



STEM CELL LABORATORY (STCL)



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UCB Processing Using the Automated Sepax 2 S-100 Cell Processing System with UCB-HES Protocol

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STCL-PROC-042

UCB PROCESSING USING THE AUTOMATED SEPAX 2 S-100 CELL PROCESSING SYSTEM WITH UCB-HES PROTOCOL

1 PURPOSE

- 1.1 The Sepax 2 S-100 system is a cell processing system intended for the separation of nucleated cells from umbilical cord blood collected in anticoagulant.
- 1.2 For parts and specific Sepax 2 S-100 Main Unit troubleshooting, manufacturer contact, warranty and parts descriptions please refer to the Biosafe® Sepax 2 S-100 Cell Processing System Operator's Manual.
- 1.3 Umbilical cord blood (UCB) units are processed to reduce volume via red blood cell and plasma reduction prior to cryopreservation. The Sepax 2 S-100 "HES" protocol is designed for routine processing of UCB to isolate the nucleated cell component (buffy coat) fraction enriched in hematopoietic stem cells using Hydroxyethyl starch (HES), a sedimentation agent.
- 1.4 The Sepax 2 S-100 Main Unit in conjunction with a CS-530 single use kit is a cell processing system that 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. This allows the automated processing of UCBs in a functionally closed and sterile environment.

2 INTRODUCTION

- 2.1 UCB is a useful source of hematopoietic stem cells for hematopoietic reconstitution in both the unrelated and related donor setting. Samples from the buffy coat are used to determine total nucleated cell count, viability and stem and progenitor cell enumeration. Samples of red blood cells and plasma are taken for sterility and samples are saved for human leukocyte antigen testing, if indicated, and future genetic or infectious disease testing.
- 2.2 UCB collections from a single donor are volume reduced to concentrate stem and progenitor cells in a small volume collection bag for cryopreservation and long-term storage under liquid nitrogen. This protocol allows processing of initial UCB volumes above 35 mL and below 320 mL (anticoagulant included, after HES addition) with optimal performance for units between 80 mL and 180 mL. The volume reduction of UCB is performed in 25 to 35 minutes and results in a predetermined volume between 20 and 50 mL.
 - 2.2.1 Specimen Requirements
 - 2.2.1.1 Umbilical cord blood units collected into Citrate Phosphate Dextrose anticoagulant via the Carolinas Cord Blood Bank or collected remotely and shipped to the Stem Cell Laboratory.

3 SCOPE AND RESPONSIBILITIES

- 3.1 The Stem Cell Laboratory (STCL) Medical Director, STCL Manager and designated STCL staff are responsible for ensuring the requirements of this procedure are successfully met.
- 3.2 Technicians shall only handle one unit per tech per hood at a time.
- 3.3 Processing of the cord blood (from collection to initiation of freezing) must be completed within 72 hours of collection.
- 3.4 Lot numbers must be recorded for all supplies, reagents and designated equipment used for processing on *STCL-FORM-050 Processing Lot Numbers – CBU Processing*. Quality control checks, cleaning and serial numbers on all equipment used must be documented prior to use for processing.
- 3.5 Equipment shall be cleaned between uses on UCB units with an appropriate cleaning solution. This includes the surface of the biological safety cabinet, manual tubing strippers, scissors, pipettes, hemostats and sealers.

4 DEFINITIONS/ACRONYMS

4.1	HES	Hydroxyethyl Starch
4.2	HLA	Human Leukocyte Antigen
4.3	UCB	umbilical cord blood
4.4	CBU	cord blood unit
4.5	CPD	Citrate Phosphate Dextrose
4.6	BSC	Biological Safety Cabinet
4.7	STCL	Stem Cell Laboratory
4.8	WBC	white blood cell
4.9	G	gauge
4.10	RBC	red blood cells
4.11	DMSO	dimethyl sulfoxide
4.12	BC	buffy coat (leuko-rich product)
4.13	ISBT	International Society of Blood Transfusion
4.14	CCBB	Carolinas Cord Blood Bank
4.15	mL	milliliter
4.16	g	gram
4.17	C	Celsius
4.18	cm	Centimeter
4.19	kg	Kilogram
4.20	µL	Microliter

