MWAC/UNSW Biospecimen Services

# Standard Operating Procedure

## Bone Marrow Aspirate Processing

Effective date: 17th May 2023

Review date: 17th May 2026

### 1. Purpose

The purpose of this document is to describe the procedure for processing bone marrow aspirates (BMA) to a frozen cell pellet and cryopreserved cells.

### 2. Scope

This SOP describes how to process BMA using the ficoll/Lymphoprep separation method to obtain mononuclear cells, stored in –80°C as cell pellet for future DNA/RNA purification and in liquid nitrogen vapour phase (VP) as cryopreserved cells.

This SOP does not cover DNA/RNA extraction from the cell pellet. See *Biospecimen Services – Extraction of DNA from a Cell Pellet, Isolation of Blood Genomic DNA/total RNA* listed in *Section 10 – Related Documents*.

This SOP does not cover research uses for the cryopreserved cells, preparing cell lines from the cryopreserved cells and research uses for DNA/RNA.

### 3. Roles & Responsibilities

The SOP applies to all Biospecimen Services personnel responsible for processing bone marrow aspirates.

| Personnel | Responsibility |
|---|---|
| Technician | • To ensure that the procedure outlined in this standard operating procedure is closely adhered to, as any changes in the methodology will jeopardise the validity of the results.<br>• To be trained in Good Laboratory Practice and be inducted into the PC2 facility.<br>• Carry out accurate pipetting and avoid cross-contamination of specimens. |

## 4. Materials & Equipment

The materials and equipment listed below are needed to perform this method.

4.1. Equipment

- Pipette controller


- Rocker (Intelli-Mixer)

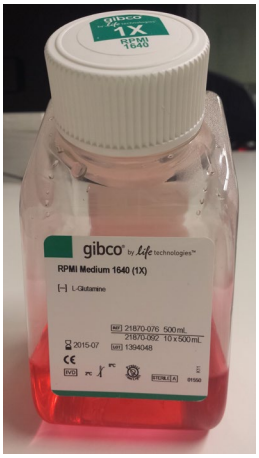

- Eppendorf Centrifuge 5810 for 50mL falcon tubes at room temperature


- Eppendorf Centrifuge 5425 for 1~2mL microfuge tubes at room temperature


- MrFrosty freezing chamber at room temperature

4.2. Consumables

- RPMI media stored at 4ºC




- 25mL pipette

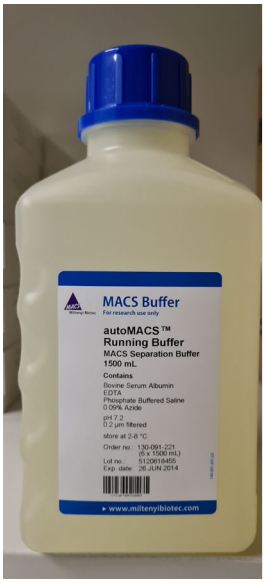

- 10mL pipette

- 50ml Falcon tubes (sterile)
- 70-100µm cell strainer


- Lymphoprep™ or Ficoll density gradient media

- 10 or 20ml syringe & cannula


- 3ml transfer Pipettes
- MACS buffer stored at 4ºC




- Fetal Bovine Serum (FBS), stored at -80ºC in 900µL aliquots. Thaw at 4ºC for cryopreserved cells.
- DMSO
- Screw cap 1.5~2mL microfuge tube (sterile)


Or


## 5. Safety Requirements

5.1. Clean back-opening gowns, eye protection and powderless gloves must be worn during all operations in this SOP.

5.2. All samples must be treated as potential infection risks and must be handled according to good laboratory procedures under PC2 requirements and methods to prevent occupational exposure.

5.3. All biohazardous material and chemical waste must be disposed in accordance with the UNSW Laboratory Hazardous Waste Disposal Guideline.

5.4. All work surfaces should be clean prior to commencing and after finishing.

## 6. Method

6.1. Bone marrow collected the previous day should be placed on a rocker at 4°C overnight to prevent coagulation (POWH cold room without rocker).

