Methodist Health Services Corporation
& UnityPoint Health Methodist
Department of Pathology
Peoria, IL 61636

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HEMATOPOIETIC PROGENITOR CELL PROCESSING, APHERESIS

Purpose

Transplantation of circulating autologous hematopoietic progenitor cells into patients facilitate the recovery of bone marrow function following administration of marrow ablative anti-cancer therapy.

Principle

Mononuclear cells are collected by apheresis in the Dialysis department by qualified personnel from the peripheral blood using the Cobe Spectra. These cells, of which hematopoietic progenitor cells are a part, are then cryopreserved with 10% DMSO, an intracellular cryoprotectant, and stored in liquid nitrogen by the cell processing laboratory.

Specimen

The hematopoietic progenitor cell (HPC) product is collected by apheresis in the MMCI dialysis department. The product is hand carried at room temperature (18-24ºC / 65-75ºF) to the laboratory as soon as possible after collection and is delivered to a clinical laboratory scientist in the laboratory by a dialysis nurse or the transplant coordinator. If more than one patient is being collected, the products, along with all paperwork (including labels) will go into a patient computer-labeled tray in the Cell Processing laboratory. The product should be in a sterile collection bag within a second sealed plastic bag. The collection bag should be labeled with the patient's name, medical number, and HPC# plus date and time collection ended. Any additives or anticoagulants and their amounts, plus volume of product should be noted on the product label. The initials of the collector should be present. The name of the product (HPC, Apheresis) and MMCI should also be visible. Visual examination of the product includes inspection for excess hemolysis, inappropriate cloudiness, or other unusual appearance. If product rejected, fill out Deviation from SOP form (CP:014) and inform collection area of the problem.

Storage requirements for hematopoietic progenitor cell product

	Temperature	Duration/Expiration	Also acceptable
Fresh	18-24ºC/65-75ºF	Up to 4 hours	1-6°C up to 24 hours*
Frozen	< or = -150°C	Indefinite	
Frozen – transport to patient floor and while awaiting thawing	< or = -80°C	Up to 1 hour 15 minutes in dry ice cooler	
Thawed or partially thawed	1-6°C	Up to 4 hours	

*A pre and post N.C. count, CD34 count, and viability need to be done if overnight storage.

Disaster plan note: If interruption occurs before addition of freezing media, use above table. If freezing media added, follow instructions at B.13 placing canisters on top of frames. If interrupted during freezing, stop program noting the section number and follow B.13 for placement of canisters.

Reagents

- Heparin (10,000 units/ml) obtain from pharmacy with miscellaneous slip – Store at room temperature. May be in equivalent 5000 units/0.5 ml vial.
- 0.9% Sodium Chloride (NaCl) Injection USP 100 ml – obtain from SPD with miscellaneous slip. Store at room temperature.
- DMSO – Store at room temperature. Protect from light.

Equipment/Supplies

Cryomed programmable freezing controller (microcomputer) and recorder
Cryostorage units with LN2 tanks attached *
Laminar Flow Hood

Cryomed Freezing Chamber	Canisters for Storage
Blunt plastic cannula (B-D 303345)	Plasma Transfer Site (4C2240)
Vial access cannula (B-D 303367)	Frames for Canisters
Blood Bank Spike Injection site (1C8333)	Origen Cryostore CS 500n freezing bags
Choraprep Solution Applicator	
1 cc syringes	2 ml Cryotubes
3 cc syringes	Kryorack
5 cc syringes	Hemastats
10 cc syringes	Roller Bar (Dowels)
20 cc syringes	Electronic Balance
30 cc syringes	Product tie tags
60 cc syringes	Heat sealer
Test Tube Racks	
Biohazard labels	16, 18, or 20 gauge needles - obtain
Sterile 50 ml Centrifuge tubes	from storeroom / warehouse

*Airgas (phone 674-3104) is our supplier of liquid nitrogen tanks. Two tanks are needed for the storage units and one is needed for the freezing chamber if collections are taking place. Tanks are ordered weekly as needed.

The LN2 tank use log monitors the psi reading on the day of delivery, plus dates connected and emptied with recording of the # of days used. Record on form CP:070

Backup equipment to use in case of equipment failure is listed after procedural notes.