5 MATERIALS

5.1 Supplies:

- 5.1.1 CS-530 single-use kit (see Figure 1)
- 5.1.2 UCB Collection bag
- 5.1.3 ChloraPrep® SEPP®
- 5.1.4 Alcohol wipes or equivalent
- 5.1.5 ISBT bar code labels
- 5.1.6 Terumo sterile welding wafers
- 5.1.7 16 G luer lock needles
- 5.1.8 19 G luer lock needles
- 5.1.9 Syringes; 3 mL, 10 mL, 20 mL, 30 mL, 60 mL
- 5.1.10 5 mL sterile snap cap tubes
- 5.1.11 5 mL phenotype tubes
- 5.1.12 Thermogenesis Demand 128 Cryo label
- 5.1.13 Cryovials
- 5.1.14 Nalgene nunc vials
- 5.1.15 Microscope slides
- 5.1.16 Microscope slide holder
- 5.1.17 BacT/Alert Aerobic Culture Bottle
- 5.1.18 BacT/Alert Anaerobic Culture Bottle
- 5.1.19 Tube Racks
- 5.1.20 Pipette tips
- 5.1.21 Transfer pipettes
- 5.1.22 Sharpie Marker
- 5.1.23 Pencil
- 5.1.24 Freezer boxes with 10 x 10 grid inserts
- 5.1.25 FTA cards
- 5.1.26 FTA bags-small zip lock bag
- 5.1.27 FTA card drying rack

5.2 Reagents:

- 5.2.1 Hespan (6% Hetastarch)
- 5.2.2 Trypan Blue Stain
- 5.2.3 Anti-A, Anti-B and Anti-D

- 5.2.4 Sysmex Cell Pack Reagent
- 5.2.5 AO/PI Stain
- 5.2.6 Cellometer disposable counting chamber

6 EQUIPMENT

- 6.1 Sepax 2 S-100 main processing unit
- 6.2 Sepax 2 Printer
- 6.3 Barcode scanner
- 6.4 Label replicator with ISBT compatible scanner
- 6.5 Biological Safety Cabinet
- 6.6 Platform Rocker
- 6.7 Sterile Tubing Welder
- 6.8 Sterile tubing sealer, adapted for both PVC and EVA tubing
- 6.9 Manual Tubing Stripper
- 6.10 Pipettes
- 6.11 Scissors
- 6.12 Serofuge Centrifuge
- 6.13 Automated Hematology Analyzer
- 6.14 Mini-Vortex
- 6.15 Ultra Low Freezer (~ -80° C)
- 6.16 Microscope
- 6.17 Cellometer Auto 2000

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, goggles, etc.

8 PROCEDURE

- 8.1 Product Endpoints
 - 8.1.1 Following completion of the automatic processing of the UCB, the whole product should have clearly separated properly into each bag component. Plasma should be in the plasma bag and not exhibit signs of WBC and RBC presence. Red blood cells should be pushed/drained back into the collection bag and BC cells should measure ~21.5 mL.
 - 8.1.2 Autologous UCB should contain >100 x 10⁶ cells and should be processed within 72 hours from collection.

- 8.1.3 Directed donor allogeneic UCB should contain $>10 \times 10^6$ cells/kg of intended recipient body weight and should be processed within 72 hours from collection (See appropriate age-specific growth charts published by the Centers for Disease Control (CDC)).
- 8.1.4 If the CBU does not meet the above criteria, but is deemed necessary for storage by the medical director, a planned deviation may be requested.
- 8.2 Place CBU on a platform rocker. Turn rocker on and let unit rock gently for at least 5 minutes to allow for adequate mixing.
- 8.3 Locate the sheet of adhesive ISBT barcode labels included in the working folder. Verify that all barcodes match with the barcode label on the CBU.
- 8.4 Label *STCL-COLL-007 FRM2 Auto/Directed CBU Receipt Form* using an ISBT barcode, handwrite the mother's name on the form and complete the form.
- 8.5 Label a sterile snap cap tube using an ISBT barcode and with a Sharpie marker handwrite "PRE" on the tube.
- 8.6 Label *STCL-PROC-042 FRM2 CBU Processing Worksheet* using an ISBT barcode and handwrite the mother's name on the form.
- 8.7 Obtain the weight/volume of the CBU using one of the following methods:
 - 8.7.1 Weigh the collection bag and subtract 65.0 g.
 - 8.7.2 Working in a BSC, disinfect the septum of the collection bag injection port and draw up the collected UCB using syringes and needles, measure volume and return blood to the collection bag.
- 8.8 Record the sample volume/weight of the bag on the *CBU Processing Worksheet*.
- 8.9 Label two FTA cards with ISBT barcode labels. Never handle the FTA cards with bare hands.
- 8.10 Obtain 1.7 mL of UCB from the syringe port of the collection bag using aseptic technique as described below. These steps must be performed in a BSC.
 - 8.10.1 After disinfecting the septum of the injection port, use a 3 mL syringe and a needle to mix the blood in the tubing between the injection site and the bag.
 - 8.10.2 After mixing 2-3 times into the 3 mL syringe, remove 1.7 mL of UCB from the collection bag and complete the following steps for inoculating the FTA cards and preparing the pre-count tube.
- 8.11 With needle bevel pointed downwards, hold the needle at a 45 degree angle and carefully drop five small drops of blood onto the card within the printed circle.

