (initials)
1.0	2006	Original document	n/a	n/a	JEC
1.2	14/11/2006	Review and reformat (version 1.1 skipped)	All	1.0	JEC
1.3	06/02/2009	Review and reformat	All	1.2	JEC
1.4	21/09/2010	Storage box info added	2 (13); 3(7)	1.3	JEC
1.5	15/07/2016	New template and details added Change of title; site IDs removed; blood holding temperature and timing clarified (2.2 & 2.3).	All	1.4	JH

Purpose

To process ABCTB blood specimens so that they are of maximal benefit for unspecified future research applications. Although local arrangements for blood collection will vary between ABCTB collection sites, consistency of blood specimen handling and processing according to this procedure should be followed at all sites. Since variance in pre-analytical conditions can impact downstream research applications, it is important that any deviations from this SOP are recorded accurately.

Scope

Blood collection from ABCTB donors according to the ethical guidelines of the project should be coordinated by Tumour Bank Officers and clinical staff following SOP 4.1.007. This procedure follows on directly from SOP 4.1.007 and the ABCTB staff member/s collecting and processing blood specimens should be familiar with both SOPs before beginning.

Materials & Equipment

- Centrifuge (Eppendorf, 5702R)
- Pasteur pipettes (1 mL)
- P100 pipette and tips
- Spray bottle of 70% Ethanol and paper wipes
- Pre-barcoded cryovials

  All blood aliquots must be stored in the correct cryovials supplied with unique 10 digit barcodes beginning with the prefix for the site where they were processed. Wherever possible, vials should be used in barcode order. In preparation for blood collection it is best to have a stored rack of empty vials in order ready for use. Use a sterile rack with a lid (e.g. a freezer box).
- Labels for aliquots and clear over-laminate labels
- Blood spot cards (Whatman 903 protein saver cards)
- Dewar of liquid nitrogen for snap-freezing

  Note: Where possible always use liquid nitrogen to snap freeze blood samples. If liquid nitrogen is not available at your site, then place samples on dry ice during processing.
- Dry ice for transfer of specimens to storage
- Serum red top tube for centrifuge balance

Method

Important: Treat all human biological material as potentially biohazardous and follow institutional safety procedures for handling and disposal accordingly.

1. Preparation

1.1. Preparation for blood processing should always be carried out in advance to keep processing times at a minimum.

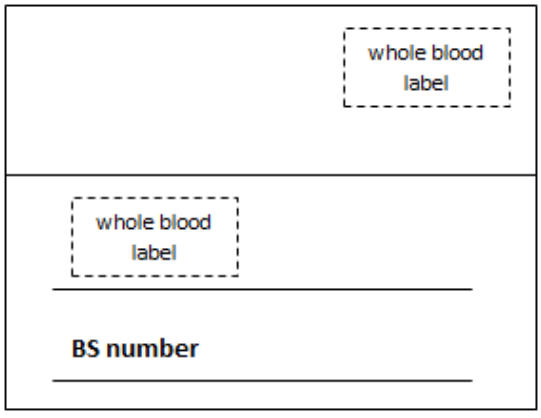
1.2. Prepare all of the materials and equipment listed above, including the printed vial labels for each of the aliquots. The table below shows all of the aliquots that will be processed in the steps that follow. See SOP 5.1.002 for label printing instructions. When you label the vials, take care not to obscure the bar code. Cover the printed labels with a clear over-laminate label and insert the coloured cap inserts as shown.

Aliquot	Average Quantity	Vial label	Cap insert colour
Blood spots	5 x 40µL	Pipetted onto blood spot card. See 1.3 below.	n/a
EDTA whole blood	2 x 0.5mL	*TBRef#* 'Whole Blood' *dd-mm-yyyy*	Pink
EDTA plasma	4 x 0.5mL	*TBRef#* 'EDTA Plasma' *dd-mm-yyyy*	Lilac
EDTA buffy coat	2 x 0.3mL	*TBRef#* Buffy Coat *dd-mm-yyyy*	Blue
Serum	4 x 0.5mL	TBRef# Serum dd-mm-yyyy	Red

Note: The total volume of blood will vary slightly between collections for EDTA plasma, EDTA white cell buffy coat and serum; see instructions for each aliquot type.