Calibration

See CP:020 QC Schedule for Cell Processing Lab Equipment, Day of Use: Cryomed Recorder.

Quality Control

LN2 Tank - Test each new tank pressure by running 2-3 minutes of program 1.1. If the tank is not putting out LN_2, double-check the pressure gauge. It should read approximately 22 psi (range 15-35). If low, check to make sure the vent is closed. If open, close it. Pressure should be built by the next day. Check program the next day. Document on CP:069. If vent was closed and LN_2 will not start, call Airgas for a new tank. Always scroll through program 1.1 when you check a new tank to make sure program has not been erased. See CP:037

Cryomed Controlled Rate Freezing –

The super cooling point (the temperature drop which occurs just prior to liquid-to-solid phase change) and freezing point (seen at end of rapid increase in temperature at formation of solid) are recorded. The difference between these temperatures can be 1-9º C. This is checked at Step B.14 and documented on CP:069

Procedure

A. Preliminary paper work and labeling can be done before product is delivered. If more than one patient is being processed, all paperwork for each patient and the product (whenever possible) is kept in a separately labeled tray. All syringes, tubes, canisters, and bags are to be labeled throughout the entire processing procedure with the patient name so as to avoid mix-ups, contamination, and cross-contamination.

1. Day 1 Only
   a. Freezer summary (CP:033) should be stapled to inside left of folder.
   b. HPC file checklist should be paper clipped to outside of folder.

2. Day 1 through last day – HPC Processing report (CP:032), HPC Daily Checklist (CP:031)
   a. Label all sheets and folder with a computer label that is generated when dialysis orders the tests.

b. On daily sheets, put HPC #_____.

3. HPC Patient File checklist (CP:035) – Initial each step when completed and date as appropriate.

a. Verify that the physician processing request (CP:034) and patient consent have been received on day 1 with the product. Call dialysis if either are missing.

b. Call dialysis for patient's weight and diagnosis. Weight is needed in Kilograms, so if given in pounds, divide by 2.2.

c. As per checklist, add patient's name to inventory file summary sheet.

d. Print infectious disease tests for patient file. Put biohazard label on product tie tag before freezing if any test positive or repeatedly reactive. Also place a biohazard label on patient's file front cover and note on cover of patient's file that biohazard label is needed daily on product before freezing. These products, plus any products with incomplete infectious disease testing, are to be stored in **vapor phase** to avoid cross -contamination. Note on patient file. These products are in quarantine and need medical director approval if to be infused.

Document on CP:033 HPC Freezer Summary for Patient File and Record Review. If, when

incomplete testing is finished, the infectious disease tests are negative, note on the front cover of patient file that vapor phase is no longer necessary. These products are no longer in quarantine as far as infectious disease testing, but may still be in quarantine awaiting microbial testing results. If and when microbial tests are negative, date of quarantine release is noted on freezer summary sheet stapled inside patient folder. These products are now available for infusion, as needed. Liquid storage can be used from now on. The infectious disease tests that apply are: a test for anti-HIV-1 is confirmed positive, a test for anti-HIV-2 is positive, a test for HBsAg is reactive, a test for anti- HCV is reactive, a test for anti-HBc is reactive, a test for HTLV type I or II is positive, or an RPR is reactive..Record on CP:043 if any results are positive.

e. Approximately 4 weeks after last collection, document post processing microbial culture results on CP:042 Results of Microbial Cultures….and have signed by cell processing lab director.

4. Daily checklist (CP:031)

a. Check balance with 147.5g. weight. Backup balances are available in Histology, if needed.

b. Label 2 cryotubes with patient's name, medical #, HPC # and date using cryopen. (You can use computer label for Hematology / Flow Cytometry tube.) One is for frozen aliquot and one for Hematology / Flow Cytometry for N. C. count, Hct, CD34+ assay, and viability. On Hematology / Flow Cytometry tube, write "post pheresis".