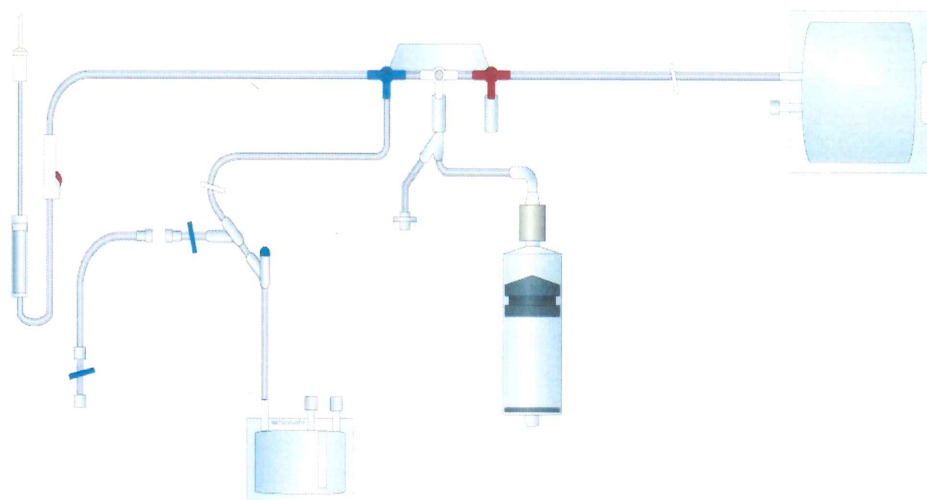


- 8.11.1 Avoid puddling of the liquid sample as it will overload the chemicals on the card.
- 8.11.2 Do not rub or smear the blood onto the cards.
- 8.11.3 Make sure that you do not touch the surface of the card with the needle tip.
- 8.12 Dispense ~0.5 mL of UCB in labeled sterile snap cap tube.
- 8.13 Split the remaining ~1.0 mL of UCB between aerobic and anaerobic culture bottles.
 - 8.13.1 Label each bottle with an ISBT barcode and handwrite the mother's name, DD UCB "PRE", the date of inoculation and the inoculator's initials.
 - 8.13.2 Remove the cap from the anaerobic bottle and disinfect the tops of both the anaerobic and aerobic culture bottles.
 - 8.13.3 Inject ~0.5 mL UCB into each bottle.
- 8.14 Place FTA cards into the polyurethane drying rack to allow to air dry.
 - 8.14.1 Blood on FTA cards must be completely dry (approximately 2 hours) before bagging each card separately.*
 - 8.14.2 Place the bagged cards in the manila envelope in the corresponding working packet.*