6.2. If the bone marrow sample is more than 2 days old then the sample should only be processed into a frozen cell pellet. Do not prepare a cryopreserved cell when the sample is more than 2 days old.

6.3. Register all participants in OpenSpecimen following the *Biospecimen Services SOP - Registering a Participant and Recording Consent in OpenSpecimen* listed in *Section 10 - Related Documents* and complete the e-processing form (Appendix A).

6.4. Book the specimen into OpenSpecimen following the instructions in the individual collection protocol.

6.5. Label the falcon tubes with the specimen number to ensure samples are not mixed up when processing more than one sample at a time.

6.6. Record the volume of bone marrow and transfer to a 50mL falcon tube using a 70-100µm cell strainer to strain out any clots in the BMA. If BMA sample is greater than 8mL separate the BMA evenly into two 50mL tubes. Add RPMI media to the sample at a 1:5 ratio (if 5ml BM add 20ml RPMI) using a 25mL serological pipette (40ml maximum volume of bone marrow/media). Mix in a swirling motion.

6.7. Add 10ml of Lymphoprep™ or Ficoll to sample carefully layering at the bottom of the tube using 10mL syringe and cannula. The syringe with cannula attached should be placed in the bone marrow media solution first and then the Lymphoprep™ or Ficoll is added to the syringe.

6.8. Spin sample in centrifuge at 400g, 35mins at 20°C (accel.5, decal.1).

6.9. For each Lymphoprep/Ficoll tube, using a 3ml transfer pipette or a cannula with syringe, remove the MNC layer and Ficoll, and transfer to a new 50ml tube. The MNCs are the fluffy opaque layer between the plasma/RPMI and Ficoll (See Appendix B). Fill each tube to 50ml with RPMI. Mix well and centrifuge at 1200rpm, 10min at 20°C (accel.9, decal.5).

6.10. Remove supernatant and resuspend pellet in MACS buffer, combining all pellets into two tubes if more than 2 tubes. Rinse the tubes with more MACS buffer and fill combined pellets (if using two 50mL Falcon tubes) to 20ml with MACS buffer.

6.11. Centrifuge at 1200rpm, 10min at 20°C (accel.9, decal.5).

6.12. Remove supernatant using a 25ml serological pipette and resuspend pellet in 2ml of MACS buffer by pipetting with P1250/P1000 and transfer into two screw cap microfuge tubes (1mL each). Repeat step 6.11. above.

6.13. Remove supernatant. Put one microfuge tube into assigned spot of –80°C for long-term storage.

6.14. Resuspend the other microfuge tube in 900uL FBS, add 100uL DMSO and mix well gently. Place in a Mr Frosty and put it into -80°C for more than 24 hours and transfer to assigned spot of VP for long-term storage.

6.15. The spots for microfuge tubes are assigned when booking the BMA specimen and deriving into Frozen Cell Pellet and Cryopreserved Cell in OpenSpecimen. See Section 7, Labelling & Storage below for the microfuge tube labelling.

## 7. Labelling & Storage

7.1. To assign a storage location, when booking the specimen into the database, select the next available box for the collection protocol in OpenSpecimen and the next available free location will be automatically assigned within that box.

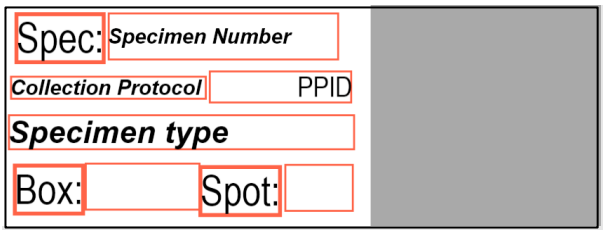
7.2. Specimens should be labelled with, at a minimum, specimen number, PPID, specimen type, box/spot location, Collection Protocol. See *Biospecimen Services SOP – Biospecimen Storage and Retrieval* in *Section 10 – Related Documents* for more information.

7.3. Labels should be printed and not handwritten.

7.4. Please see how to print the tube thermal labels in file *LTB-WI-006-V3 Print Permanent label from LabelMark5.pdf*

7.5. Use the following label template:

Template:




## 8. Monitoring of compliance to this SOP is ongoing.

8.1. The Biospecimen Services Manager or their delegate is responsible for ongoing monitoring of biobank operations to verify compliance with this SOP.

