STEM CELL LABORATORY (STCL)

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Processing Bone Marrow Using the Sepax 2 Instrument

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STCL-PROC-046
PROCESSING BONE MARROW USING THE SEPAX 2 INSTRUMENT

1 INTRODUCTION

1.1 The Sepax 2 RM system is a cell processing system intended for the separation of nucleated cells from cellular products, including bone marrow. The system uses a rotating syringe technology that provides both separation through rotation of the syringe chamber (centrifugation) and component transfer through displacement of the syringe piston. An optical sensor measures the light absorbance of the separated components and manages the flow direction of each of them in the correct output container. The system allows the fast, automated and reproducible separation of cellular products in a functionally-closed and sterile environment with a single-use separation kit.

2 PURPOSE

2.1 This protocol is designed for routine volume reduction of bone marrow and extraction of the enriched nucleated cells. Input/output volume ratios are determined with consideration of intended recipient size, ABO/Rh incompatibilities, and/or desired final volume for cryopreservation or infusion.

3 SCOPE AND RESPONSIBILITIES

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

4 DEFINITIONS/ACRONYMS

4.1	QSU	Quality System Unit
4.2	BSC	Biological Safety Cabinet
4.3	HIS	Hospital Information System
4.4	STCL	Stem Cell Laboratory
4.5	QC	Quality Control
4.6	RBC	Red Blood Cell
4.7	mL	milliliter
4.8	ISBT	International Society Blood Transfusion
4.9	BM	Bone Marrow
4.10	RFLP	Restriction Fragment Length Polymorphism
4.11	SCD	Sterile Connection Device

5 MATERIALS

5.1 SmartRedux protocol with the CS-490.1 kit

5.2 Bone Marrow cellular product input bag

5.3 150, 300 or 600 mL transfer bags

5.4 Heatsink accessory (only applicable with initial volume greater than 1 liter)

5.5 Sterile Needles, various sizes

5.6 Syringes, 3 mL, 5 mL, 10 mL, 20 mL, 30mL, or 60 mL

5.7 Alcohol Prep Pads

5.8 Sample Tubes

5.9 Sampling Site Coupler

5.10 SBT Barcodes

5.11 Demand Label

6 EQUIPMENT

6.1 Sepax 2 RM

6.2 BSC

6.3 Heat Sealer

6.4 Sterile Tubing Welder (if applicable)

6.5 Scale

6.6 Tubing stripper

6.7 Heatsink accessory (only applicable with initial volume greater than 1 liter)

7 SAFETY

7.1 Wear all appropriate personal protective equipment when handling any/all potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coats, etc.

8 PROCEDURE

8.1 All work in this procedure should be performed in a BSC whenever possible using aseptic technique at all times.

8.2 Throughout the procedure, record lot numbers and expiration dates for all appropriate reagents/disposables on the bone marrow processing lot number record (*STCL-FORM-045Processing Lot Numbers-Bone Marrow Processing*).

8.3 Prior to processing the cells, confirm that the physician orders for processing have been signed and a copy is available in the recipient's laboratory file. A printed copy of the recipient's (and donor's if applicable) information and blood type from Duke Hospital Information System (HIS) should also be in the file along with laboratory generated recipient (and donor) labels containing the recipient (and donor) name, history number, date of birth, blood type, product type and facility location.

8.4 Weigh the bone marrow collection bag and record the value on the worksheet. NOTE: If the bone marrow arrives to the STCL in multiple collection bags, combine the bags prior to processing.

8.5 In a BSC, obtain a sample (~1.5 mL) to perform initial pre-processing QC to include; cell count, trypan blue viability, ABO/Rh, manual differential slide preparation, flow analysis and RFLP (if indicated).

8.6 Obtain a sample to perform pre-processing sterility (~2 mL).

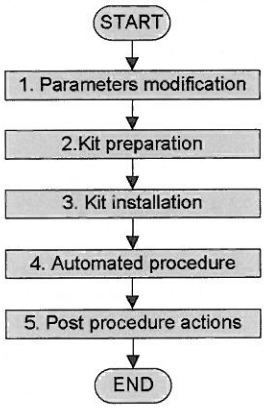
8.7 Obtain a sample (~5 mL in a syringe) for Colony Forming Unit (CFU) analysis.

8.8 **Sepax 2 General description**

8.8.1 This protocol is used to reduce the volume of a generic product (bone marrow, peripheral whole blood, cord blood) ranging from 30 to 3300 mL. The volume collected in the buffy-coat (output) bag can be chosen in two ways:

8.8.2 ***Proportional:*** The final volume corresponds to a predetermined percentage of the total volume remaining in the processing chamber after the plasma fraction has been extracted (volume of cells).

