



STEM CELL LABORATORY (STCL)



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Enumeration of Viable CD34+ Stem Cells in Fresh Umbilical Cord Blood Using the BD Stem Cell Enumeration Kit: FACSCanto II Clinical Software Version

DOCUMENT NOTES:

Document required for the BLA.

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FLOW-GEN-039

ENUMERATION OF VIABLE CD34+ STEM CELLS IN FRESH UMBILICAL CORD BLOOD USING THE BD STEM CELL ENUMERATION KIT: FACSCANTO II CLINICAL SOFTWARE VERSION

1. PURPOSE

- 1.1. This procedure will instruct the user to perform BD FACSCanto II flow cytometer instrument setup and optimization for test samples prepared using the BD Stem Cell Enumeration Kit (SCE). This procedure will further instruct the user to perform process control testing using cells with known assayed CD34+ content. Process controls are used to assure cell staining and all testing systems are performing as designed. Once all quality control specimens are deemed to meet expected results, then patient specimens are cleared for testing

2. INTRODUCTION

- 2.1. The BD™ SCE kit provides a single tube viable CD34 assay for fresh umbilical cord blood. The reagent is combined with test samples in individual BD Trucount Tubes to obtain absolute cell counts. When a sample is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to the cell surface. Additionally, the lyophilized pellet in the BD Trucount tube dissolves, releasing a known number of fluorescent beads. The dye 7-AAD is added to assess viability of the cells. Cells that are 7-AAD+ are not viable. Ammonium chloride is added to lyse erythrocytes before the sample is acquired on a flow cytometer. During analysis of the sample, the concentration of viable CD34+ cells, viable CD45+ cells, and the percentage of viable CD34+ cells in the viable CD45+ cell population are calculated.

3. SCOPE AND RESPONSIBILITIES

- 3.1. This procedure should be used when performing flow cytometric assays to enumerate viable CD34+ stem cells in fresh UCB specimens. It is the responsibility of the laboratory director, manager, and flow cytometry staff, to ensure the requirements of this procedure are successfully met.

4. DEFINITIONS/ACRONYMS

- 4.1. SCE- Stem Cell Enumeration
- 4.2. CCBB- Carolinas Cord Blood Bank
- 4.3. BD- Becton Dickinson, manufacturer of flow cytometry instruments, software, and reagents.
- 4.4. Single-platform method – This is a flow cytometry method for directly enumerating cells of interest (CD34) by using a known number of beads/volume of sample. Results are reported as number of CD34+ progenitor cells per µl of specimen tested.
- 4.5. Test specimen- The test tube that holds the UCB or dilution of UCB to be added to the testing tubes.
- 4.6. Testing Tubes- The tubes used in the staining step in which antibodies and test specimen are mixed.

- 4.7. Reverse pipetting – A method of pipetting using an adjustable air displacement pipette that allows for a more accurate volume measure upon dispense, particularly with viscous fluids. The plunger is pressed all the way down prior to inserting the tip into the fluid, then, released all the way up. The plunger is then pressed only to the first stop to dispense the volume dialed into the pipette.
- 4.8. UCB - Fresh (not thawed) umbilical cord blood that has been red cell depleted and volume reduced.
- 4.9. SIP – Sample injection probe where the testing tube is installed on the flow cytometer.
- 4.10. Dot Plot - A two parameter box used to display events acquired by a flow cytometer.
- 4.11. Acquisition region (or gate) – The region drawn around the designated population of events/cells that determine when the acquisition will stop, signaling that the data file with the information needed to perform an analysis has been stored and that the testing tube can be uninstalled from the SIT.
- 4.12. Region - A line or box drawn on a dot plot to define a specific population of events/cells during flow cytometry acquisition or analysis.
- 4.13. Gate - used to define events/cells of interest on a flow cytometry plot. Gates may be defined by one or more regions and may be used to sequentially define events/cells from enumeration of a specific population such as CD34+ progenitor cells. The logical operators AND, OR, and NOT are used to define logical gates.
- 4.14. Sequential gating - The process of using regions and gates around populations of events based on various parameters including light scatter and fluorescent antibody staining intensity. This can also be referred to as hierarchical gating in which gates (parent population) can contain sub-gates (subpopulations).
- 4.15. CCBB Worksheet Packet – The packet of forms delivered with the UCB specimen includes, Graft Characterization Form, the Progenitor Assay Form, Flow Cytometry Worksheet, and additional bar code labels with lab letter ID.

