Brisbane Breast Bank

Blood Collection and Processing SOP

Breast Pathology Laboratory
University of Queensland Centre for Clinical Research

Blood Collection

We collect 30ml of blood from patients who have consented to take part in the study. Blood is collected in two EDTA tubes (10ml) and one plain tube (10ml). Blood is collected and placed on ice before transportation to the Breast Pathology Laboratory.

Blood Processing

Overview

Log the sample in the tissue bank log book and provide the sample with the next consecutive blood number (B#) to de-identify the sample. The following aliquots are required:

1 x Guthrie Card

3 x Plasma (clear cap) aliquots

3 x Serum (yellow cap) aliquots

1 x Buffy coat (clear cap) aliquots

5 x Whole blood (clear cap) aliquots

Blood Tubes	Aliquots
First **purple** cap 10ml EDTA tube	Plasma in three 1.8ml clear capped tubes. Buffy coat in one 1.8ml clear capped tube.
Second **purple** cap 10ml EDTA tube	Guthrie card Whole blood in five 1.8ml clear capped tubes.
Red cap 10ml plain Tube	Serum in three 1.8ml yellow capped tubes.

Safety Procedures

Treat all blood samples as potentially infections.

- Wear personal protective equipment (PPE) at all times: lab coat, safety glasses and double gloves.
- All processing of unscreened blood is to be performed in the biohazard in room 620.
- All non-liquid waste is to be double bagged and sealed.
- Liquid blood waste is discarded in blood waste containers that contain biodyne.
- Use two layers of bench coat.

Materials Required for Blood Processing

All materials required for processing can be found in the drawers near the biohazard hood. Place all items (tubes, racks, pipettes etc.) to be used into the hood **BEFORE YOU BEGIN**.

List of items to be placed inside the hood (per patient):

- Two sets of bench coats
- 50ml tube (1) on a rack
- Sterile pasteur pipettes with bulb - one pack
- 1.8mL Tubes: yellow (3) and clear (9) in tube rack (label the tubes before starting or label during the centrifugation steps)
- Guthrie card (1)
- Blood waste container containing ~10mL biodyne
- a set of double-lined plastic bags
- 1X TE buffer
- Rack for the original blood tubes

Items to place next to the hood:

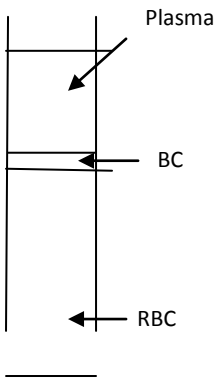
- 70% ethanol and cavicide for cleaning the hood
- esky with ice
- tissues and paper towel
- gloves

Occasionally, there is less than 10ml of blood in the blood tubes. In this case, use the EDTA tube with the largest volume for whole blood aliquots and guthrie card. We prioritise whole blood over plasma aliquots. Process the EDTA tube for plasma first since plasma should be frozen quickly to preserve protein integrity.

EDTA tubes (purple cap)

First EDTA tube is for Plasma and Buffy coat

Centrifuge at 3000rpm (1500g) for 10min. Handle with care as shaking disturbs the fractions.



For Plasma (P):

- Plasma fraction is the top layer after centrifuging
- Aliquot into 3 clear capped tubes labelled with the B# (lid and side of tubes), date and aliquot number (P1, P2 & P3) on the side of the tubes.
- Store at -80 in the plasma box.
- Record number of aliquots and any other details i.e. Green, cloudy etc. in the tissue bank log book.
- Record the location of the tubes on the plasma map sheet.

For Buffy Coat (BC):

- This is the middle layer and is a white colour.
- Using a sterile pasteur pipette remove this layer into a 50ml tube containing approximately 50mls of 1x TE.
- Spin at 3000rpm (1500g) for 10min.
- Carefully pour off the supernatant into the blood waste container with biodyne, taking care that the pellet does not dislodge.
- Repeat this TE wash and spin.
- Carefully pour off the supernatant into the blood waste container with biodyne, taking care that the pellet does not dislodge leaving about 1ml of TE buffer.
- Transfer the pellet with 1ml of TE into a 1.8ml clear capped tube labelled with the B# (lid and side of tube), date and BC on the side of the tube.
- If the buffy coat is very red you will need to do the 1 x TE wash one more time.

- Record number of aliquots and any other details in the tissue bank log book.
- Store BC in -80 freezer in BC box and record the location of the tube on the paper map sheet.

Second EDTA tube is for whole blood aliquots and Guthrie card

Do not spin this tube

You can process this tube during the centrifugation steps of the first tube.

For Guthrie Card

The purpose for the Guthrie card is for quality assurance. If there is a concern that there may have been an error then the DNA can easily be extracted from the Guthrie card and cross referenced with the existing DNA. All blood processed requires a Guthrie card. It makes no difference if the patient has given a previous blood sample.

- Label the card with the B# and the date.
- Invert the second EDTA tube to ensure that the blood is mixed and then dot the card across one line (six dots in total).
- Allow the Guthrie card to dry overnight in the biohazard hood.
- Store the dried guthrie cards in the guthrie card storage box at room temperature in numeric order.

For Whole Blood

- Aliquot blood into five 1.8ml clear capped tubes labelled with B# (lid and side of tubes), date and aliquot number (B1, B2, B3, B4 & B5) on the side of the tubes.
- Store at -80 in the blood box.
- Record number of aliquots and any other details in the tissue bank log book.
- Record the location of the tubes on the blood map sheet.

Plain Tube (red cap)

For Serum (S)

- Centrifuge at 3000rpm (1500g) for 10min.
- Serum fraction is the top layer after centrifuging.
- Aliquot the serum into 3 X 1.8ml tubes with a yellow cap labelled with B# (lid and side of tubes), date and aliquot number (S1, S2 & S3).
- Record number of aliquots and any other details i.e. Green, cloudy etc. in the tissue bank log book.
- Record location on the serum map sheet.
- Store at -80 in the serum box.

Cleaning up

- Spray pipettes, bottles etc with 70% ethanol when they come out of the biohazard hood.
- Spray centrifuge with 70% ethanol after use.
- Throw out the bench-coat into the double-bagged blood solid waste (containing blood tubes etc). Tie the double-bagged solid waste with autoclave tape and place it in the yellow-lined biological waste bin.
- Use cavicide to wipe the biohazard hood, rinse with water, then spray with 70% ethanol and wipe again. Press down arrow on the hood to turn the shield down. Then turn the hood off.

Blood DNA Extractions

- DNA extractions from whole blood aliquot tubes are only done if the samples are requested for research projects.
- Extract the DNA using the Qiagen QIAmp Midi Blood Extraction Kit.
- Quantify the DNA using Nano drop and also check integrity of the DNA on a 1.5% agarose gel.
- Record the storage and concentration information in the tissue bank database.
- Store at -80 in the blood DNA box and record the map location.

Solutions for Blood Processing

1X TE

To prepare 1L of 1 X TE, add 10ml of 1M Tris HCl ph8.0 and 2mL of 0.5M EDTA pH 8.0, then make it up to 1L with milliQ water.