c. Label cap for frozen aliquot with patient's initials.
d. Leave a third cryotube unlabeled for freezing probe.
e. Label a canister with patient's name, medical #, HPC #, date, Bag label # and bag _____ of _____ using cryopen. Put canister into refrigerator.
A second canister may be labeled later, if needed.
f. Fill out a product tie tag using a cryomarker. (Patient name, HPC, Apheresis, Medical # / HPC #, date, bag ___ of ____, and tech initials. The tag is checked by a 2nd Clinical Laboratory Scientist for accuracy. Add time collection completed when product arrives. Volume is written on tag when final volume is known after product is transferred to the freezing container. A second product tie tag can be made later, if needed. The patient's daily ABORh is recorded on the tag. A copy of the report is obtained from Blood Bank who performs crosscheck with historical records. See "ABO and Rh Tube Testing" in BB SOP.
g. Put freezer bag number on freezer summary sheet with date and HPC #.
h. Write bag number on processing report.
i. To order blood and fungal cultures for Microbiology, see CP:002.
j. For details on equipment checks of laminar flow hood, cryomed recorder, programmable freezer, and freezing chamber, see Quality Control schedule in Standard Operating Procedure manual. Record checks on CP:069 Quality Control for Recorder, Freezing Chamber, LN2 Tank, Tube Sealer, Balance, Cryomed Freezing Process.

k. As steps are completed, check off on daily checklist with your initials.
l. When processing report is completed, a 2nd Clinical Laboratory Scientist will double check results.

5. Preparing freezing media (working solution)
NOTE: Use sterile procedure under hood.
a. Clean hood and countertop with disinfecting towelettes and mark on Hood,Benchtop/Cleaning/ Temperature/Humidity Record (CP:073) along with temperature and humidity from gauge next to Cell processing door.
b. Do not use any reagent that has evidence of bacterial growth or turbidity, or in any way has had its' sterility compromised. Any reagent or supply rejected shall be documented on "Reagent/Supply Problem log".
c. Using following table, make desired amount of media to correspond with expected volume of product.

Freezing media total	**25 ml**	**30 ml**	**35 ml**	**40 ml**	**45 ml**	**50 ml**
0.9% NaCl	15 ml	18 ml	21 ml	24 ml	27 ml	30 ml

DMSO	10 ml	12 ml	14 ml	16 ml	18 ml	20 ml

d. Using sterile procedure, enter Chloraprep swabbed port of 0.9% NaCl bag with 16, 18, or 20 gauge needle and appropriate syringe. Remove needle and place into a sterile 50 ml centrifuge tube. Use a new bag daily. Put current date on bag.

e. Swab top of bottle with Chloraprep. Pull DMSO into syringe using syringe cannula. Remove cannula from syringe before adding the DMSO to the NaCl.

f. Date and initial the DMSO bottle when opened. Put expiration date of 1 week later on the bottle.

g. Cap the 50 ml tube and mix by inversion several times. Label with date and "freezing media". Refrigerate until used. It must be cold before adding to the cells.

h. If more media is needed during the procedure, any amount may be made in a ratio of 3 parts 0.9% NaCl to 2 parts DMSO. Do not make more than is needed. Be sure to put in fridge until cold before adding to cells. Final concentration of working solution in progenitor cell product: 15% 0.9% NaCL and 10% DMSO.

NOTE: Complete processing report, file checklist, and procedure checklist as items are completed.

6. Processing report (CP:032)
   a. Fill out patient's diagnosis, actual weight (Kg), machine used is Spectra, volume processed is 12 L unless dialysis states otherwise.
   b. Fill in lot number and expiration date for heparin.
   c. For pheresis kit lot number, "See Nursing Notes" is filled out.
      1) Dialysis will bring a daily log sheet that contains the collection kit lot numbers and signature of collection nurse when the specimen is delivered. Put in patient's file.
   d. Freezing bag lot number and expiration date can be taken off box or bag.
   e. Record DMSO and 0.9% NaCl lot numbers and expiration dates.

B. <u>All underlined steps following are performed under the hood</u>.
1. When the progenitor cell product is received into the laboratory, check for correct labeling as stated under "Specimen" on page one. Check visually for bag sterility and heat seal the tubing several times leaving approximately one inch of tubing. Make sure seals are complete. Cut off excess tubing and remove plastic clip. The product should be at room temperature in the laboratory until refrigerator storage in step B.7b. Backup for heat sealer is metal clips and sealing instrument.