8.2. The Biospecimen Services Manager or their delegate is responsible for obtaining annual updates to this SOP and for communicating these changes to all personnel.

## 9. Definitions

| Term or Abbreviation | Definition |
|---|---|
| BMA | Bone Marrow Aspirate |
| RPMI Media | RPMI 1640 Medium was originally developed to culture human leukemic cells in suspension and as a monolayer. Gibco RPMI Medium 1640 (1x) [-]L-Glutamine is suitable for a variety of mammalian cells, including HeLa, Jurkat, MCF-7, PC12, PBMC, astrocytes, and carcinomas for a range of cell culture applications. |
| MNCs | Mononuclear cells |
| Ficoll/ficoll tube | A neutral, highly branched, high-mass, hydrophilic polysaccharide which dissolves readily in aqueous solutions. |
| Lymphoprep™ | Is a density gradient media for isolating human mononuclear cells (lymphocytes and monocytes), especially from blood. It has the same specifications as Ficoll(R)-Paque and Histopaque(R)-1077 and is manufactured to essentially equivalent quality standards. As compared to top brands, Lymphoprep™ can save up to 50% on gradient medium. |
| MACS Buffer | AutoMACS™ Running Buffer is a Rinsing Solution for preparation of a preservative-free MACS Separation Buffer. It contains Bovine Serum Albumin, EDTA, Phosphate Buffered Saline and 0.09% Azide. |

| | |
|---|---|
| FBS | Fetal bovine serum |
| DMSO | Dimethyl sulfoxide (DMSO) is a cryoprotectant used when freezing cells in ultra-low temperature environment to prevent intracellular and extracellular crystals from forming in cells during the freezing process. |
| PPID | Unique code or number assigned to the participant. |
| Specimen Number | Unique code or number assigned to individual specimens. |
| Collection Protocol | A Collection Protocol is an ongoing accrual and storage of specimens, undertaken by a Principal Investigator (PI) in association with Biospecimen Services. A Collection Protocol in OpenSpecimen has defined specimen collection groups, time points and a predetermined specimen type and processing protocol. |

## 10. Related Documents

| Document | Description |
|---|---|
| Biospecimen Services SOP – Biospecimen Storage and Retrieval, Version 3, 12/05/2023 | SOP that describes how to store, retrieve and ship biospecimens. |
| Biospecimen Services SOP - Extraction of DNA from a Cell Pellet, Isolation of Blood Genomic DNA/total RNA, Version 1, 15/05/2023 | SOP that describes the procedure for extracting DNA/RNA from a blood cell pellet. |
| Biospecimen Services SOP - Registering a Participant and Recording Consent in OpenSpecimen, Version 1, 26/04/2023 | SOP that describes the data entry procedure to follow when registering participants and recording consent in Open Specimen. |
| LTB-WI-006-V3 Print Permanent label from LabelMark5 | Instructions on how to print labels saved on the DVCR drive. |
| NSW/CTRNet Required Operational Practice 9: Biospecimen Collection and Processing | ROP that describes the key principles regarding biospecimen collection and processing that should be adhered to, to meet the current best practice standards. |
| NSW/CTRNet Required Operational Practice 10: Biospecimen Storage and Retrieval | ROP that describes the key principles regarding biospecimen storage and retrieval that should be adhered to, to meet the current best practice standards. |
| NSW/CTRNet Required Operational Practice 13: Safety and Waste Disposal | ROP that describes the key principles regarding safety and waste disposal that should be adhered to, to meet the current best practice standards. |
| UNSW Laboratory Hazardous Waste Disposal | UNSW Guideline for disposing of hazardous waste in laboratories. |
| UNSW Biosafety Policy | UNSW Procedure for identifying biohazardous material and meeting legislative and regulatory requirements. |
| UNSW Personal Protective Equipment Guideline | UNSW Guideline for selecting, using and maintaining PPE. |

