

THE CHILDREN'S HOSPITAL AT WESTMEAD

TUMOUR BANK

STANDARD OPERATING PROCEDURE

BONE MARROW ASPIRATE AND TREPINE SAMPLE PROCESSING			
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1. PURPOSE

The purpose of this document is to outline standardised procedures to follow when snap freezing bone marrow samples in order to provide maximum benefit to the end users.

2. SCOPE

This protocol covers all bone marrow samples collected from oncology or non-oncology patients at The Children's Hospital at Westmead (CHW), for whom the Haematology department has residual samples, and subsequently contacts the Tumour Bank (TB) for processing and storage.

3. RESPONSIBILITIES

The TB Clinical Research Associate (CRA), Project Officer and Research Assistants (RA) must ensure that these protocols are adhered to at all times when processing and storing bone marrow samples.

4. MATERIALS, EQUIPMENT AND FORMS

- 2.0mL Cryovials – with clear lid
- Cryovial lid inserts – white
- Disposable plastic transfer pipettes
- Racks to hold tubes while processing
- Tongs
- Freezer storage boxes
- Laboratory Bench Sample Record Sheet form
- Permanent waterproof marker
- Metal cup for aliquotting liquid nitrogen into
- 4 decimal place balance
- Small dewar for liquid nitrogen transportation
- Personal protective equipment - gowns, gloves, safety glasses

5. METHOD

5.1 Sample Processing and Labelling

Aliquot and snap freeze bone marrow aspirate and/or trephine samples as soon as possible after retrieval.

5.1.1 Bone Marrow Aspirate

- a. Aliquot (into cryovials with clear lid) the BMA sample into 2.0mL lots using a disposable plastic transfer pipette.
- b. Press a white insert into the lid.
- c. Label the side of the cryovial with the following information
 - Patient medical reference number (MRN)
 - Date of collection
 - Type of specimen (BMA)
- d. Label the insert of the lid with the following information
 - Volume of sample in millilitres

5.1.2 Bone Marrow Trephine

- a. Using the 4 decimal place balance in the chemical room, weigh an empty cryovial with yellow lid. Record the weight.
- b. Place bone marrow trephine sample into the cryovial which has been weighed and replace lid.
- c. Reweigh the cryovial containing bone marrow trephine sample.
- d. Record the weight of the tube with a yellow lid plus the bone marrow trephine sample.
- e. Subtract the weight of empty cryovial from the weight of the cryovial plus sample. Record the weight.
- f. Press a white insert into the lid.

- g. Label the side of the cryovial with the following information
 - Patient medical reference number (MRN)
 - Date of collection
 - Type of specimen (trephine)
- h. Label the insert of the lid with the following information
 - Volume of sample in grams

5.2 Sample Freezing and Storing

- a. Decant liquid nitrogen from the large dewar (in the liquid nitrogen store room on Level 3) into a small dewar and transport to laboratory.
- b. In the laboratory, aliquot liquid nitrogen into a metal cup.
- c. Using tongs, immerse cryovial containing sample into liquid nitrogen for approximately 15 seconds or until sample changes colour.
- d. Record the volume of each cryovial on request form.
- e. Place frozen cryovial containing sample in next available freezer box space in Tumour Bank freezer, according to the reference sheet to the right of the laboratory bench space.
- f. Record box number, row and column position on request form.

5.3 Sample Recording

Record the following details of sample in the Tumour Bank Laboratory Bench Sample Record Sheet form for entry into the TB database.

- Patient MRN
- Surname and first name
- Date of birth
- Sample type
- Date of sample collection
- Number and weight/volume of sample
- Freezer box number and position
- Any additional notes

6. SAFETY

- Adhere to all local chemical and sharps policies.
- Dispose empty bone marrow tubes, pipette tips and soiled gloves in accordance with local regulations for handling of potentially infectious biological material.