2. Mix product well. Using Chloraprep applicator to clean spike injection site inserted into bag, remove approximately 0.9 ml aliquot for hematology and flow cytometry, using 1 cc syringe and blunt cannula. Be sure aliquot represents a well-mixed specimen.
   a. Hematology aliquot should be put into labeled cryotube. Hematology performs a WBC count (which we call a nucleated cell count) and a hematocrit. Two techs each do a 100 cell differential which we average for our report. Nucleated red blood cells are included in the 100 cell differential. Hematocrit should be less than 50%. If it is 50% or greater, contact dialysis to see if there is an explanation. Contact laboratory director with findings and document on SOP deviation log the decision made as to whether product should be processed.
   b. Flow cytometry uses the tube after Hematology is finished for the CD34+ count and viability.

3. Fold bag in half to weigh the product, subtract 44 grams for specimen bag (40.5g) and spike injection site (3.5 g) if bag has clear tabs. If it doesn't, see note on counter for weight of bag and injection site to subtract. Convert weight to volume by dividing by 1.03. When the nucleated cell count is received from Hematology, calculate the total nucleated cell count by multiplying the cell count/ml by the volume. Fill in processing report as calculations are completed. These numbers go under "harvested" column on the report. Starting with collection #2 and subsequent collections, check previous days total N. C. count. If today's results are not within ±50% of the previous day's total N.C. count, performed on the hematopoietic progenitor cell sample, the sample can be re-run if desired. Document on patient worksheet that the sample was double-checked if it was. If any discrepancies are found, such as wrong count reported, fill out an RL SolutionsReport online and have reviewed by the Cell Processing Laboratory Director. Patient's peripheral WBC count, whether or not patient took Neupogen as ordered, or the collection process can all have an effect on the N. C. counts, thus a large deviation from day to day can sometimes be seen. In any case, the product is still processed as usual. Record on DiscrepantResults log (CP:040) if not within 50% of previous collection and the reason.

4. There are two criteria to consider when deciding whether or not the product needs to be diluted and/or divided into two or more bags.
   a. The first criteria is the nucleated cell count. The product must not have a concentration of more than 3×10^8 nucleated cells (N.C.) per ml. Less concentrated is fine. To decide whether or not the product needs to be diluted or left as is, divide total N.C. $\times 10^8$ by 3, then multiply by y 0.75. Then divide by the volume. If the resulting number is less than or equal to 1, no dilution may be necessary. See Criteria 2. If the

resulting number is more than 1, dilution with 0.9% NaCl is necessary. Bring volume to whatever is necessary so that there is approximately 3 x 10^8 cells/ml. See sample calculation sheet (CP:036) following this procedure, keeping in mind the 55-120 ml volume limit per bag. Additional freezing media may have to be made.

b. The second criteria is the product volume. The product volume plus freezing media needs to be between 55 and 120 ml per bag. If the product volume plus freezing media is greater than 120 ml, more than one bag is needed. The reason it is not allowable to freeze more than 120 ml per bag is because the infusion takes too long and the cells die at room temperature. Also, the freezing process may not be as effective with large volumes. Bring the volume to whatever is necessary (see c. below) so that there is 55-120 ml / bag, keeping in mind the 3 x 10^8 cells / ml, if also applies. See sample calculation sheet following procedure. Additional freezing media may have to be made.

c. If dilution is necessary, use 0.9% NaCl with Chloraprep swabbed injection site and add to product. Weigh the product. Subtract 44g for weight of bag and calculate volume for decision to be made on the number of bags necessary and number of ml per bag once freezing media is added. This volume does not go on the processing report.

5. Mix the final product well.

a. Using 1 ml syringe with vial access cannula, sterilely add 10 units/ml of Heparin to the stem cell product. (Be sure to swab the top of the Heparin vial with the Choraprep.)