NOTE: (*) Indicates that these steps do not have to be completed at this point in the process.
- 8.15 Using *STCL-EQUIP-002 Sysmex XS-1000i Hematology Analyzer for the Stem Cell Laboratory*, run automated complete blood count using sample in pre-count tube.
- 8.16 Once count is complete the results will print automatically. Verify the barcode on the printout to the barcode on your labeled tube.
- 8.17 Circle the Platelet (PLT) Count and verify that the count is $\geq 100,000$. If the count is $< 100,000$, an estimated platelet count must be ordered. A manual differential must be ordered on all UCBS.
- 8.18 Verify ISBT barcode labels, transcribe the white blood cell count (WBC) onto *STCL-PROC-042 FRM2 CBU Processing Worksheet*, and calculate total volume and total nucleated cell count of the UCB.
 - 8.18.1 Autologous CBUs should contain $> 100 \times 10^6$ cells.
 - 8.18.2 Allogeneic CBUs should contain $> 10 \times 10^6$ cells/kg of intended recipient body weight (see *STCL-PROC-044 JAI Growth Charts for Girls and Boys* for appropriate age-specific growth charts published by the Centers for Disease Control (CDC)).
 - 8.18.3 If the CBU does not meet the above criteria, notify the medical director of results. The medical director will determine if CBU should be processed and stored. A planned deviation will be required.

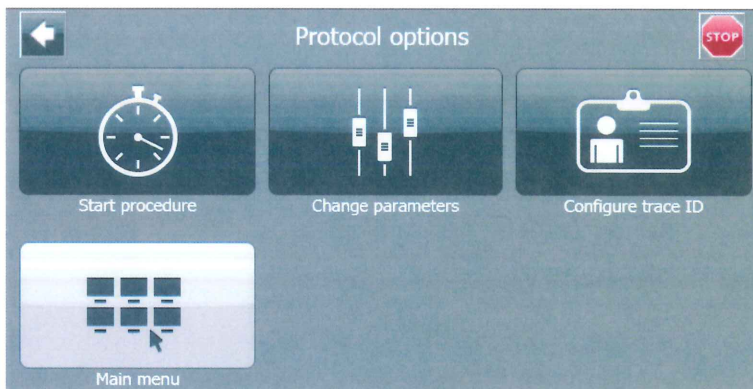
- 8.19 Hespan (6% Hetastarch) is added to help sediment the red cells and concentrate the unit. Calculate the volume of Hespan to be added to the CBU using the following formula:
$$\text{Total Volume (Blood + CPD) mL} / 5 = (\text{Total Hespan}) \text{ mL}$$
- 8.20 Record the calculated volume onto *STCL-PROC-042 FRM2 CBU Processing Worksheet*.
- 8.21 In the BSC, attach a needle to the appropriate syringe size to accommodate calculated Hespan volume.
- 8.22 Disinfect the septum of the injection port of the Hespan bag, insert the needle and withdraw the calculated amount of Hespan.
- 8.23 Disinfect the injection port of the UCB collection bag and slowly inject the Hespan into the bag at a rate of approximately 2 mL/second. Manually agitate the CBU with one hand, while injecting the Hespan with the other hand, to rock the bag back and forth during infusion to gently mix the contents.
- 8.24 Note time of Hespan addition on the collection bag or on *STCL-PROC-042 FRM2 CBU Processing Worksheet*.
NOTE: After Hespan addition, the CBU/Hespan mixture must incubate in a vertical state for a minimum of 20 minutes but no more than 120 minutes prior to RBC and plasma reduction on the Sepax 2 S-100. Record the hanging and start times as appropriate.
- 8.25 Switch on the Sepax 2 S-100 by using the main switch located at the lower right of the rear panel.
- 8.26 On the display choose “UCB applications”.
- 8.27 On the display choose current “UCB-HES vXXX”.
- 8.28 Kit verification: Before opening the kit blister pack, ensure that the sterility indicator on the Tyvek® cover is green, indicating that the kit is sterile and that the kit is within expiration date. If the sterility indicator is dark brown, the kit should not be used and you should notify a supervisor immediately. Supervisor will collect any necessary information and report findings to manufacturer. Kit will not be used for processing.
- 8.29 Kit opening: Open the blister of the kit and Prepare CS-530 kit by closing the **red roller clamp** on the input UCB line and the **blue clamp** on the DMSO injection line and tubing. Leave the other two white clamps open. Keep the unattached DMSO tubing extension for future use in cryopreservation steps.
- 8.30 Kit inspection before docking: Spread the kit out to identify the tubing lines and components. Always do visual checks 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 a supervisor immediately. The supervisor should notify Biosafe of the lot number of any defective kit.