5. Materials

- 5.1. BD Stem Cell Enumeration Kit
 - 5.1.1. CD34 PE/CD45 FITC reagent
 - 5.1.2. 7-aminoactinomycin-D (7AAD) reagent
 - 5.1.3. 10X ammonium chloride lysing solution
 - 5.1.4. 50 BD Trucount tubes (2 foil pouches of 25)
- 5.2. Reagent grade deionized water
- 5.3. Specimen Dilution reagent-PBS/ 1% BSA, Gibco BRL
- 5.4. 12 mm x 75 mm, 5 ml non sterile polystyrene test tubes and caps, Fisher brand or equivalent
- 5.5. Test tube rack
- 5.6. Traceable timers-VWR or equivalent
- 5.7. Bleach – Clorox household or equivalent for waste tank.
- 5.8. FACSFlow Sheath fluid – BD
- 5.9. Colored Markers-Sharpie

6. EQUIPMENT

- 6.1. FACSCanto™ II Flow Cytometer, Becton Dickinson

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 Stem Cell Laboratory, DUMC
 Durham, NC

- 6.2. Vortex mixer, VWR or equivalent
- 6.3. Adjustable pipettes and tips 20, 200, 1000 microliter (µl), Rainin
- 6.4. Automated pipette and tips 2000 microliter, Rainin

7. SAFETY

- 7.1. Use universal precautions and wear appropriate personal protective equipment (PPE) when working with the biological materials described in this procedure.
- 7.2. Use particular caution with the following reagents used in this procedure. Refer to Material Safety Data Sheet for these products or Duke Occupational and Environmental Safety Office website for more information on these warnings.
 - 7.2.1. Monoclonal antibodies used in testing.
 - 7.2.2. Sodium azide warning
 - 7.2.3. 7-AAD
 - 7.2.4. BD Trucount tubes
 - 7.2.5. Cobalt chloride warning
 - 7.2.6. Silica warning
 - 7.2.7. Ammonium Chloride


8. PROCEDURE

- 8.1. UCB Specimen Receipt:
 - 8.1.1. Upon receipt of the specimen and worksheet packet at the designated drop off location in the STCL flow cytometry section, open the worksheet packet to the FLOW-GEN-012 (FRM5) Stem Cell Laboratory Flow Cytometry Worksheet. The example below shows how the specimen information section of the worksheet should be completed. The dilution factor (Dil Fac.) is entered by the STCL staff at the staining step.

InfoCard #: FLOW-GEN-012 FRM5 Rev. 04 Effective Date: 12 Sep 2012
Accession # _____
(Not Required for banked UCB Units)

FLOW-GEN-012 FRM5 STEM CELL LABORATORY FLOW CYTOMETRY WORKSHEET

Circle or write NA when request is not applicable

Sample ID (Attach labels with donor, recipient or patient sample info) <u>NA</u>	Bar Code label <u>NA</u>  W1582 14 491061 3 Y UCB LAB ID: <u>A</u>
Recipient wt. <u>NA</u> kg	

If NMDP Product: ☐ OUTGOING ☐ INCOMING NA

Sample classification (✓): ☐ Adult ☐ Pediatric ☐ Allogeneic ☐ Auto ☒ UCB Bank
☐ To be concentrated ☐ Other _____

Sample source (circle the appropriate test condition):
☒ UCB ^{fresh} Thawed infusion Other _____
☐ PBSC Midpoint Preliminary Final Thawed infusion Other _____
☐ Bone Marrow OR Bag Post process Other _____
☐ PB mobilized Immune Reconstitution (IR) _____ (Provide IR time point if known)
☐ Donor lymphocyte infusion (DLI)
☐ Control Cell Lot# _____ Exp. Date _____

