STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Control Rate Freezing Using CryoMed

DOCUMENT NOTES:

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STCL-EQUIP-005
CONTROLLED RATE FREEZING USING CRYOMED

1 PURPOSE

1.1 The objective of controlled rate freezing of cellular products is to minimize damage during the cryopreservation process. Cells are frozen gradually, at a specified rate that has been determined to preserve cellular integrity.

2 INTRODUCTION

2.1 During cryopreservation, the extent of cellular damage depends on the amount of free water in the cells and the ability of that water to crystallize during freezing. Ice formation initiates in the extracellular environment, resulting in increased salt concentrations as water is removed to form ice. This results in an osmotic imbalance. Water leaves the cells by osmosis, resulting in cellular dehydration.

2.2 One important way these detrimental effects can be minimized is by controlling the cooling rate using a controlled rate freezer. Cells are frozen at a rate preprogrammed into the freezer's computer memory. By decreasing the temperature gradually, the "heat of fusion" is overridden, preserving the viability of the cells. At the end of this freezing procedure, the cells will be at a temperature of approximately -90°C.

3 SCOPE AND RESPONSIBILITIES

3.1 The Medical Directors, Laboratory Manager, and laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	SOP	Standing Operating Procedure
4.2	°C	degrees Celsius
4.3	LN2	liquid nitrogen
4.4	CRF	control rate freezer

5 MATERIALS

5.1 Supplies

5.2 Cryogenic protective gloves

5.3 Cryogenic storage bags and vials

5.4 Freezing presses

5.5 Sample racks

5.6 Liquid Nitrogen

5.7 Thermal printer paper

6 EQUIPMENT

6.1 ThermoScientific CryoMed controlled rate freezer

7 SAFETY

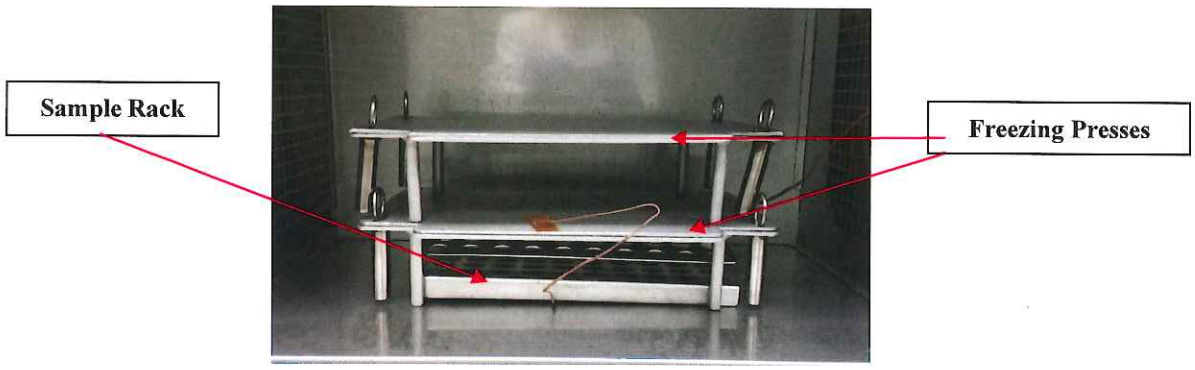
7.1 Use all applicable personal protective equipment when handling any potentially hazardous blood and body fluids to include, but not limited to, lab coats, gloves, cryogenic gloves, and protective eye wear / face shield.

7.2 **NOTE: Use EXTREME caution when working with liquid nitrogen**.

8 PROCEDURE

8.1 Preparation

8.1.1 Place the appropriate amount of presses and vial holder inside the CRF before pressing RUN.



8.1.2 Select the desired freezing profile by scrolling through the selections using the UP/DOWN arrow keys. If the profile list is not on the display, press the Back arrow until the list appears.