Kit Diagram CS-530






- 8.31 Connect the UCB input bag (with HES added) to the CS-530 kit using a sterile tubing welder.
 - 8.31.1 The sterile connection should be made between the pre-installed spike connection and the bubble chamber of the UCB input bag line.
 - 8.31.2 If a sterile connection device is not available, the CS-530 kit can be connected to the UCB input bag using the pre-installed spike connection of the UCB input bag line inside the biological safety cabinet.
- 8.32 Using Zebra cryo label printer, scan the ISBT barcode label on the CBU collection bag to print a cryo label set.
 - 8.32.1 Affix the large cryo label onto the 80% fraction of CBU product cryobag and the small label onto the 20% fraction.
 - 8.32.2 Affix the other small 6 digit label onto the clip of a Thermogenesis cryo canister.
- 8.33 Kit installation on the Sepax 2 S-100:
 - 8.33.1 Hang the cord blood unit collection bag on the bag holder above the Sepax 2 S-100 unit.



NOTE: After Hespan addition, the CBU/Hespan mixture must incubate in a vertical state for a minimum of 20 minutes but no more than 120 minutes prior to RBC and plasma reduction on the Sepax. Record the hanging and start times as appropriate.
 - 8.33.2 After 20 minutes, check that the following screen is displayed:



- 8.33.3 Open the two-separation chamber pit covers.
- 8.33.4 Install the separation chamber in the pit by pushing it down firmly and verify that it is well inserted.
- 8.33.5 Insert the separation chamber tubing into the optical sensor and ensure that the tubing is correctly inserted.
- 8.33.6 Verify that the stopcocks are aligned in the T-position.

- 8.33.7 Open the stopcock holder by pushing down the two levers, place the stopcocks firmly on the pistons and close the holder by pushing the levers up.
- 8.33.8 Close chamber pit covers and tighten the centrifuge cover lock screwing it clockwise.
- 8.33.9 Connect the pressure sensor line (luer connection/filter) to the pressure sensor port on top of the Sepax. Tighten luer lock firmly.
- 8.33.10 Hang the plasma bag on the hook provided on the right side of the Sepax unit.
- 8.33.11 Hang the cryobag on the hook provided on the left side of the Sepax unit.
- 8.33.12 Insert the bubble chamber (clot filter) in its support, ensure tubing doesn't kink.
- 8.34 Press "Start procedure".
- 8.35 Next the display will prompt you to enter the following (order may vary with each protocol version); click on the  following each entry.
 - 8.35.1 Hematocrit – Enter the hematocrit of the umbilical cord blood unit
 - 8.35.2 Operator - Scan Duke Unique ID on badge
 - 8.35.3 Initial bag - Scan ISBT barcode label on collection (input) bag
 - 8.35.4 Final bag - Scan Demand 128 Cryolabel on cryobag

- 8.35.5 Kit ID - Scan lot number barcode on Sepax kit cover
- 8.35.6 Expiration date - Verify kit expiration, enter correct date, if necessary
- 8.35.7 Center - Should be listed as "DUKE"
- 8.35.8 Labo - Should be listed as "STEM CELL LAB"
- 8.35.9 Plasma bag - Scan ISBT barcode
- 8.35.10 Next - Press to return to previous screen
- 8.35.11 Press "Input done, continue"
- 8.36 After pressing Input done, continue the Sepax unit will start the automatic kit test.
 - 8.36.1 If the kit test is unsuccessful, troubleshoot, take corrective action and restart.
 - 8.36.2 If successful, continue.
- 8.37 Following kit test, display will read "Open input bag clamp".
 - 8.37.1 Open red roller clamp below bubble chamber (clot filter) on kit and pinch sterile dock weld to open tubing at connection and press  .
- 8.38 Sepax unit will automatically start processing the CBU. Avoid touching the Sepax or moving kit parts during automated procedure.