Proficiency Test
 Date/Time PB or Product Collected 05/05/2014 @ 1420 NA
 Date/Time Product Sampled for tests 05/06/2014 @ 1129 By mw NA
 Cell Concentration 33.1 x10⁶/ml Product volume 2.50 ml
To be completed by STCL flow cytometry staff for stem cell product collections:
 Date/Time stained 5-6-14 @ 1242 BY JS Dil. Factor X 1
 For Banked UCB: Sample stained within 4hr of sampling time ✓ tech/date: JS 15-6-14 NA

- 8.1.2. If any information is missing, contact the CCBB laboratory manager or designee and request the required information.
- 8.1.3. Observe the recorded sampling date/time (05/06/2014@1120 in the example provided) of each specimen to determine if staining will be within 4 hours of the sample draw, then initial and date that this was checked in the space provided “For Banked UCB” under Date/Time Stained.
- 8.1.4. For quality assurance purposes, if the specimen is >4 hours past sampling at the time of staining, note this by writing “Specimen >4 hours post sampling at time of staining” on the comment line below the reagent lot information section of the Flow Cytometry Worksheet.
- 8.1.5. Arrange the worksheet packets in order of lab letter ID (i.e. alphabetical order) and remove a bar code label from each packet to place in the specimen receipt log book. Complete the log book entry after staining the test samples.
- 8.1.6. Verify that the specimen identification matches that of the corresponding worksheet packet for both the barcode label and for the lab letter ID.
- 8.1.7. Verify that the reagents to be used for testing have met quality control requirements (This may be determined in advance of specimen receipt).
- 8.1.8. For the reagents used in this testing, enter the lot and expiration date in the designated fields on the Flow Cytometry Worksheet prior to specimen staining.
- 8.2. Specimen Staining:
 - 8.2.1. Prior to running test samples on any day of testing, low and high process controls with known assayed CD34+ absolute count values are run to confirm staining and system integrity. The results of this testing must be acceptable prior to running test samples.
 - 8.2.2. Gather staining supplies:
 - 8.2.2.1. BD SCE kit (component lots marked in current use).
 - 8.2.2.2. 12 x 75 mm non-sterile polystyrene test tubes with caps
 - 8.2.2.3. 1x working solution of red cell lysing reagent:
 - Determine volume of 1x lysing agent that will be required for the day and make a 1:10 working solution from the 10x Ammonium Chloride reagent using 1 part 10x reagent and 9 parts reagent grade DI water. Make only enough for each day of use.
 - 8.2.2.4. PBS with 1% BSA used for sample dilution.
 - 8.2.2.5. 1000, 200 and 20 µl Adjustable pipettes and tips
 - 8.2.2.6. 2000 µl Automated pipette and tips
 - 8.2.2.7. Test tube staining rack
 - 8.2.2.8. Traceable timers (2)

8.2.3. The following tube setup is shown as a guideline only. Other designs may be used as long as specimen bar code is matched with staining tubes.

8.2.3.1. Arrange (up to 3 specimens /rack, one specimen per row horizontally) the testing tubes and specimen in order as illustrated below and in alphabetical order from the forward facing side of the rack by lab letter ID (i.e. row one = lab ID “A” specimen, row three = lab ID “B” specimen, row 5 = lab ID “C” specimen, or row one = lab ID “X” specimen, row three = lab ID “Y” specimen, row 5 = lab ID “Z” specimen).:

	Col 1	Col 3 open	Other	Other	Col 5 open	Col 6 open	Col 7	Col 8			
Row 5	SCE						Dil	Sp.			
Open row 4											
Row 3	SCE						Dil	Sp.			
Open Row 2											
Row 1	SCE						Dil	Sp.			

Forward facing side of rack

- SCE= Trucount tube (vertical column 1st column position from left to right)
 - Open position (i.e. open column 3rd position)
 - Dil=Dilution tube 12x75mm polystyrene tube (If needed)
 - Sp=The UCB Specimen tube
 - Once the specimen is placed next to the row of tubes, match the bar code labels to the specimen and apply one barcode label to each tube in the row.
- 8.2.4. Dispose of unused labels in the labeled “Shred-it” drawer at each cytometer table. The contents must be shredded at the end of each work day.
- 8.2.5. UCB specimens with cell concentrations of 40.0 x10⁶ cells/ milliliter or greater must be diluted using the specimen dilution reagent. Bring the concentration below this mark using whole value dilutions (i.e. x 2, x 3 etc.). The final diluted sample volume should be no less than 300µl in order to provide enough volume to complete testing. The original specimen volume used in the dilution should not be less than 50µl. Table 1 provides guidelines for making dilutions.
- 8.2.6. The dilution factor must be entered in the designated field (Dil Fac.) on the Flow Cytometry Worksheet.