8.1.2.1 Select profile 5.1 when freezing products in the 250ml storage bags.

Freezing Program " 5.1 "

(Used for 31-70ml PBPC or BM cryopreservation procedures)

Section	Freezing Rate
1	Wait at 4°C
2	25°C/min until **chamber** reaches **-40°C**
3	50°C/min until **chamber** reaches **0°C**
4	**HOLD chamber** temp at **0°C** for **15** minutes
5	1°C/min until **sample** reaches **-10°C**
6	30°C/min until **chamber** reaches **-65°C**
7	15°C/min until **chamber** reaches **-20°C**
8	1°C/min until **chamber** reaches **-70°C**
9	10°C/min until **chamber** reaches **-90°C**
10	Hold **chamber** for 6 min at **-90°C**
END	**Chamber** stays at **-90°C** until BACK is pressed

8.1.2.2 Select profile 1.1 when freezing products in the 50 ml storage bags.

Freezing Program " 1.1 "

(Used for 10-30ml PBPC or BM cryopreservation procedures)

Section	Freezing Rate
1	Wait at 4°C
2	25°C/min until **chamber** reaches **-40°C**
3	50°C/min until **chamber** reaches **0°C**
4	**HOLD chamber** temp at **0°C** for **5** minutes
5	1°C/min until **sample** reaches **-11°C**
6	30°C/min until **chamber** reaches **-50°C**
7	15°C/min until **chamber** reaches **-20°C**
8	1°C/min until **chamber** reaches **-70°C**
9	10°C/min until **chamber** reaches **-90°C**
10	Hold **chamber** for 6 min at **-90°C**
END	**Chamber** stays at **-90°C** until BACK is pressed

8.1.3 Close and latch the door.

8.1.4 Check the thermal printer paper supply and verify that the paper is advancing correctly.

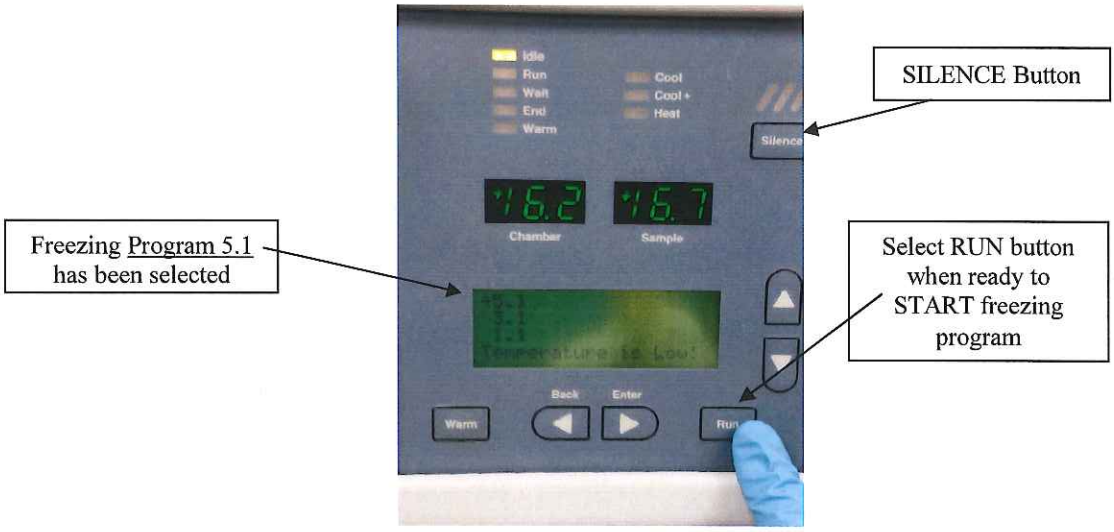
8.1.4.1 To advance the printer paper, press the Online/Offline button. The orange offline light will light up.

8.1.4.2 Press the feed button to advance the paper.

8.1.4.3 Press the Online/Offline button again to place the printer back online. The green online light will light up.

8.1.5 Press RUN to start the freezing program. The chamber will cool to 4°C and hold that temperature until RUN is pressed a second time.

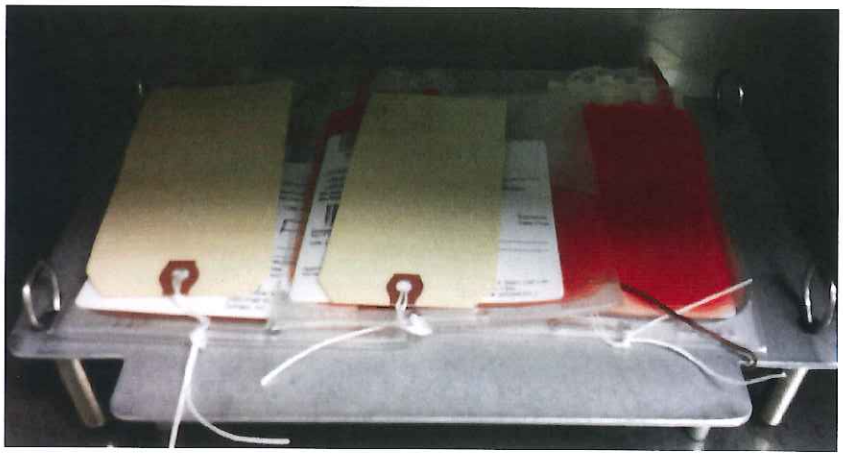
8.1.5.1 Start the CRF before you begin adding DMSO to the product.