8.8.3 ***Fixed: The final volume corresponds to a predetermined amount selected between 1 and 1500 mL.***



ONLY users trained on this protocol by Biosafe or by an official trainer are authorized to use this application.

Note: This protocol can only be used with the following single-use kit:

- **CS-490.1**

8.9 **Parameters modification**

8.9.1 Switch on the Sepax 2 device.

8.9.2 Select and load the Protocol "SmartRedux" (formerly named GVR v310) in "BM applications".

8.9.3 Select "Change parameters".

8.9.4 Reference job aids *STCL-PROC-046 JA2 Sepax 2 Guidelines – BM Processing, RBC and Plasma Depletion* and *STCL-PROC-046 JA3 Sepax 2 Guidelines –BM Processing, Plasma Depletion Only* to determine input/output volume ratios (ie. 5X, 10X, 15X) and final calculations of all data parameters that will be entered in the SmartRedux protocol.

8.9.4.1 Initial volume

8.9.4.1.1. Volume processed with the Sepax 2. The number of processing cycles is limited to 15, thus the initial volume should not exceed 3300 mL (in best conditions). This volume can be lower depending on the "Reprocessed BC" and "Reprocessed Plasma" volumes (described below). An automatic check is done at the beginning of the procedure to verify the coherence of these inputs. The initial volume can be selected between 30 and 3300 mL.

Reprocessed BC	Reprocessed Plasma	Maximal Input volume
0	0	3300 mL
10	10	3020 mL
20	20	2740 mL
30	30	2460 mL
40	40	2180 mL
50	50	1900 mL

8.9.4.2 Proportional volume

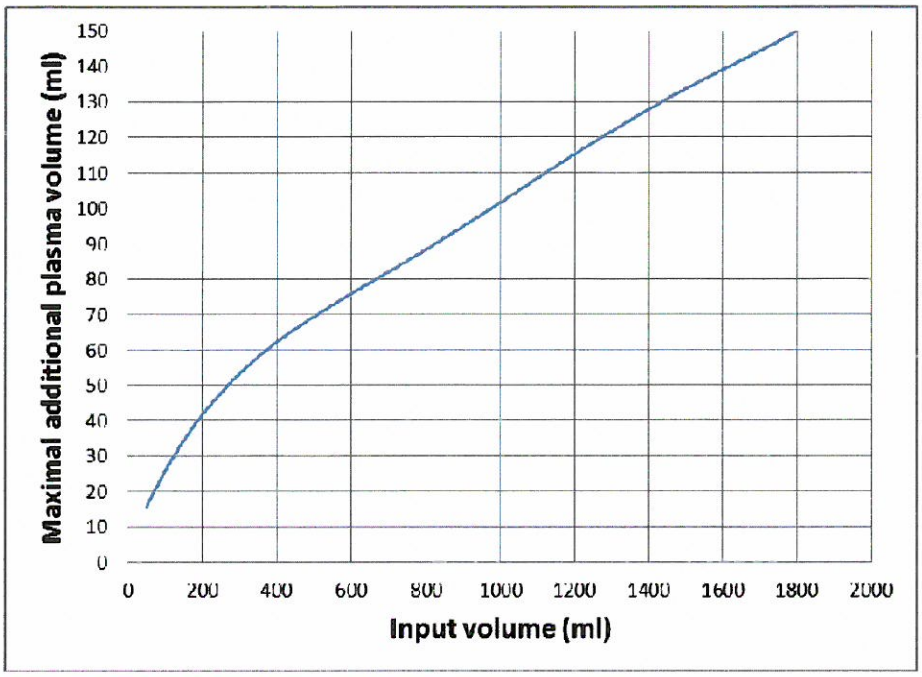
8.9.4.2.1. The final volume corresponds to a predetermined percentage of the total volume remaining in the processing chamber after the plasma fraction has been extracted. This value can be chosen between 1 and 100%, the default value being 80%. (NOTE: Proportional volume will typically not be used in the STCL).

8.9.4.3 Fixed volume

8.9.4.3.1. The final volume represents the volume that shall be collected as the final product in the output bag. This value can be chosenbetween 1 and 1500 mL, the default value being 100 mL.