## 11. References, Regulations & Guidelines

| Article | Source |
|---|---|
| Isolation of mononuclear cells from human bone marrow aspirates by density gradient centrifugation | https://www.miltenyibiotec.com/_Resources/Persistent/462c1fdb5e9346fe673da97fa40cca8c9d29320e/SP_MC_BM_density_gradient.pdf |

| | |
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| Density Gradient Centrifugation Compromises Bone Marrow Mononuclear Cell Yield | https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3516517/pdf/pone.0050293.pdf |
| CD34+ Purification from Human Bone Marrow Samples | Adult Cancer Program Labs, Lowy Cancer Research Centre, UNSW Sydney |

## 12. Appendices

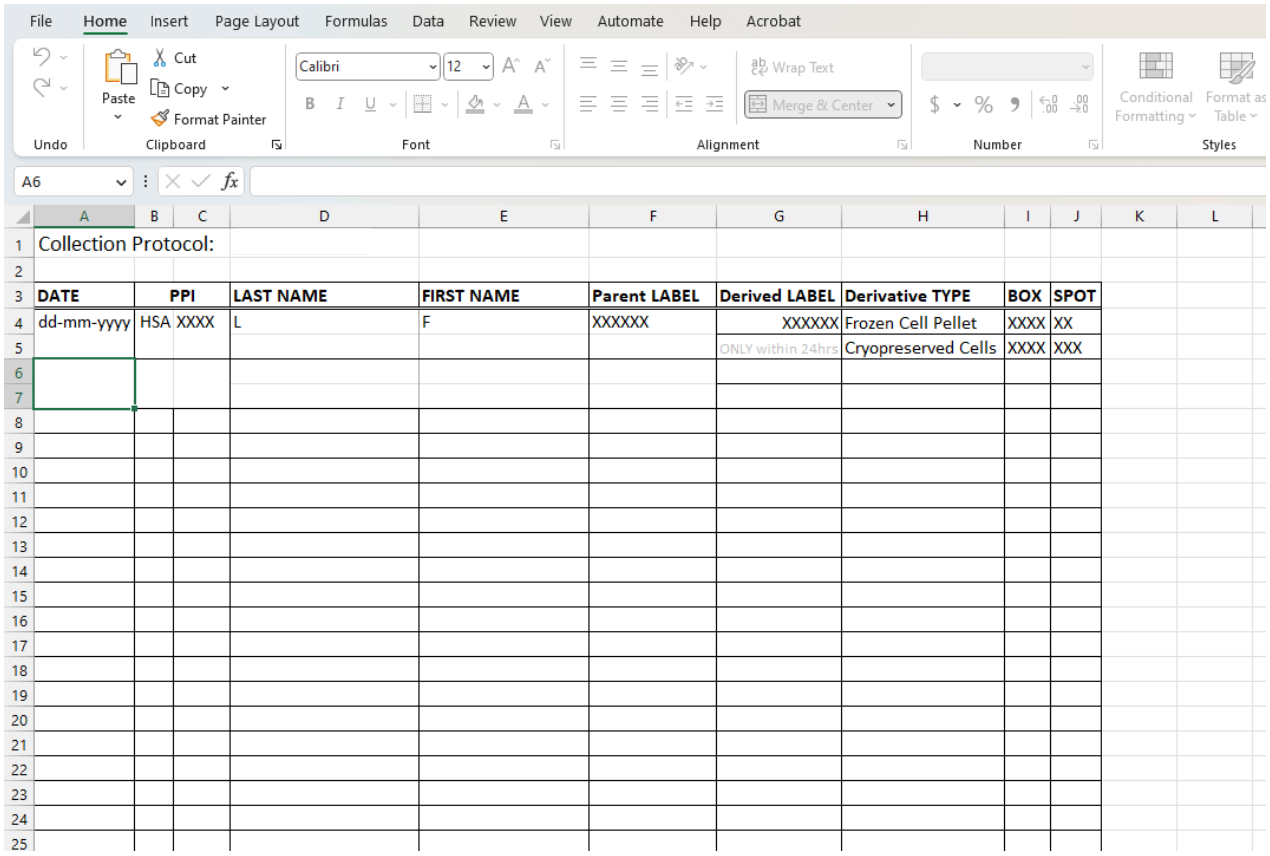
Appendix A: e-Processing Form

Appendix B: Density gradient separation diagram

## 13. Version History & Authorisation

| Version | Date | Author | Summary of Changes | Authorised By: |