Table 1 Use the following guidelines when preparing dilutions:

DILUTION TYPE		SPECIMEN VOLUME (Reverse Pipette method)	DILUTION REAGENT (Reverse Pipette method)
2-FOLD	1:2	150 µl	150 µl
3-FOLD	1:3	100 µl	200 µl
4-FOLD	1:4	100 µl	300 µl
5-FOLD	1:5	100 µl	400 µl
10-FOLD	1:10	50 µl	450 µl
15 FOLD	1:15	50 µl	700 µl
20 FOLD	1:20	50 µl	950 µl

- 8.2.7. Refer to table 2 reagent volume requirements when adding the antibodies for testing. Use the antibodies in the designated “IN USE” box or if a replacement vial is required, replace from the vial compartment labeled with green “Ready For Use” labels.

NOTE: If a new lot of reagent must be opened it must first pass laboratory established quality control testing before being put into use for test specimens.

Table 2: Use the following guidelines when preparing process controls and test samples as recommended by the manufacturer:

1. Using the pipetting guidelines in 8.2.8 below, prepare the process controls or test sample according to the following table. Prepare and run the process controls prior to staining test samples to complete the instrument setup and assure reagent performance qualification.					
Sample Type	Tube Type	Reagent (μl) Normal pipetting		Cells (μl) Reverse pipetting	
		CD34	7-AAD	High	Low
7-AAD control	Polystyrene	20	20	100 of either	
High Control	Trucount	20	---	100	---
Low Control	Trucount	20	---	---	100
Test Sample	Trucount	20	20	100 test sample	
2. Cap the tubes, vortex gently 2-3 seconds, and incubate for 20 minutes in the dark at room temperature.					
3. Add 2 ml of 1X ammonium chloride lysing solution to each tube.					
4. Cap the tubes, vortex gently 2-3 seconds, and incubate for 10 minutes in the dark at room temperature.					
5. Immediately put the tubes on wet ice and acquire within 1 hour of lysing.					

NOTE: Store BD Trucount tubes at 2-25° C. These tubes must be used within 1 hour of removal from the foil pouch. Remaining tubes must be used within 1 month of opening the foil pouch. Carefully reseal the foil pouch immediately after removing a tube. Examine the desiccant each time the pouch is opened to determine if color has changed from blue to lavender. Discard tubes if color has changed.

- 8.2.8. Use the following pipetting guidelines when adding antibodies and specimen:
 - 8.2.8.1. Adjust the pipette volume setting to the correct volume.
 - 8.2.8.2. Keep the pipette upright and hold the Trucount tube in the hand opposite the pipetting hand while dispensing the antibody being sure to avoid touching the bead pellet with the pipette tip.
 - 8.2.8.3. Use the same tip when adding the same antibody reagent to multiple testing tubes if the addition is to a clean tube.
 - 8.2.8.4. Use a clean tip between different antibody vials or if the tube receiving antibody contains another agent.
 - 8.2.8.5. Touch the end of the tip to an interior side of the tube just above the metal barrier to dispense the reagent so that it runs down and into the bottom of the tube. Normal pipetting may be used.
 - 8.2.8.6. Use the reverse pipetting method when making specimen dilution or adding 100µl of specimen (or specimen dilution if required) to Trucount tubes.
 - 8.2.8.7. Hold the Trucount tube in the opposite hand to the pipetting hand.
 - 8.2.8.8. Touch the pipette tip opposite the interior side that was used to add antibody reagent approximately 2/3rds the distance down the tube (i.e. 1/3rd from the bottom of the tube).
 - 8.2.8.9. Residual specimen may be dispensed back into the original specimen tube unless the tip was contaminated. In this case the tip with residual specimen should be disposed of.
 - 8.2.8.10. Use a clean tip each time specimen is added to a Trucount tube.
- 8.2.9. Cap all testing tubes, color code them by row using colored markers.
- 8.2.10. Place the original UCB specimen tube on the sample storage rack corresponding to the day of testing. Test specimens for the week are disposed of the following Monday prior to start of testing.
 - 8.2.10.1. Dispose of the dilution tube, if applicable.
- 8.2.11. Record the staining time information on the Flow Cytometry Worksheet based on the timer setting compared to the cytometer computer clock time.
- 8.3. FACSCanto II Instrument Setup:
 - 8.3.1. Instrument setup should be run once each work day. Section 8.3 may be omitted if the setup has already been performed.
 - 8.3.2. Power up the FACSCanto II flow cytometer and computer.
 - 8.3.3. Log into windows: Administrator and enter the required password.

