STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-PROC-012

DOCUMENT TITLE:

Hetastarch Depletion Procedure

DOCUMENT NOTES:

Initial Release to Master Control. Prior hard copy signatures on file. See Doc Control for previous revisions.

Document Information

Revision: 03	**Vault:** STCL-Processing-rel
Status: Release	**Document Type:** Processing

Date Information

Creation Date: 30 Apr 2014	**Release Date:** 20 Jun 2014
Effective Date: 20 Jun 2014	**Expiration Date:**

Control Information

Author: WATE02	**Owner:** WATE02
Previous Number: STCL-PROC-012 Rev 02	**Change Number:** STCL-CCR-193

STCL-PROC-012
HETASTARCH DEPLETION PROCEDURE

1 PURPOSE

1.1 This procedure may be used to deplete Red Blood Cells (RBCs) from granulocyte collections, Umbilical Cord Bloods (UCB), bone marrow, or any other product requiring RBC depletion.

2 INTRODUCTION

2.1 Hespan is composed of 6% hetastarch (hydroxyethyl starch) in 0.9% sodium chloride. When added to products containing large amounts of RBCs, it increases the erythrocyte sedimentation rate. This procedure also incorporates the use of centrifugation in conjunction with the sedimentation properties to speed up the process of RBC depletion. RBC depletion is commonly indicated when a granulocyte product is transfused to an ABO incompatible patient, when a cord blood is processed, and when a bone marrow backup volume is too low for processing on an automated instrument. RBC depletion is also employed when a product is designated in a written order by the medical directors or designees.

3 SCOPE AND RESPONSIBILITIES

3.1 The Medical Directors, Laboratory Manager, STCL staff, and the Quality Systems Unit (QSU) are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	RBC	Red Blood Cell
4.2	UCB	Umbilical Cord Blood
4.3	mL	milliliters
4.4	ABO	Blood Groups
4.5	SOP	Standard Operating Procedure
4.6	QSU	Quality Systems Unit
4.7	PPE	Personal Protective Equipment
4.8	QC	Quality Control
4.9	WBC	White Blood Cell
4.10	STCL	Stem Cell Laboratory
4.11	RPM	Revolutions per minute
4.12	HCT	hematocrit
4.13	CRP	Component Rich Plasma (Plasma + non-red cell fraction)
4.14	HCT	hematocrit

5 MATERIALS

5.1 Supplies

5.1.1 Syringes: 60 cc, 10 cc, 20 cc or 30 cc

5.1.2 Counting Vials

5.1.3 Sterile cannula or needles

5.1.4 Gloves

5.1.5 Transfer bags, 150 ml or larger

5.1.6 Culture bottles

5.2 Reagents

5.2.1 0.9% sodium chloride, USP

5.2.2 Hespan, 6% hetastarch in 0.9% NaCl

5.2.3 Plasmalyte-A

6 EQUIPMENT

6.1 Biological Safety Cabinet

6.2 Automated hematology analyzer

6.3 Calculator

6.4 Sterile Welder

6.5 Heat Sealer

6.6 Refrigerated centrifuge

6.7 BacTAlert 3D instrument

7 SAFETY

7.1 Wear appropriate personal protective equipment (PPE) when handling any/all potentially infectious blood or body fluid to include, but not limited to, lab coats, gloves, goggles, etc.

8 PROCEDURE

8.1 After the product arrives in the lab, do the following QC:

8.1.1 WBC count

8.1.2 Hematocrit

8.1.3 Viability

8.1.4 Sterility

8.1.5 Coverslip (if applicable)

8.1.6 Measure the total volume of the product

8.1.7 Determine the total number of WBCs

8.1.8 Record this information on appropriate worksheets

8.2 Document supplies, reagents and equipment used in processing of the cells on the appropriate Processing Lot Number worksheet.