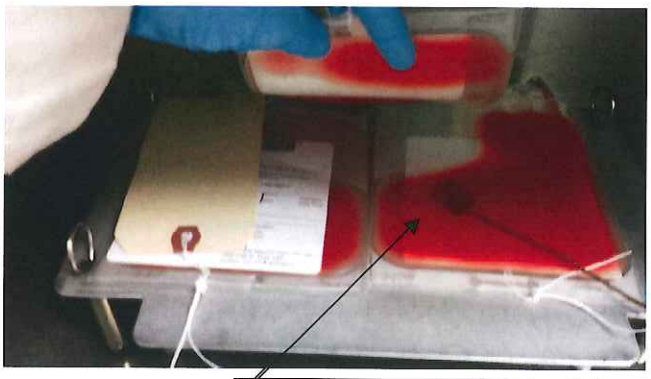
8.2 Cryopreservation

8.2.1 When the product freezing bags are ready to be placed in the control rate freezing chamber, open the door.

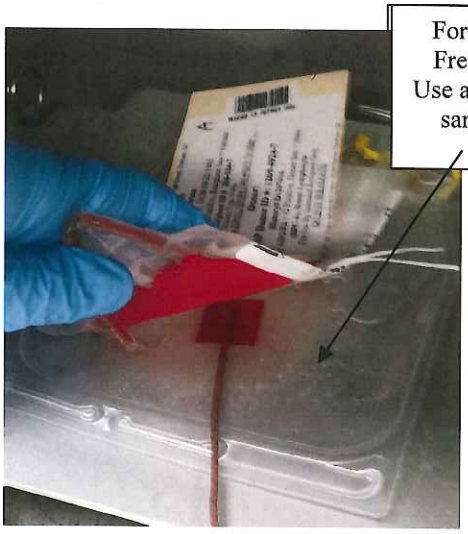
8.2.2 Arrange the product freezer bags on the freezing presses so they are evenly distributed.



8.2.3 Position the temperature probe so that it rests in between the thickest portions of two freezing bags. The probe must not come in contact with the metal of the freezer press. Place the top plate of the freezer press onto the rack and clamp it in place to hold the bags securely on the press.



Temperature probe being "sandwiched" between two (2) cryopreservation bags



For 50ml Cryo Freezing bags. Use a water bag to sandwich the probe.

8.2.4 If freezing sample vials, place the vials in a rack under or on top of the freezer press(es).

8.2.5 Close and latch the control rate freezer door.

8.2.6 Press RUN.

8.2.6.1 Record the date and time you press RUN (2nd time) and initial on the freezing graph.

8.2.7 Label the freezing graph with the patient's name, Duke medical record (history) number, procedure number, barcode, type of cells being frozen, and CRF number used.

8.2.7.1 The thermal printer paper will have a date and time stamp. Verify that this is correct and do NOT cover the date and time with a label.

8.2.7.2 The CRF numbers are 154 or 155, based on the last 3 digits in each freezer's serial number. Serial numbers are located on the back, left side of the freezer.

8.2.7.3 Do **NOT** walk away from the control rate freezer(s) until verifying that the program has advanced from Step 1 to Step 2.

8.2.8 Fill out the Freezer Book and vial location book with where you will be storing the product and vials.

8.2.8.1 Place the assigned canisters in a LN2 vapor freezer while the product is freezing in the CRF. This will allow the canisters to chill so product coming out of the CRF is not placed in warm canisters. Normally the canisters are placed on top of racks in LN2 freezers VF3 and VF4.

8.2.9 If the controller detects an abnormal step during the freezing run, an alarm will sound. Turn the auditory alarm off by pressing the SILENCE key on the pad. Confirm that the run is progressing correctly. Trouble-shooting may be indicated.

8.2.10 When the freezing run has ended, an alarm will sound. The sound of the alarm is not loud so be aware of the approximate time that the freezing program will be complete.