8.9.4.4 Additional plasma

8.9.4.4.1. The additional plasma volume is the amount of pure plasma contained into the final volume (used to decrease the final hematocrit). **This volume is included in the volume set with the Fixed volume** (e.g. a "Fixed volume" of 30 mL and an "Additional plasma" of 5 mL correspond to 25 mL buffy-coat plus 5 mL of pure plasma). This value can be chosen between 1 and 150 mL. A warning will appear if the user selects an additional plasma volume that is too large for the actual input volume. The chart below shows approximate possible additional volumes as a function of the input volume.



8.9.4.4.2. **Note:** For units larger than 2 liters, the maximal additional plasma volume is 150mL.

8.9.4.5 Reprocessed BC

8.9.4.5.1. Indicates the volume after the buffy-coat extraction that is extracted back to the input bag and reprocessed at the next cycle (only active if the initial volume is processed in more than one cycle). This value is selectable between 0 and 50 mL, the default value being 30 mL. Confirm this value before proceeding.

8.9.4.6 Reprocessed plasma

8.9.4.6.1. Is the amount of plasma extracted back to the initial bag in order to equilibrate the hematocrit increase due to the "Reprocessed BC" parameter. To be used in conjunction with the latter parameter. This value is selectable between 0 and 50 mL, the default value being 30 mL. Use typically the same volume as the "Reprocessed BC" parameter. Confirm this value before proceeding.

8.9.4.6.2. **Note:** The last 2 parameters are useful to improve cell recovery performances in case of a high volume reduction ratio (e.g. 15 times – from 450 mL to 30 mL).

8.9.4.6.3. Corresponding to an approximate input/ output volumes ratio, some values for "Reprocessed BC" and "Reprocessed Plasma" are suggested in the table below: (See *STCL-PROC-046 JA3*)

Input/Output vol. ratio	Reprocessed BC	Reprocessed Plasma
approx. 5	0 mL	0 mL
approx. 10	20 mL	20 mL
approx. 15	30 mL	30 mL

8.9.4.7 BC detection parameter

8.9.4.7.1. This parameter influences the detection of the buffy-coat. It should be changed only under the supervision of a Biosafe engineer. **Always keep the default value of 30%.**

8.9.5 Press on  . The single-use kit can be prepared and installed on the Sepax 2 main processing unit.

8.10 **Kit preparation**

8.10.1 Use only single-use kit CS-490.1.

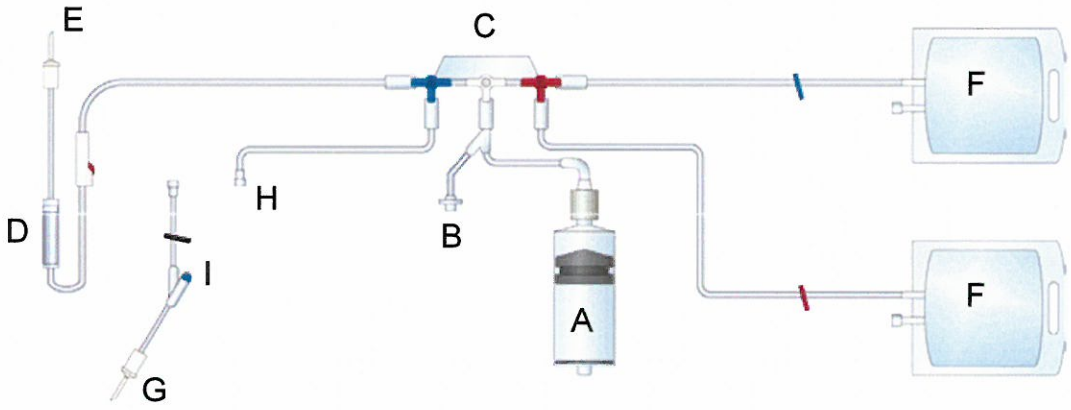


Fig. 1: CS-490.1

A: Separation chamber 220 mL
B: Line pressure monitor luer with microbial filter
C: Stopcock manifold
D: Bubble chamber
E: Spike to input bag
F: 500 mL PVC collection bags
G: Spike to output bag
H: Female luer lock to output bag
I: Injection site

The tube between the injection site (I) and the spike (G) is double-coated type and may be difficult to seal with certain types of sealers. That portion of tube is not compatible with sterile connection device (SCD).

8.10.2 Kit verification

8.10.2.1 Before opening the kit blister pack under sterile conditions, ensure that the sterility indicator on the Tyvek® cover is green, indicating that the kit is sterile.

8.10.2.2 If the sterility indicator is purple, the kit should not be used and the operator should inform Biosafe of the kit lot number in question.