NOTE: If any errors or problems occur, the Sepax will display the error codes on the display. Refer to the Biosafe® Sepax 2 Operator's Manual for troubleshooting and corrective action steps.
- 8.39 At the end of the automated procedure, the Sepax will automatically generate and print the Sepax report. Check report to see that "PROCEDURE COMPLETE" displays at the end of the report. Verify all information on the Sepax protocol summary. If any other errors display, do not remove the unit and contact a supervisor for instructions regarding how to proceed.
- 8.40 With the kit still installed on the Sepax, the display will show "Remove bags and air filter". Remove all three bags (collection, plasma and CBU product cryobag (BC)) from their hooks and unscrew air filter. Allow them to hang down from machine then press  .
- 8.41 The message "Strip RBC Line" appears on the display. The RBC line consists of the line from the left stop cock (blue) to the input bag. Always hold the line with one hand while stripping. Press  when complete.
 - 8.41.1 DO NOT strip the line between the blue stopcock and the bubble chamber (clot filter) closer than 3 cm from the latter.
 - 8.41.2 Be careful when stripping the line between the bubble chamber (clot filter) and the collection bag as this line can detach from the bubble chamber if not properly held during stripping.

- 8.42 The message “Strip BC Line” appears on the display. Strip the BC line all the way down to the point where the line connects to the bag.
- 8.43 Before validating, manually remove the air from the BC bag by squeezing it. Do not push cells up past the point of the syringe port if possible. If cells go past the port, use tubing strippers to pull cells back below the port. While holding the cryobag, to not allow air to reenter, in one hand, use the other to press .
- 8.44 The BC bag can now be released as the stop cock on the Sepax has been closed preventing the return of air.
- 8.45 The message “Close all clamps, Dismount kit” appears on the display. Close all clamps then press .
- 8.46 Remove Sepax kit from Sepax unit.
- 8.47 Using a heat sealer seal the tubing to each bag three times. Cut center seal removing bag from the rest of the kit. After all three bags (Red Blood Cells, Plasma, and BC) have been removed throw the remainder of the kit in the biohazard trash. Retain the non-attached DMSO line.
- 8.48 Sepax protocol summary will be retained in the working packet.
- 8.49 Using ISBT barcodes label the following forms and handwrite the mother’s name on each form:
- 8.49.1 FLOW-FORM-012 Graft Characterization
 - 8.49.2 STCL-SOP-052 (FRM1) Progenitor Cell Assay Form
 - 8.49.3 FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet
 - 8.49.4 STCL-PROC-035 (FRM1) Manual Differential (Slide Method) – CBUs for CCBB Program
 - 8.49.5 STCL-PROC- 045 FRM1 CBU Cryopreservation
 - 8.49.6 STCL-FORM-050 Processing Lot Numbers – CBU Processing
- 8.50 Using ISBT barcodes label the following:
- 8.50.1 1 sterile snap cap tube (post processing sample)
 - 8.50.2 5 phenotype tubes (1 post count tube, 4 tubes for ABO/Rh typing)
 - 8.50.3 14 nunc vials (6 for cord blood RBCs and 8 for cord blood plasma)
 - 8.50.4 2 cryovials (DMSO nuncs)
 - 8.50.5 1 aerobic culture bottle
 - 8.50.6 1 anaerobic culture bottle
 - 8.50.7 2 ISBT barcodes for BacT-Alert Log form and -80° C Freezer Log form
 - 8.50.8 10-14 ISBT barcode sheet for use by HPCA and flow

