BC Generations Project
Processing ACD Vacutainers

Table of Contents

1.0 PURPOSE

The BC Generations project is collecting whole blood in Becton Dickenson ACD tubes for future EBV immortalization of the B-cells contained within the white blood cell fraction. This protocol follows that of the UK Biobank where whole blood is frozen in place of isolated white blood cells. The immortalized B-cells will serve as an unlimited source of genomic DNA. These samples are collected by a certified phlebotomist either during the participant's visit to an assessment centre or to a community laboratory.

2.0 MATERIALS, EQUIPMENT AND FORMS

Officially received ACD samples	Biological Safety Cabinet Class II type A2
Lab gown & gloves (nitrile)	Cryovial racks
NUNC Cryovials with yellow cap inserts containing 750 µL of RPMI + 20% DMSO	P1000 pipette & filtered tips
Biohazard waste bag	Storage boxes with 9x9 inserts
Sharps bucket	Isopropanol
Mr. Frosty™	-80°C freezer
50 mL conical tubes	Dry ice
RPMI	Serological pipettes (25 mL)
RPMI/20% Batch Tracking Sheet	DMSO
Red jerry can	Batch Labels
Yellow highlighter	Pipette repeater & tips
Vacutainer racks	MSDS (Isopropanol, DMSO)
	Computer with access to Laboratory Information Management System

3.0 PREPARING THE MR. FROSTY™

3.1 Read the Isopropanol MSDS sheet to become familiar with its hazards and necessary safety steps.

3.2 Uncap the Mr. Frosty™ and remove the cryovial holder insert. Leave the foam pads inside the Mr. Frosty™.

3.3 Fill each Mr. Frosty™ with 250 mL of isopropanol. The isopropanol should reach the fill line. Mark off on the Mr. Frosty™ usage record the corresponding day with a yellow highlighter on the Month's calendar (see appendix 1).

3.4 Return the cryovial holder insert and cap. Ensuring the correct number (1 – 10) cap matches the base number.

3.5 Store at Room Temperature.

3.6 Each time the Mr. Frosty™ is used mark off the corresponding day with an "X" on the Month's calendar (see appendix 1).

3.7 After 10 usages the isopropanol needs to be replaced. Discard the used isopropanol into a red jerry can appropriately labelled.

4.0 PREPARING THE RPMI/20% DMSO SOLUTION

4.1 Read the DMSO MSDS sheet to become familiar with its hazards and necessary safety steps.

4.2 RPMI/20% DMSO will be prepared in a biological safety cabinet (BSC) (Class II type A2).

4.3 Prepare enough RPMI/DMSO aliquots for 3-5 days.

4.4 For each box of 81 cryovials prepare enough RPMI/20% DMSO solution. You may find it easiest to prepare it in batches of 45 mL (fits in one 50 mL tube and provides enough room for mixing). Each box of 81 cryovials is ~1.5 batches of 45 mL (i.e. 1 box = 1.5 tubes of 45 mL, 2 boxes = 3 tubes of 45 mL).

4.4.1 Record the RPMI lot # and DMSO lot # on the tracking sheet (appendix 2).

4.4.2 Transfer 36 mL of sterile RPMI media using a serological pipette to a 50 mL conical tube.

4.4.3 Using a serological pipette add 9 mL of DMSO. This will give a final volume of 45 mL with a 20% (v/v) DMSO concentration.

4.4.4 Mix well.

4.5 Uncap the yellow cap cryovials.

4.6 Using a pipette repeater transfer 750 uL to each cryovial.

4.7 Recap the cryovials and return them to the storage box.

4.8 Complete the documentation form for the batch record (appendix 2).

4.9 Complete the batch label (appendix 3) and attach to the box.

4.10 Transfer these prepared tubes to the 4^{o}C refrigerator adjacent to the sample processing.

4.11 Clean up the BSC.

5.0 ACD SAMPLE PROCESSING

5.1 You will receive <u>one</u> ACD Solution B tube (6.0 mL) per subject. Samples will arrive at room temperature (22^{o}C). Proceed using officially received ACD tubes only.

5.2 Process samples in batches of 6 tubes (6 participants) or less, maintaining the order in which they were <u>collected</u> and starting with the oldest. Collection time can be obtained from the lab requisition that accompanies the sample. Sample processing and storage will be documented using the Laboratory Information Management System (LIMS).