1. To calculate the amount of Heparin to add to the product, multiply what the total volume with media and any extra for dilution (if needed) will be by 10, then divide by 10,000. There are either 5000 units per 0.5 ml or 10,000 units per ml Heparin in the vials we use. Either way, the calculation is the same. Mix well.

b. If dilution of product was necessary, using 3 cc syringe and blunt plastic cannula, remove 1.5 ml sample. One ml is for another nucleated cell count and hematocrit. Label cryotube with patient's name, medical number, date, HPC #, and "diluted".

c. Put 1 ml of sample into cryotube. The remaining 0.5 ml is left in the syringe for a microbial culture. Label the syringe with the correct Microbiology label.

d. If no dilution was necessary, use same 1 ml syringe used to add Heparin and withdraw sterile 0.5 ml sample for micro culture. (No specimen is taken off for Hema counts.)

6. Prior to their use, visually check the freezing bag(s) for damage or evidence of contamination. This includes breakage of seals and abnormal color. Do not use bag if any problem is evident. Record any problem on action log for

reagent/supply problems. Before transferring product, label the bag pocket area with: HPC, Apheresis, Cryopreserved, Patient Name, Medical Record # HPC-A #, and bag #. Weigh the product and transfer into freezing bag. If freezing more than one bag, divide evenly into the number of bags needed. You can use 60 cc syringes and blunt plastic cannula, if possible. If more than 60 ml per bag, use transfer tubing with spike and needle adapter.

a. Before removing spike injection site from product bag, use hemostat right below port to seal the opening, being careful not to puncture the bag. Attach spike end of tubing to product bag and needle adapter end to freezing bag. Zero the freezing bag on the scale and allow the correct amount of the product to flow into that bag. Heat seal the tubing two or three times near the "Y" above the junction, not below, as the other side of "Y" is used to add the freezing media.
b. Cut off excess tubing. Be sure to cover needle adapter whenever it is exposed (if not being reconnected right away) to avoid contamination.
c. Fill subsequent freezing bags using scale when necessary. Heat seal the tubing. Again, inspect bags for any problem with sterility or damage.
d. Weigh each bag (whether there is 1 or more bags), making sure scale is still tared for the freezing bag. If it was not zeroed, subtract 21 g from each for weight of bag. Convert weight to volume by dividing by 1.03 on processing report. Record individual bag weights and volumes under cryopreserved column.
e. Carefully check the identifying information and attach the product tie tag on the freezing bag with cable tie and initial the "Tech Box".

7. Divide each product volume by 3. The result is the volume of freezing media to add (3 parts product + 1 part media). This number goes in cryopreserved column on the processing report.
   a. Write on corner of each bag the volume of that bag plus the amount of freezing media to be added.
   b. Put product in 1-6ºC refrigerator until well chilled (at least 15-20 minutes) up to four hours. (up to 24 hours in an emergency)
   c. Assign each canister a storage position in the cryostorage freezer units. Units III and IV have openings as detailed in the freezer position inventory file. Vary the canisters between the two units. Position "a" or "b" in each frame for unit III should be used for vapor phase storage. "c" and "d" are used for liquid storage. Unit IV is all vapor phase.
   d. Measure correct amount of freezing media into separate syringe(s) using blunt plastic cannula. Mark volume on (each) syringe and keep in fridge until ready to use.
   e. Record quality control for recorder, freezing chamber, and LN2 tank before proceeding.

8. Take one syringe and the corresponding bag out of the fridge when ready.
   a. Add the cold freezing media (using syringe hooked to male adapter) to the product slowly while mixing well on the Kryorack (approximately 45-60 seconds).

   b. The product should be frozen as soon as possible once the media is added. Most of the air bubbles should be expelled from the freezing bags to avoid breakage of the bag after freezing. This can be accomplished after most of the freezing media is added to the bag.

   c. Roll the bag around the dowel and hold the port of the bag up to push the air back through the tubing to the syringe. Use hemostats to keep air from lodging in the two ports on the bag. Work quickly to get the majority of the bubbles expelled from the bags.

   d. Whether freezing in one or more bags, save approximately 0.5 ml sample syringe from the LAST bag filled. Fill one labeled cryotube to the 0.5 ml line to be frozen. This aliquot is kept frozen in storage Unit IV in case the microbial culture is positive and needs to be repeated. A one ml aliquot is also needed in a cryotube for the temperature probe. You may use sample from the discarded tubing if careful to keep sterile

9. Close roller clamp and heat seal the tubing two or three times, close to the bag. If the heat sealer is not working, use Hemodialysis' heat sealer attached at the back of one of their Cobe Spectra machines, if time allows. Otherwise, use metal clips and store in vapor phase. Cut off excess tubing.