8.50.9 4 ISBT barcode sheet for microscope slides

- 8.51 Remove the pink striped maternal sample labels from the envelope in the packet and label seven nunc vials with the pink striped maternal sample labels. Place "S" on one of the vials with a Sharpie to indicate that it is the serum nunc.
- 8.52 Place any extra ISBT barcode labels in the manila envelope in the working packet.
- 8.53 Post CBU processing, and working in a BSC, mix the cells of the buffy coat bag well.
- 8.54 Disinfect the syringe port of the buffy coat bag.
- 8.55 Using a 3 mL syringe and a needle, remove 0.5 mL and place into a second sterile labeled snap cap tube. Confirm the labels by observation.
- 8.56 For post processing cell count sample, use a pipette to extract 160 µL of cell pack reagent and 40 µL (1:5 dilution) of buffy coat sample from the sterile snap cap tube and dispense into the labeled phenotype tube and mix the dilution well.
- 8.57 Using *STCL-EQUIP-002 Sysmex XS-1000i Hematology Analyzer for the Stem Cell Laboratory*, run an automated complete blood count. Results will print automatically. Verify the barcode of the printout to the barcode on the labeled tube. Record on Sysmex printout the dilution performed and show calculations.
- 8.58 Record and calculate the total nucleated cell count and percent recovery using the *STCL-PROC-042 FRM2 CBU Processing Worksheet*.
NOTE: If the total nucleated cell count recovery is <60%, contact a supervisor to see if the CBU requires reprocessing.
- 8.59 Perform a viability count using *STCL-SOP-022 Viability Counts via Trypan Blue Dye Exclusion* or *STCL-PROC-057 Performing automated Viability Counts using the Cellometer Auto 2000*.
- 8.60 Calculate and complete applicable sections of forms *STCL-SOP-052 FRM1 Progenitor Cell Assay Form* and *FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet*.
- 8.61 Label four microscope slides with ISBT barcode labels. Using a pencil, label two of the slides with "PRE" (collection bag, pre-processing sample) and two with "POST" (buffy coat, post processing sample).
- 8.62 Using a BSC and a pipette, remove 8 µL of sample from the pre-count tube taken from the original collection bag. Split the 8 µL between the two slides labeled "PRE" by placing a small drop on each slide. Then use a clean slide to smear blood across slide.
- 8.63 Repeat step 8.62 with the buffy coat, post processing sample and "POST" slides.
- 8.64 Allow slides to air dry and then place into a slide holder.
- 8.65 Stem Cell Laboratory analysis of post processing sample:

- 8.65.1 Take buffy coat post processing sample tube, flow and progenitor forms and 10-12 ISBT barcode labels to HPCA for plating and flow analysis.

- 8.65.2 Take slides and *STCL-PROC-035 Manual Cell Differential (Slide Method) – CBUs for CBBB Program* to HPCA for staining. Stain one “PRE” and one “POST” slide with the Wright-Giemsa stain using the automated stainer. Leave the other slide unstained.
- 8.65.3 Once slides are stained and read and testing is complete, the slides and all associated paperwork will be returned for filing with the CBU packet.
- 8.66 ABO/Rh typing of the CBU
 - 8.66.1 Follow procedure *STCL-SOP-049 ABO Rh Typing* and record results on blood bank worksheet and on *STCL-PROC-042 FRM2 CBU Processing Worksheet*.
- 8.67 RBC distribution:
 - 8.67.1 Using a BSC and aseptic technique, attach a needle to a syringe.
 - 8.67.2 Disinfect the syringe port on the residual RBC containing bag and insert needle. Extract ~18 mL of RBCs.
 - 8.67.3 Remove cap from anaerobic culture bottle, disinfect the tops of both the anaerobic and aerobic culture bottle. Inject ~2.5 mL of RBCs into each bottle.
 - 8.67.4 With remaining RBC volume, fill six nunc vials with 1.5 mL each of RBCs. Confirm ISBT barcodes by observation.
- 8.68 Plasma Distribution
 - 8.68.1 Using a BSC and aseptic technique, attach a needle to a 30 mL syringe.
 - 8.68.2 Disinfect the syringe port on the plasma bag and insert the needle. Extract 30 mL of plasma.
 - 8.68.3 If applicable, disinfect the tops of both the anaerobic and aerobic culture bottles. Inject ~7.5 mL of plasma into each bottle.
 - 8.68.4 With remaining plasma, fill 8 nunc vials with 1.5 mL each. Confirm ISBT barcodes by observation.
- 8.69 Sterility Testing
 - 8.69.1 Enter CBU information and load sterility culture bottles per *STCL-EQUIP-011 Sterility Culture Using the BacT/Alert Microbiology System*.
 - 8.69.2 Place CBU ISBT barcode label and bottle ID labels onto *STCL-EQUIP-011 FRM1 BacT Alert Log Sheet*.
 - 8.69.3 Bottles will be incubated for a minimum of 7 days. Any positive bottles are flagged by the analyzer. Positive and negative bottles will be unloaded and results printed for each unit. Results will be filed in the working packet.

8.69.4 Any positive bottles will be forwarded to clinical microbiology for organism identification and sensitivity testing. Final microbiology results will be filed in the working packet along with all other test results.