8.10.3 Kit opening

8.10.3.1 Open the blister of the kit under sterile conditions and close only the roller clamp of the input line.

8.10.3.2 Under sterile conditions spread the kit out to identify the tubing lines and components. Always do a visual check of the kit before starting - If ruptures or kinks are detected or components are missing (clamps, caps, etc.), the kit should not be used and the operator should inform Biosafe of the kit lot number in question.

8.10.4 Output bag connection

8.10.4.1 The output bag can be connected by luer-lock ("H" in Fig. 1), sterile connection device (SCD) or spike ("G" in Fig. 1). If connected with an SCD, the sterile connection should be made between the luer-lock and the blue stopcock ("C" and "H" in Fig. 1). All types of connection should be done under sterile conditions.

8.10.4.2 NOTE: with an SCD or luer lock connection, you will lose the sampling port on the output extension line. Be sure that a sampling port is available on your output bag.

8.10.5 Input bag connection

8.10.5.1 Using a sterile connection device (SCD)

8.10.5.1.1. Connect the input bag to the CS-490.1 kit with an SCD under sterile conditions, if possible.

8.10.5.1.2. The sterile connection should be made between the spike and the bubble chamber of the input bag line ("D" and "E" in Fig. 1).

8.10.5.2 Using the spike connection

8.10.5.2.1. The CS-490.1 kit can be connected to the input bag using the pre-installed spike connection of the input bag line ("E" in Fig. 1) **under sterile conditions.**

8.10.5.2.2. The kit is now closed and can be taken out of sterile conditions. The kit is ready to be installed on the Sepax 2 device.

8.11 Kit installation

8.11.1 Check that the following screen is displayed:

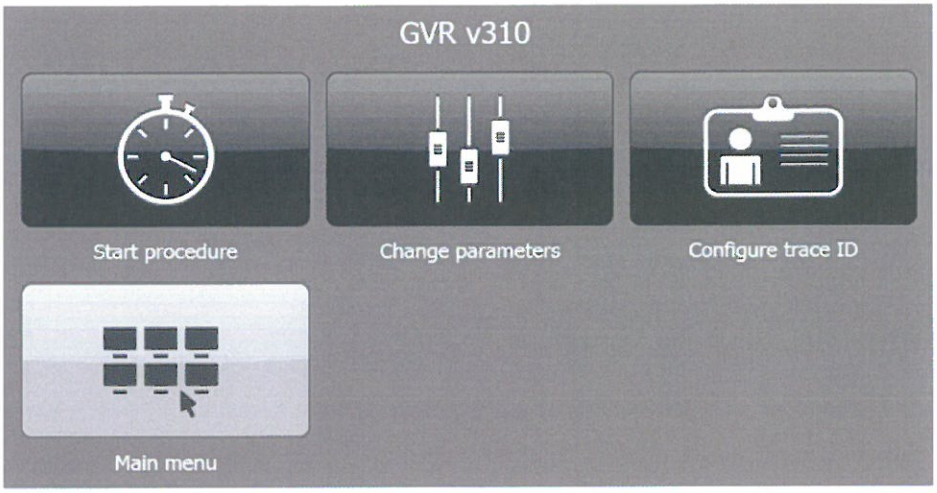


Fig. 2

8.11.2 Open the two separation chamber pit covers.

8.11.3 Install the separation chamber in the pit by pushing it down firmly and verify that it is well inserted.

8.11.4 Insert the separation chamber tubing line into the optical sensor and ensure that the tubing is correctly inserted.

8.11.5 Verify that the stopcocks are aligned in the T-position: ⊤ ⊤ ⊤

8.11.6 Open the stopcock holder by pushing down the two levers; install the stopcocks on the SePAX rotary drive pins by pushing them down firmly and close the holder by pushing down the levers.