1.3. Label a blood spot card as shown in the diagram:

- The 'BS number' is a unique identifier for the blood spot card in the format:
  Site prefix + 'BS' + *number*, where the number is four digits starting from 0001 at each site.
  Write the next BS number on the bottom of the card.
- Attach two printed whole blood labels for the TBRef#, specimen type and date. Alternatively, write these details on the card with the TBRef# at the top right corner.



2. Blood collection and handling

2.1. Always handle blood specimens with care and avoid agitation when they are transferred to prevent hemolysis.

2.2. The serum collection tube (red top) needs enough time to clot before processing since the clotting separates cellular components and clotting factors from the serum that is aliquoted. It is best to let it clot at room temperature for at least 30 minutes after the blood is drawn. If blood is received less than 30 minutes after being drawn, allow the extra time necessary for clotting before processing.

2.3. If it is not possible to process blood specimens immediately after receipt in the processing laboratory, then they should be held at 4°C (with consideration of point 2.2. above) and processed within 4 hours.

2.4. Check that the top section of the Blood Tracking Form has been completed according to 4.1.007 Method '4. Delivery and processing of blood samples'. A copy of the Blood Tracking Form is attached. If any deviation to the specified collection tubes, transfer or holding conditions occurs then make a note of this.

2.5. When you begin to process the bloods by steps 3 to 5 below, record the 'Time blood processed' on the tracking form.

3. Blood spot card and whole blood aliquots

Note: Blood spot cards are stored at room temperature both before and after use. Always handle blood spot cards wearing gloves and do not touch the circles where the blood is deposited.

3.1. The blood spots and whole blood aliquots are both taken from the purple top EDTA blood tubes. Check the level of blood in both tubes before starting and aliquot from the one with a larger volume so that they are roughly balanced for the centrifugation step that follows.

3.2. Mix the EDTA blood gently by inverting the tubes several times or with an electronic blood mixer/rotator if available.

3.3. Wipe the top of the EDTA blood tubes with ethanol before opening. Using sterile pipette tips entirely fill the circles on the blood spot card taking care not to saturate the paper. Each circle will take approximately 40µL.

3.4. Leave the blood spot card open to air-dry thoroughly in the hood or in a covered container before folding over the cover for storage. Drying time will vary according to ambient conditions but will take at least 30 minutes. Cards may be left overnight to dry.

3.5. Using a Pasteur pipette, transfer two 0.5mL aliquots of EDTA whole blood into pre-barcoded vials.

3.6. Record the two whole blood vial numbers and volumes on the tracking form and snap freeze them in liquid nitrogen (or place them on dry ice if liquid nitrogen is not available at your site).

4. EDTA plasma and buffy coat

4.1. Centrifuge the remainder of the two EDTA blood tubes at 568g for 20 minutes at 20°C. Handle the tubes very carefully when removing from the centrifuge so as not to disturb the separation of layers.

4.2. Using a sterile Pasteur pipette, transfer 0.5mL aliquots to the four EDTA plasma vials, taking care not to disturb the white cell layer.

- If there is more than 4 x 0.5mL of plasma, add the remaining amount in ≤ 0.5 volumes to the vials remembering the final volume of each one. No vials should be more than 1.0mL; use a fifth vial if necessary.

- If there is less than 4 x 0.5mL of plasma, the last vial may have less than 0.5mL and you may use less than four vials. Remember the final volume in each vial and discard any unused vials.

4.3. Record the EDTA plasma vial numbers and aliquot volumes on the tracking form and snap freeze them in liquid nitrogen (or place them on dry ice if liquid nitrogen is not available at your site).

4.4. Plasma should be clear and yellow. If you noticed hemolysis (pink/red tinge) or anything unusual about the sample (e.g. cloudiness or blue dye), then note this on the tracking form.

4.5. Using a new sterile Pasteur pipette, transfer the buffy coat (layer of white blood cells and platelets sitting on top of the red cells) from the first EDTA collection tube into the first buffy coat vial. Repeat for the second buffy coat. As you transfer each buffy coat aliquot, estimate the volume from the pipette markings (usually 0.3 – 0.4mL).

Note: The buffy coat aliquots will include a small amount of residual plasma and red blood cells but try to minimize disturbance and inclusion of these layers. If the buffy coat layer has been disturbed you may need to repeat the centrifugation step 4.1. Note this on the tracking form.

4.6. Record the buffy coat vial numbers and aliquot volumes on the tracking form and snap freeze them in liquid nitrogen (or place them on dry ice if liquid nitrogen is not available at your site).