8.2.10.1 A typical freezing program takes approximately 1 hour and 45 minutes to complete.

8.2.11 The freezer will hold the final temperature of -90°C until the control rate freezer door is opened or the run is discontinued.

8.2.12 Press the Back arrow key to end the program.

8.2.13 **NOTE**: NEVER end the freezing program unless the cells are immediately going to be removed from the control rate freezer and relocated to a designated LN2 freezer.

8.2.14 Open the door and relocate all of the freezer bags and sample vials to the appropriate designated LN2 freezer(s) as quickly as possible.

8.2.15 Remove the freezing graph, record the time, date and initial for when the freezing programmed was ended, record the heat of fusion on the appropriate paperwork, and give the freezing graph to the Laboratory Manager or designee so it can be reviewed and then filed in the designated location.

8.2.16 The Medical Director, Laboratory Manager, and Laboratory Technologist IIIs are approved to review the freezing graphs for heat of fusion, cooling rate, and unexpected peaks in temperature.

NOTE: If no heat of fusion is displayed on the freezing graph, a segment (from each cellular patient product cryopreserved in that run) must be removed, thawed, and tested for viability. If the thawed viability is <85%, notify the Lab Manager or designee in case troubleshooting is required and/or additional cells must be collected from the patient). Record the thawed viability on the freezing graph.

8.2.17 Place the empty freezer presses and vial rack in the CRF and close the door. Press WARM on the display.

8.2.17.1 The chamber warms to 24°C. An alarm sounds at completion.

8.2.17.2 The freezer will continue to run until the BACK arrow is pressed. Setting a timer as a reminder to discontinue the warming cycle is advised. Set the clock for approximately 25 minutes.

8.2.17.3 If the control rate freezer is not going to be used for another run, you can forego selecting the WARM cycle by just leaving the control rate freezer door open.

8.2.18 Store the freezer presses and sample racks in a manner that will allow them to air dry. Typically the presses and sample racks are stored on top of the control rate freezers on towels that absorb the condensation that accumulates as they warm up.

8.2.19 Do **NOT** use damp freezer presses. Make sure they are dried thoroughly before use. If they are not dried adequately, cryopreservation bags could stick to the freezer presses which could result in the segments on the bags breaking off, when removing them from the freezer, which could compromise the integrity of the bag and the cells housed within that bag

8.2.20 Thoroughly dry the freezer door and gasket prior to initiating another run.

8.2.21 Maintenance is performed according to the STCL-EQUIP-005 FRM1.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-EQUIP-005 FRM1 Control Rate Freezer Semi-Annual, Annual QC – PM Record

9.2 STCL-EQUIP-005 JA1 Review of Control Rate Freezing Graphs

10 REFERENCES

10.1 CryoMed Controlled Rate Freezer 7452 Series Operating and Maintenance Manual 7007452 Rev.10.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
06	Barbara Waters-Pick	Section 3.1 Modified to read "The Medical Directors, Laboratory Manager, and laboratory staff is responsible for ensuring that the requirements of this procedure are successfully met". Section 4 – Added Definitions/Acronyms to procedure. Section 7 - Added "to include, but not limited to, lab coats, gloves, cryogenic gloves, and protective eye wear / face shield." Section 8 - Added 8.1.18.4 If the control rate freezer is not going to be used for another run, you can forego selecting the **WARM** cycle by just leaving the control rate freezer door open. Section 8 - Added 8.1.19 Store presses and sample racks in a manner that will allow them to air dry. Typically the presses and sample racks are stored on top of the control rate freezers on towels that absorb the condensation that accumulates as they warm up. Section 8 – Added freezing programs in 8.1.2 Section 8 – Deleted 8.2.17.1 "It is not necessary to close the door during the warming cycle". Section 8 - Added 8.1.20 Do **NOT** use damp freezer presses. Make sure they are dried thoroughly before use. If they are not dried adequately, cryopreservation bags could stick to the freezer presses which could result in the segments on the bags breaking off, when removing them from the freezer, which could compromise the integrity of the bag and the cells housed within that bag Section 8 – Added pictures and text boxes. Section 9 – Added reference STCL-EQUIP-005 JA1 Review of Control Rate Freezing Graphs. Section 11 – Added section to this procedure.

Signature Manifest

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All dates and times are in Eastern Time.

STCL-EQUIP-005 Control Rate Freezing Using CryoMed

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