10. Place the bag into its appropriate canister, making sure neither the tubing nor the bag is caught. If more than one bag, place first canister back into the refrigerator while adding media to the second bag. Put into its canister as well as additional bags into their canisters after media is added, if applies.

11. Stagger the canisters in the freezer chamber along with the tubes.
    a. Place the probe in the center of the 1.0 ml aliquot making sure the probe is not touching the bottom or sides of the cryotube.

12. Open the valve on the liquid nitrogen tank. You should still be able to see the indicator on the gauge of the tank.
    a. Flip the power, the recorder switch, and the chart drive on.
    b. Uncover the pen and place the arm in a down position.
    c. Touch the key pads: **ALRM, FAN, COOL** (Located in the upper left corner.)
    d. Under **PROGRAM DISPLAY,** put in the program number. Touch 1, 1 again, ·, then · again.
    e. Touch **RUN** under mode selection (the nitrogen will start).

f. Under **TC Scanner**, touch **SCAN**. Under **Display**, touch **PROG** then **CHAM**
ACT SAMP
so the actual sample temperature is displayed. Make sure the "SAMPLE" and "ACTUAL" lights are lit.

g. When the actual sample temperature is -4ºC to -5ºC, touch **RUN** again. The program will now go into Section 2 and automatically continue through section 6.

h. Use a computer label to label the chart paper with the patient's name, hospital number, date, HPC #___, and your initials.

13. If the freezer will not start, refrigerate the products until the problem can be determined. If the program has been erased, see Cryomed Model 1010 Microcomputer program outline (CP:037) and "Programming Cryomed Computer 1010" (CP:038) following this procedure. If the program is fine and the problem cannot be fixed, canister(s) may be placed in vapor phase on top of the frames in the cryostorage units by first placing 2 styrofoam sheets around each canister and securing with tape. This is to slow the freezing process. The next morning, place the unwrapped canisters into their assigned cryostorage unit location. If the program starts fine, but a problem develops before the heat of fusion takes place, use above method of placing in cryostorage units. If something happens during the freezing program after Section 3 (heat of fusion) when the product goes from the liquid phase to the frozen phase, immediately place the canister directly into the assigned cryostorage unit location.

14. During section 7, the "END" light will be on. When the program is complete, the "END" light will flash and an alarm will sound continuously. Check for chamber temperature reading end point of –100 C on graph. See QC for Cryomed controlled rate freezing on page 3.
    a. Turn the alarm, the recorder, the chart drive, and the power off.
    b. Cover the pen.
    c. Close the valve on the liquid nitrogen tank and check the gauge to see if a new tank is needed.

15. Remove the canister(s) and tube. Quickly put the canister(s) into the designated frame(s) in the liquid nitrogen storage units. Put the cryotube into the box in the LN2 Unit IV. The canister's location should be noted on the patient's process report and (in pencil) on the freezer inventory list. LN2 storage is at -196°C liquid phase and -150°C or lower vapor phase.

C. Completing the processing report.

1. The two 100 cell differentials (CP:039) from hematology are averaged and

put on the processing report.

2. If the product was diluted, record the second nucleated cell count and Hct on processing report under Cryopreserved column.
   a. If no dilution was made, use numbers from harvested column for cryopreserved column for N.C. and Hct.

3. In either case, calculate new total N.C. using cryopreserved volume. Use the lower value total N.C. count for the rest of the calculations.

4. At the center of the report, follow calculations to obtain mononuclear cells (MNC) count for patient. Complete small boxed area at bottom left of report. Be sure to add the day's MNC count into previous counts for collection total.

5. The 'VOLUME EACH BAG" is the average of all the bags as is the "TOTAL N.C. PER BAG".

6. Put the results from processing report into HLAB. See CP:002.
   a. Obtain a copy of the CD34+ and viability results from flow cytometry and record on processing report. Total CD34+ as appropriate.

7. Give the processing report, Hematology counts (including differential), freezing curve, nursing notes, and daily checklist to pathologist for review.