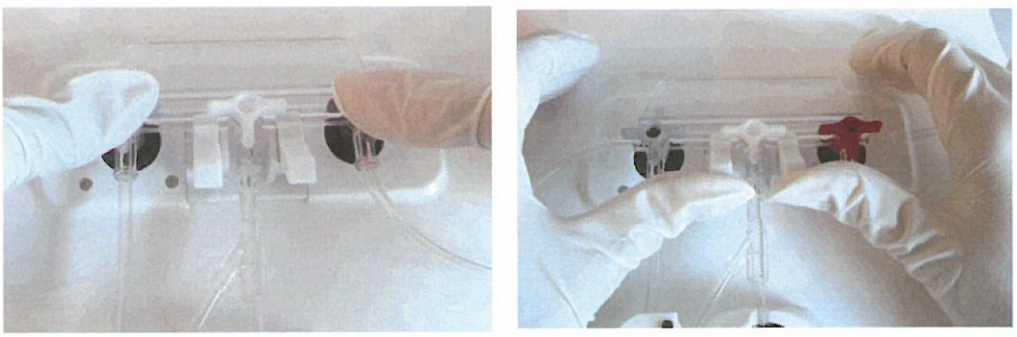
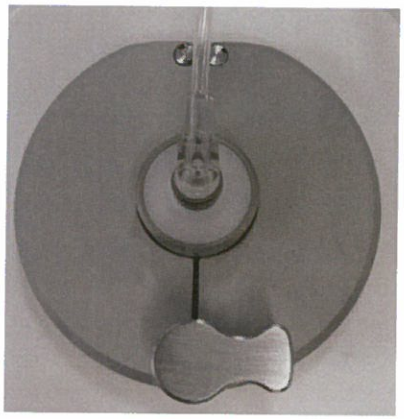


Fig. 3

8.11.7 Close the centrifuge cover and tighten the centrifuge cover lock screwing it clockwise



Open

Closed

Fig. 4

8.11.8 Connect the pressure sensor line (luer connector/ filter) to the pressure sensor port on the top of the Sepax. Tighten the luer lock firmly.

8.11.9 Hang the plasma and RBC bags on the hook provided on the Sepax.

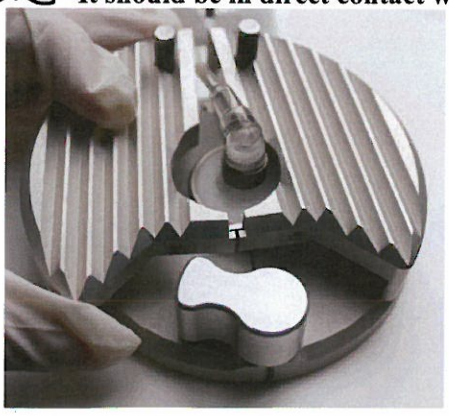
8.11.10 Hang the input bag on the bag holder.

8.11.11 Put the bubble chamber in its support.

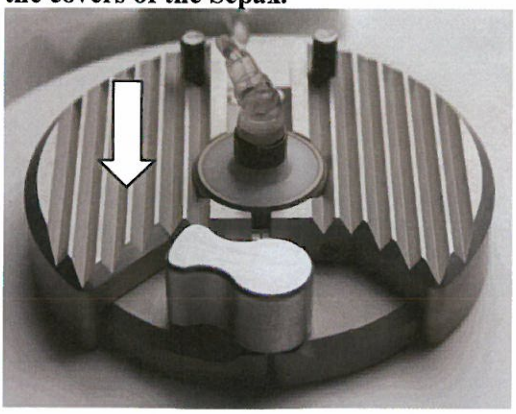
8.11.12 Mount the heatsink accessory only with initial volumes greater than 1 liter, see Fig. 5. Skip this step for initial volumes smaller than 1 liter.

The heatsink accessory is needed for <u>input volumes greater than 1 liter only</u>, when there is a longer processing time.

The heatsink accessory improves the heat dissipation by increasing surface area. It should be in direct contact with the covers of the Sepax.



Place heatsink accessory from above, with the tube between its opening



Push down the accessory firmly to establish direct contact with Sepax cover

Fig. 5

8.11.13 Press "Start procedure" to validate and to start the kit test.

8.11.14 The operator is then asked to choose the wanted volume calculation (fixed or proportional). Only the corresponding parameter (explained in section 1) will be taken into account.

8.11.15 An automatic check is done at this point to verify the waste bags capacity.

8.11.16 If the traceability feature is available, enter the traceability IDs (either with the barcode reader or using the keyboard).

8.11.17 After having entered all traceability IDs, select "Input done, continue" to start the automatic kit test.

Do not close the separation chamber pit covers until the kit has been positioned in the separation chamber pit and the tubing has been placed in the optical sensor.

To avoid any risk of leakage, the tubing connector on top of the separation chamber should not be rotated once the pit covers have been closed.

8.12 **Automated procedure**

Avoid touching the Sepax and the kit during the automated procedure. Moving bags, tubes, stopcocks and covers may cause the process to be interrupted.

8.12.1 An automatic kit test is performed. At the end of the test, the procedure pauses for 10 seconds to stabilize the pressure in the separation chamber. After the automated kit test, the operator is asked to open the roller clamp.