5. Serum

5.1. Spin the red top serum tube at 2749g for 10 minutes at 4°C, using a spare serum tube filled with water to balance the centrifuge. Be careful not to disturb the clot when you remove the serum tube after spinning.

5.2. Wipe around the lid of the serum tube with ethanol before opening. Using a sterile Pasteur pipette, transfer 0.5mL aliquots into the four pre-barcoded serum vials. If there is more or less than 4 x 0.5mL of serum, do the same as for the EDTA plasma vials in 4.2.

5.3. Record the serum vial numbers and aliquot volumes on the tracking form.

5.4. Serum should be clear and yellow. As in 4.4 above, note on the tracking form if the serum looks hemolyzed or if there is anything else anything unusual about the sample.

5.5. Snap-freeze the serum vials in liquid nitrogen and record the final freezing time on the tracking form. Alternatively, if liquid nitrogen is not available at your site and you are using dry ice for transfer, record the final freezing time as the time you place the samples at -80°C in step 6 below.

6. Specimen storage, data entry and filing

Note: Whole blood, EDTA plasma and serum are stored at -80°C and buffy coat is stored in the vapour tank (where available). As a precaution in case of equipment failure, frozen blood specimens from each donor are split into different storage locations. Each freezer box (-80°C) and vapour tank box should have a 'B' backup box for later transfer to another ABCTB collection site for long term storage; see SOP 5.1.016 for storage box naming formats.

6.1. Check the database for the next storage locations for the frozen blood aliquots and note the boxes and positions on the tracking form for each vial.

6.2. Transfer all of the frozen blood vials from liquid nitrogen to their allocated locations. Take a box of dry ice to keep specimens and freezer/vapour tank boxes as cold as possible whilst you are moving the specimens into their storage locations.

6.3. Check the database for the next blood spot card storage location and note on the tracking form. When the blood spots are dry (at least 30 minutes after processing), fold over the card and transfer it to the allocated box.

6.4. Enter all of the details recorded on the blood tracking form into the database following SOP 5.1.006. Sign the bottom of the tracking form when complete.

6.5. Note: if you have a barcode reader and/or computer set up in your laboratory, you will be able to start the data entry while processing the bloods during the centrifugation steps.

6.6. File the tracking form away with the donor's other paperwork.

Safety

Institutional safety requirements must be adhered to at all times.

Personal protective equipment such as gowns and gloves must be worn at all times when in the laboratory.

All human sample material must be handled in a Class II Biohazard Cabinet.

All local chemical and sharps policies must be adhered to.

Ordering Information

Supplies for blood processing and storage boxes are ordered and stored at the Central Processing Laboratory at Westmead. Contact the TBO at Westmead when stocks are getting low or expiry date for consumables is approaching.

Item	Manufacturer	Supplier	Product name	Catalogue number
Protein Saver Cards	Whatman	Interpath Services	903 Protein Saver Cards	10534612
Pre-barcoded vials	Greiner	Interpath Services	Greiner per bar coded cryovials	122263-128C
Labels	GA-International	Molecular Solutions	CryoLabel, White, 25.4 x 12.7mm	TTCL-7
Clear label cover	GA-International	Molecular Solutions	Vial Clear-Wraps 51 x 25mm	CF-1
Freezer boxes	Greiner	Interpath Services	Greiner Cryobox Pc - 2Ml 10X10 - Assorted	575019S

Australian Breast Cancer Tissue Bank

Stick addressograph sticker here OR

Patient's name: .....

Patients MRN: .....

Hospital: .....

Blood Tracking Form

Blood Collector Use –

Tissue Bank Ref#_____

Date blood taken: _____ **Time blood taken:** _____

Number of tubes: EDTA _____ Clotted_____Gel tube? Y / N Other _____

Blood holding condition (circle one): room temperature / fridge / on ice

Tumour Bank Use –

Date blood processed:_____ **Guthrie Card:** Y / N BS Number:_____

Time blood processed: _____ Blood Spot Box No:_____

Time blood frozen: _____ **Freezing method (tick):** ☐ Aliquots snap frozen in liquid nitrogen
☐ Aliquots transferred to storage on dry ice

Vial No.	Type (Serum, Plasma etc.)	Vol (mLs)	Storage Box	Position	Freezer/Shelf/Rack

Notes:

Entered in database by:_____