8.3 **If the hematocrit is greater than 60%**, adjust to 45% using the following formula: (actual HCT / desired HCT) x Total volume of sample = Final Product Volume (including amount of Plasmalyte-A needed to adjust (lower) HCT).

NOTE: This step must be used for products with HCT >60%.

NOTE: Ex: Product volume = 250 mLs; actual HCT = 61%; desired HCT = 45%. Calculate as follows: (61/45) x 250 mLs = 338.9 mls final product volume. Volume 338.9 – 250 mls = 88.9 mLs of Plasmalyte-A to ADD to the product in order to adjust the HCT to ~ 45%.

8.4 Place the product into a transfer bag, choosing the size bag that is closest to the total volume of the sample. Sterile connect, using the tubing, to another bag of the same size.

8.5 Add 1 ml of Hespan for every 5 mls of product. Divide the total volume of the product by 5 to calculate this amount.

NOTE: (eg. Product volume = 250 mLs; 250 / 5 = 50 mLs of hespan is needed)

8.6 Mix product thoroughly.

8.7 Allow the product to hang on plasma expressor, undisturbed, for at least 30 to 60 minutes.

8.8 If the interface is not clearly defined after 30-60 minutes, centrifuge the product for 10 minutes at a speed of 700 RPMs at 2-10°C. Some additional gravity sedimentation time may be needed after centrifugation if the interface is not clearly defined. If necessary, hang the product bag, post centrifugation, back on the plasma expresser and allow it to settle until a clear interface is visible; this make take an additional 10-15 minutes.

8.9 Express the component-rich plasma (CRP) into the connected bag and perform the following QC testing:

8.9.1 WBC count

8.9.2 Hematocrit

8.9.3 Viability

8.9.4 Measure the total volume of sample

8.9.5 Determine the total number of WBCs in the product

8.9.6 Calculate the total nucleated cell recovery.

8.9.7 Perform sterility testing.

8.9.8 Record this information on the appropriate worksheet

8.10 The product is now RBC depleted and may be cryopreserved or transfused to the patient. Be sure any additional QC testing, that might be required, is completed

before the product is cryopreserved or distributed for infusion. Label the product appropriately.

8.11 If cryopreservation is necessary, follow the appropriate standard operating procedure for cryopreservation based on the product type.

8.12 If the product is designated for immediate infusion, label the bag appropriately following STCL labeling guidelines. Communicate with the clinical team to coordinate the timing of the infusion. Store the product appropriately until it is ready for distribution / infusion.

8.13 Limitations of Procedure

8.13.1 This procedure does NOT remove all unwanted RBCs.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-FORM-048 Processing Lot Numbers – Granulocyte Processing

10 REFERENCES

10.1 Pablo Rubenstein,MD Director Placental Blood Bank, New York Blood Center, NY; conversations and supervision of procedure.

10.2 Rubenstein, P, Dobrila, L, Rosenfield, RE, Adamson, JW, Migliaccio, G., Migliaccio, AR, Taylor, PE, and Stevens, CE. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA 92:10119-10122, Oct 1995.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
03	B. Waters-Pick	Reformatted the entire document. Section 4.4 Added a few abbreviations. Section 5.1 Added culture bottles to supply list. Section 6.7 Added BacTAlert 3D instrument to equipment list. Section 8.3 Modified to better clarify instructions and added an example Section 8.5 Added an example Section 8.8 Added more detail to better clarify instructions Section 9.1 Added number and name to lot number form used Section 8.10 Added wording to ensure add'l testing, if needed, is completed before product is cryopreserved or distributed. Section 8.12 Slightly modified wording to better clarify instructions. Section 11 Revision History added to this document.

Signature Manifest

Document Number: STCL-PROC-012 **Revision:** 03
Title: Hetastarch Depletion Procedure

All dates and times are in Eastern Time.

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Barbara Waters-Pick (WATE02)		07 May 2014, 08:01:57 PM	Approved

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Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		11 Jun 2014, 08:36:12 PM	Approved