Calculations

1. See separate calculation worksheet (CP:032) for processing report.

Anticipated Results / Objective

Ideal viability >70%

To collect approximately 5 x 10^6 CD34+ Cells/Kg body weight over one or more days.

Endpoint

5 x 10^6 CD34+ cells/Kg body weight unless physician requests otherwise.

Reporting Results

1. See CP:002 for entering results into Sunquest.. Double check entries before

verifying.

2.. Mike or dialysis informs the transplant director of the CD34+ results daily and they make a decision about subsequent collections for the patient. Mike or dialysis informs the cell processing laboratory of the decision. The cell processing lab is aware of when the endpoint has been met. Record the person's initials who is notifying the physician of the day's count on the daily checklist. If the endpoint has not been reached and no more collections are to be performed, for whatever reason, document this notification and any remedial action if taken, on the freezer summary page at front of chart.

Procedural Notes/Problem-Solving Tips

- ★ Any object or container (including original product freezing bags returned after infusion, syringes, etc.) that have come into direct contact with the progenitor cell product must be disposed in a red biohazard bag.
- ★ Sharps and glass must be disposed of in a Sharps container.
- ★ Any deviations form this SOP must be recorded on "Deviations from SOP" log (CP:041)and signed by Cell Processing Laboratory Director.
- ★ In the event of a post processing microbial culture showing positive results, see SOP CP:007.
- ★ In the event of emergency overnight storage, pre and post storage N. C. counts, CD34+ counts and viability must be performed.

- ★ Backup equipment in case of equipment failure:
  Cryomed freezing controller / chamber - no alternate equipment - See B. 13 of this procedure.
  Cryomed Recorder – Manually record sample temperature at designated times as listed on sheet in file labeled "Manual graphs for freezing when Cryomed printer down"
  Laminar Flow Hood – Pharmacy IV room has a horizontal hood we have approval to use. Call ext. 5779 to get specific instructions first. Will need to wear scrubs, shoe covers, hat, mask.
  Heat Sealer - Cobe Spectra in Hemodialysis has a heat sealer we can use.
  Electronic Balance – replacement in Histology

References

University of Nebraska Bone Marrow Processing Lab, October 1989.

FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing and Administration, Fourth Edition, October 2008

Author: Deb Rinne CLS (ASCP) July 1990

Laboratory Director Approval: DMcKnight MD **Date**__7/1990_____

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
March 4, 2017	Elizabeth A. Bauer-Marsh, M.D.	Elizabeth A. Bauer-Marsh MD
SECTION MEDICAL DIRECTOR		
May 10, 2016	Julia Adams, M.D.	Julia Adams, M.D.

REVISION HISTORY			
Rev	Description of Change	Author	Effective Date
.1	Eliminated reference to Pathnet , eliminated printing/sending chart , changed 2x10 NC/ml to 3x10, added ABORh BB SOP reference	DRinne	
.2	Added document numbers. Added New medical director	Deb Rinne	2/23/16
.3	Changed fridge temp, changed form name on p.5, omitted red barrel for glass	Deb Rinne	1/22/17

Reviewed by

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
Deb Rinne, CLS	12/1/03			Douglas McGrady, M.D.	12/03
Deb Rinne, CLS	6/10/04			Douglas McGrady, M.D.	6/10/04
Deb Rinne, CLS	12/5/05			Douglas Mc Grady, M.D.	12/05
Deb Rinne, CLS	10/25/06			Douglas McGrady, M.D.	10/6
Deb Rinne, CLS	10/1/07			Douglas McGrady, M.D.	10/7
Deb Rinne, CLS	8/19/08			Douglas McGrady, M.D.	8/19/08
Deb Rinne, CLS	4/02/09			Douglas McGrady, M.D.	4/02/09
Deb Rinne, CLS	4/16/10			Douglas McGrady, M.D.	2/2010
Deb Rinne, CLS	5/22/12			DMcGrady MD	6/26/12
Deb Rinne, CLS	4/11/14			DMcGrady MD	4/11/14
D. Rinne	2/23/16	Kathy L. Turpin	6/2/16	James Adams, M.D.	7/11/16
D. Rinne	1/22/17	June Bembenek	2/3/17	James Adams, M.D.	4/19/17