8.12.2 After each sedimentation cycle an automatic check is done to verify the waste bags capacity.

8.12.3 If one or both waste bags will be overfilled in the next cycle, a warning message is displayed to specify which bag needs to be changed and the minimum capacity of the new bag to be connected by sterile connection device.

8.12.4 Once done, the user should confirm which bag has been replaced by pressing one of the three icons.

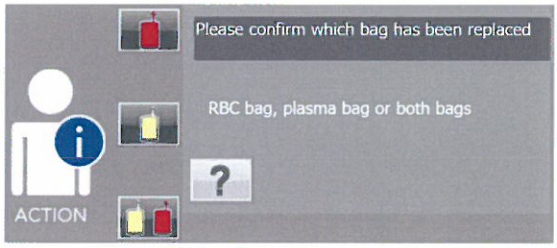
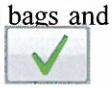


Fig. 6

8.12.5 After each sedimentation cycle, the procedure pauses and the machine begins to beep. The operator is asked to mix the input bag. Once it is done press  to resume the procedure.

8.13 Post procedure actions

8.13.1 At the end of the automated procedure, with the kit still installed on the Sepax, the message "Remove bags and air filter" appears on the display. Follow the instructions and press 

8.13.2 The message "Strip BC line" appears on the display. Strip the BC line as shown in Fig. 7 and press  . Always hold the line with one hand while stripping.

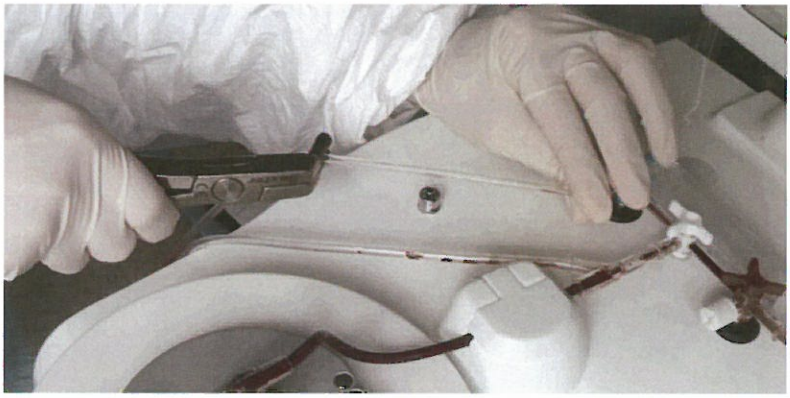


Fig. 7

8.13.3 The message "Strip RBC line" appears on the display. Always hold the line with one hand while stripping.

8.13.4 The message "Strip plasma line" appears on the display. Always hold the line with one hand while stripping.

8.13.5 The message "Close all clamps, Dismount kit" appears on the display. Close all clamps and press  .

8.13.6 Close the clamp of the output line just above the Y connection if applicable.

8.13.7 The protocol is unloaded and the Sepax is ready to start a new procedure

8.13.8 Heat seal the output line just above the clamp and detach from processing kit.

8.13.9 Heat seal the RBC and plasma lines and detach from processing kit.

8.13.10 Discard the processing kit.

8.14 **Sampling of final product**

8.14.1 In a BSC, obtain a sample (~0.7 mL) to perform post-processing QC to include: cell count, trypan blue viability, flow analysis, differential, and Colony Forming Unit.

8.14.2 In a BSC, aseptically using a syringe and needle withdraw ~5 mL of product from each of the RBC and plasma waste bags to use for sterility inoculation.

8.14.3 Save plasma and rbc waste bags until all QC testing is complete in case additional processing is deemed necessary based on recovery, etc.

9 RELATED DOCUMENTS/FORM

9.1 STCL-PROC-046 JA1 SePax 2 Product Data Sheet CS-490-1

9.2 STCL-PROC-046 JA2 Sepax 2 Guidelines – BM Processing, RBC and Plasma Depletion

9.3 STCL-PROC-0-46 JA3 Sepax 2 Guidelines – BM Processing, Plasma Depletion Only

10 REFERENCES

10.1 SePAX 2 RM Operator's Manual

11 Revision History

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1	B. Waters-Pick	New procedure

Signature Manifest

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STCL-PROC-046 Processing Bone Marrow Using the Sepax 2 Instrument

Author Approval

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Medical Director Approval

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Linda Sledge (SLEDG006)		28 Aug 2013, 09:19:23 AM	Approved

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