5.3 Document the following attributes in LIMS for each ACD tube: presence of clotting, processing note.

5.4 Label each yellow capped cryovial with each own unique label generated by LIMS. *Note that the cryovials for the whole blood sample have a solution of RPMI / 20% DMSO which are pre-prepped and stored in the 4^{o}C refrigerator.*

5.5 Samples will be batch processed and done so within a biological safety cabinet (BSC) (Class II type A2).

5.6 Ensure the Mr. Frosty™ has been used no more than 10 times and contains sufficient amount of isopropanol (i.e. reaches the fill line). If this will be the 11th time, then proceed with step 3.0. If the ispropanol level is low add more as required.

5.7 Aliquot each ACD vacutainer tube into 3 yellow capped cryovials.

5.7.1 Fully mix the ACD tubes by gently inverting the tube a minimum of 7X.

5.7.2 Uncap <u>all</u> labelled cryovials (a maximum of 18) containing the RPMI/20% DMSO that you will be pipetting into, also uncap all ACD tubes to be pipette from – this is to speed up the time between the whole blood contacting the DMSO solution and it reaching the freezer. *When opening the ACD tube you may wish to use a kim-wipe overtop of the plug as these types of tube sometimes spray when being opened. <u>Double check</u> to make sure the correct ACD tube is being aliquot into the correct cryovials.*

5.7.3 Using the P1000, pipette 750µl of whole blood to each of the 3 corresponding cryovials to give a final DMSO concentration of 10%, changing tips between different ACD source tubes.

5.7.4 Recap the cryovials, mix by inversion 5X, and place in the room temperature Mr. Frosty™ beginning at position 1, then position 2, then position 3, etc. If it`s the start of the day use Mr. Frosty™ 1, then 2, then 3 etc.

5.7.5 Place the Mr. Frosty™ at -80°C within 5 minutes of the first sample being aliquoted. Document processing in LIMS.

5.7.6 Recap and discard the ACD tube with any remaining blood into the sharps bucket.

5.8 Repeat steps 5.2 to 5.7 for any additional batches.

5.9 Record the Mr. Frosty™ usage by marking an "X" on the Month's calendar corresponding to the correct Mr. Frosty™ number and day. (see appendix 1).

5.10 Clean up the BSC.

6.0 FILLING AND INVENTORY OF THE PERMANENT STORAGE BOX: DAY 2

6.1 All samples will be kept on dry ice when outside the freezer.

6.2 Retrieve the Mr. Frosty`s™ containing the ACD samples from the -80°C and place on dry ice. Each subject has 3 cyrovials now containing frozen whole blood in RPMI/10% DMSO.

6.3 Select either the last partially filled storage box or a new storage box and place on dry ice.

6.4 Physically transfer the samples from the Mr. Frosty™ location to its permanent boxes. Samples will be transferred to 3 storage boxes to be stored in 3 separate freezers. Maintain the same sample order.

6.5 Inventory the samples in LIMS.

6.6 Transfer the permanent boxes to the -80°C freezer.

6.7 Transfer full permanent boxes to a -190°C vapor phase liquid nitrogen freezer. Document move in LIMS.

7.0 REFERENCES

7.1 Amoli MM, D Carthy, H Platt. EBV Immortalization of human B lymphocytes separated from small volumes of cryo-preserved whole blood. Int. J. Epi. 2008; 37:i41-i45.

7.2 Cryopreservation Technical Manual. Nalgene, Nunc in partnership with ATCC. 2006.

APPENDIX 1: MR FROSTY USAGE RECORD

Mr. Frosty Isopropanol Record

Month: _____ Year: _____

Mr. Frosty ID #

Day	1	2	3	4	5	6	7	8	9	10
1										
2										
3										
4										
5										
6										
7										
8										
9										
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25										

Instructions: Mark-off the square with an "X" corresponding to the day when the Mr. Frosty™ is used.
If the Mr. Frosty™ has been refilled with isopropanol, highlight the day with a yellow highlighter.

APPENDIX 2: RPMI/DMSO (20%) BATCH RECORD

RPMI 1640/20% DMSO Batch Record

Manufacture Day (MMDDYY)	# of boxes Produced (81 tubes/box)	RPMI Lot #	New RPMI bottle (Y/N)	DMSO lot #	New DMSO Bottle (Y/N)	Person who produced the batch	Usage Start Date (MMDDYY)	Usage End date (MMDDYY)

APPENDIX 3: RPMI/DMSO BATCH LABEL

RPMI 1640/20% DMSO

Manufacture Date: _____
Box #: _____
Usage Start Date: _____
Usage End Date: _____