

ISHAGE 2001 – TECHNICAL BREAKFAST 1 & 16 STORAGE and CRYOPRESERVATION of CELL PRODUCTS

CRYOPRESERVATION – DMSO Freeze Solution

1.0 PRINCIPLE:

Hematopoietic graft cryopreservation is required to preserve viable hematopoietic progenitor cells during the patient's cytoreductive preparative regimen. Dimethylsulfoxide (10%) is the cryoprotectant used to reduce the freeze-thaw injury to these cells. Alterations in cryopreservation techniques may alter progenitor cell survival.

2.0 SPECIMEN:

2.1 Hematopoietic progenitor cell products

3.0 SUPPLIES AND EQUIPMENT:

- 3.1 Freeze Bags - Fenwal 4R2216
- 3.2 Dimethylsulfoxide
- 3.3 Plasmalyte A - Baxter 282544
- 3.4 Administration Sets - Cutter 20-5614
- 3.5 Sampling Site Couplers - Fenwal 4C22405
- 3.6 60 ml syringes - BD 309663
- 3.7 16 gauge needles - BD 305198
- 3.8 3 way stopcock - Medex 24G0611117
- 3.9 Lifecell Septum and Cap - Fenwal 3L2100
- 3.10 Combi cap - Medi-dose 1V2100
- 3.11 Control Rate Freezer - Forma
- 3.12 Laminar Flow Hood
- 3.13 Heat Sealer
- 3.14 Freeze Racks

4.0 PROCEDURE:

- 4.1 DMSO releases heat after it is added to media. Therefore, this mixture should be prepared before use and the solution refrigerated. Warm freeze solution should never be added to cells. Autologous plasma should be added to the solution before use. If the cells have been purged, the plasma should be irradiated (3000 rad) before it is added to the cryopreservation solution.
- 4.2 Freeze solution preparation:
 - 4.2.1 Add 30 ml of DMSO to 100 ml of Plasmalyte A.
 - 4.2.2 Cool the solution.
 - 4.2.3 Add 15 ml of plasma before use.
 - 4.2.4 If larger volumes of solution are required increase the proportions as necessary.
- 4.3 Based on the nucleated cell count obtained after the concentration procedure, calculate the cell volume to be frozen.
 - 4.3.1 Initial volume x cell count = total cells.
 - 4.3.2 $\text{Total cells} / 6 \times 10^8 = \text{freeze volume}$.
 - 4.3.3 To obtain the final volume, round the freeze volume to the next highest multiple of 25.
 - 4.3.4 Aseptically add autologous plasma to the cells to obtain the final volume.
 - 4.3.5 Mix the cells.
 - 4.3.6 Label the appropriate number of freeze bags with the following information:
 - 4.3.6.1 Patient's name, history number, unique number, processing date, volume, cryoprotectant, storage temperature, expiration date, product type, and treatment.
 - 4.3.7 Aseptically transfer 25 ml of cells to each bag.
 - 4.3.8 Use the Lifecell septum and cap to close the freeze solution bottle. Insert the other end of the administration set into the middle port of a 3 way stopcock. Attach a 60ml

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syringe to the port of the stopcock and the freeze bag to the remaining port on the stopcock.

- 4.3.9 Add 25 ml of freeze solution to each bag.
- 4.3.10 Remove the air from the freeze bags. Leave 0.5 inch of tubing filled with cells in order to prepare a segment.
- 4.3.11 Seal the bags. Prepare a segment on 1 of the bags. Check the ports for leaks prior to freezing.
- 4.3.12 Place the bags flat on the freeze presses. Make sure the bag is covered by the plate and that there is no folds in the bag. A maximum of 4 bags can be placed on each layer. Place the flat ends of the temperature probe between a bag and the top plate. Turn the rings on the plate corners to secure the bags.

4.4 CRYOMED PROCEDURE

- 4.4.1 The Cryomed freezer and controller should be attached to a nitrogen tank that is more than 1/2 full with adequate pressure (>20 psi). Open the valve on the nitrogen tank prior to use.
- 4.4.2 Place the racks in the freezer chamber. Insert the end of the probe into the socket on the side of the freezer chamber. Close the door.
- 4.4.3 Turn the power switch on (which is located on the back of the controller). All the indicators on the front panel will light as a means of a visual inspection. The controller will also perform self -tests.
 - 4.4.3.1 An error in any of these tests will result in the activation of the alarm for 10 seconds and the appearance of the message "ERR #" in the middle display.
 - 4.4.3.2 Explanation of the error codes can be found in the Cryomed manual. When there are no system problems the indicator will change and reflect the parameters for the particular section displayed.
- 4.4.4 Turn on the chart recorder and place the pen in the holder.
- 4.4.5 Verify the calibration of the strip recorder by using the CHAM and SAMP keys in the TC SCANNER cluster.
 - 4.4.5.1 Press the CHAM keypad to force the chart recorder pen to mark the zero degree level.
 - 4.4.5.2 If the recorder pen does not reach 0 degrees, adjust the knob on the recorder until 0 degrees is achieved.
 - 4.4.5.3 Press the SAMP keypad to force the strip chart recorder to mark -180. With recorder calibration now complete, press the SCAN keypad to alternate which temperature is recorded.
- 4.4.6 Press the number keypad (1) followed by the CLR keypad to select the program. The use of the CLR will add a period after the program number. This indicates the program was user specific. The program is as follows:

4.4.6.1 Program #	Function	Temperature
1.1	WAIT	0°
1.2		1 °C/min -6 °C (sample)
1.3		25 °C/min -60 °C
1.4		15 °C/min -20 °C
1.5		1 °C/min -45 °C
1.6		10 °C/min -90 °C
1.7	END	

- 4.4.6.2 The COOL+ feature should be added to every section. Consult the Cryomed Technical Manual for details concerning the program function.

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- 4.4.7 Use the program advance key to move to section 1. The UP arrow will present the next section and the DOWN arrow will present the previous section.
- 4.4.8 Press the ALARM keypad to activate the alarm.
- 4.4.9 Press the RUN keypad in the MODE SELECT cluster. The freeze will begin.
- 4.4.10 Section 1 is a WAIT section and the chamber temperature will hold at 0 indefinitely. When the specimen temperature is +4, press the RUN keypad to advance to section 2.
- 4.4.11 The program will run without controller assistance until Section 7 is reached. This is the END section and the alarm will sound. Press the ALARM keypad to silence the alarm. The chamber temperature will remain at -90. When the sample temperature has reached -80, press the RUN keypad and transfer the cells to the liquid nitrogen storage tanks (-196 °C). Turn the power switch and chart recorder off. The freeze chart (from either control rate freezer) should be observed and marked with the following: patient's name, history number, and date. Complete all forms and attach the chart to the Processing Form.

5.0 PROCEDURE NOTES: NA

6.0 REFERENCES:

- 6.1 Rowley SD. Hematopoietic stem cell cryopreservation: A review of current techniques. Journal of Hematotherapy 1992; 1:233-250.