8.70 Storage and Allocation of RBC Pellet and Plasma Nunc Vials

8.70.1 All nunc vials will be stored in cardboard freezer boxes and assigned a location on the -80° C Freezer Log sheets. These specific freezer locations will be recorded on *STCL-PROC-042 FRM2 CBU Processing Worksheet* form for ease in locating, should they be needed in the future.

8.70.2 Twenty slots are assigned for each CBU: six slots for RBC nunc vials, seven slots for plasma nunc vials and seven slots for maternal nunc vials (see *STCL-COLL-007 JA10 Auto/Directed Maternal Blood Sample Processing*). Place the RBC and plasma nuncs in the assigned location for storage. Place any empty maternal sample nuncs in a sample tray and hold until maternal samples are received and processed. Maternal nuncs will be placed into assigned location following processing.

8.70.3 The remaining plasma nunc vial will be placed in the freezer box labeled "CMV" located in the ultralow freezer in the CCBB lab.

8.70.4 Follow *STCL-PROC-045 Cryopreservation and Storage of CBU*.

9 RELATED DOCUMENTS/FORMS

- 9.1 STCL-PROC-042 FRM2 CBU Processing Worksheet
- 9.2 STCL-FORM-050 Processing Lot Numbers – CBU
- 9.3 STCL-COLL-007 FRM2 Auto/Directed CBU Receipt Form
- 9.4 FLOW-FORM-012 Graft Characterization
- 9.5 STCL-SOP-052 (FRM1) Progenitor Cell Assay Form
- 9.6 FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet
- 9.7 STCL-PROC-035 (FRM1) Manual Differential (Slide Method) – CBUs for CCBB Program
- 9.8 STCL-PROC- 045 FRM1 CBU Cryopreservation
- 9.9 STCL-SOP-049 ABO Rh Typing
- 9.10 STCL-SOP-022 Viability Counts via Trypan Blue Dye Exclusion
- 9.11 STCL-EQUIP-002 Sysmex XS-1000i Hematology Analyzer for the Stem Cell Laboratory
- 9.12 STCL-EQUIP-011 Sterility Culture Using the BacT/Alert Microbiology System
- 9.13 STCL-EQUIP-011 FRM1 BacT Alert Log Sheet
- 9.14 STCL-COLL-007 JA10 Auto/Directed Maternal Blood Sample Processing

- 9.15 STCL-PROC-045 Cryopreservation and Storage of CBU
- 9.16 STCL-PROC-057 Performing Automated Viability Counts using the Cellometer Auto 2000.

10 REFERENCES

- 10.1 Sepax Cell Processing System Operator's Manual
- 10.2 American Association of Blood Banks (AABB). Standards for Hematopoietic Progenitor Cell and Cellular Product. Current edition.
- 10.3 Foundation for the Accreditation of Cellular Therapy (NetCord-FACT) International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release Current edition.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
03	Ann Kaestner / Barbara Waters-Pick	<ul style="list-style-type: none"> Section 1 – Updated the description and changed the instrumentation from Sepax S-100 to Sepax 2 S-100. Section 2 – Moved portion from Section 1 to Section 2. Section 5 – Updated materials to include Chloraprep SEPP, Cellometer stain, and Cellometer disposable counting chambers. Section 6 – Changed Sepax S-100 to Sepax 2 S-100, dedicated printer, and added Cellometer Auto 2000 instrument (when applicable). Section 8 – Changed references from CBU to UCB throughout the document. Section 8.25 thru 8.45 – Updated steps associated with use of Sepax 2 S-100 and pictures. Section 8.49 – Deleted STCL-PROC-042 FRM1 CBU Processing FRM1. Section 8.59 – Added reference for STCL-PROC-057 Performing Automated Viability Counts using the Cellometer Auto 2000. Section 8.65.3 – Removed sentence regarding recording manual differential results on STCL-PROC-042 FRM1. Section 9 – Deleted STCL –PROC-042 FRM1 CBU Processing form and added reference for STCL-PROC-057 Performing Automated Viability Counts using the Cellometer Auto 2000.

Signature Manifest**Document Number:** STCL-PROC-042**Revision:** 03**Title:** UCB Processing Using the Automated Sepax 2 S-100 Cell Processing System with UCB-HES Protocol

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STCL-PROC-042 UCB Processing Using the Automated Sepax 2 S-100 Cell Processing System with UCB-HES P**Author**

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