|---|---|---|---|---|
| 1 | March 2020 | Anusha Hettiaratchi | Original | Anusha Hettiaratchi |
| 2 | 17/05/2023 | Pearl Zhu/ Ussha Pillai | Updated to new SOP format | Manager, Biospecimen Services: Anusha Hettiaratchi |

## Appendix A: e-Processing Form



| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Collection Protocol: | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | **DATE** | **PPI** | | **LAST NAME** | **FIRST NAME** | **Parent LABEL** | **Derived LABEL** | **Derivative TYPE** | **BOX** | **SPOT** | | |
| 4 | dd-mm-yyyy | HSA | XXXX | L | F | XXXXXX | XXXXXX | Frozen Cell Pellet | XXXX | XX | | |
| 5 | | | | | | | ONLY within 24hrs | Cryopreserved Cells | XXXX | XXX | | |
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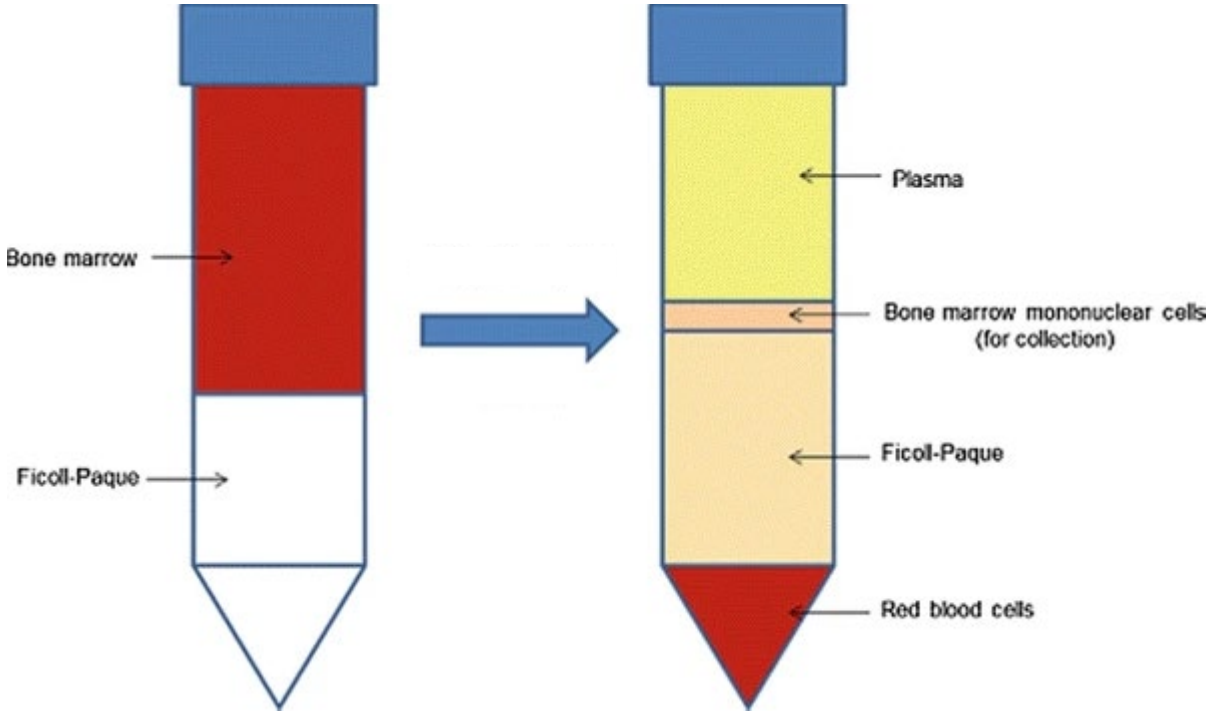
**Appendix B: A density gradient separation**

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