- 8.3.4. Launch BD FACSCanto Clinical software from the desktop icon.

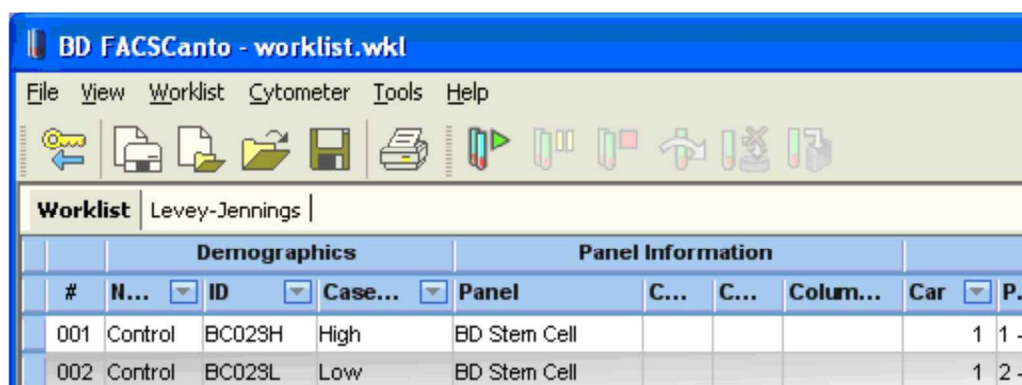



- 8.3.5. From the drop down window find your name as user and enter your password if required.
- 8.3.6. Check all fluidics levels and replace as needed.
- 8.3.7. Empty waste container if required.
- 8.3.8. Add approximately 500 mls of bleach to waste container prior to reinstallation on the fluidics cart.
- 8.3.9. Click on the “Cytometer” menu and run fluidic start up.
- 8.3.10. Allow cytometer to warm up for 30 minutes before running setup.
- 8.3.11. Prepare FACS 7-Color Setup Beads, by first removing the tube from the foil pouch, then adding the diluent to the line marked on the side of the setup tube. (Approximately 1 ml)
- 8.3.12. Vortex the tube of beads on medium speed 2-4 second.
- 8.3.13. Select Cytometer>Setup>Standard Setup.
- 8.3.14. In the Setup lot information window:
- 8.3.14.1. The Lot ID should match the lot in use. If new lot information is required, click on “New lot ID” button, enter the target values in “Target” tab, the spectral overlap values in “Spectral overlap” tab and the expiration date. *This information is on the label supplied with the 7-color Setup Beads.*
 - 8.3.14.2. Verify that the beads are not expired.
 - 8.3.14.3. Verify that the value in the Targets and Spectral Overlap Factors tab match those for the BD FACS 7-color setup beads you are using.
- 8.3.15. Click Next, verify Run “setup in manual mode” is selected, and then click Next.
- 8.3.16. Load the bead tube on the cytometer when prompted and click OK. The software acquires data to set up the cytometer.
- 8.3.17. Remove the tube when prompted.
- 8.3.18. Verify that setup was successful and all parameter results read “PASS”. If setup was not successful, click “Cancel” button and see Troubleshooting section in the Application Guide.
- 8.3.19. Print the report.
- 8.3.20. At the Cytometer Setup Wizard, click “Next” button.
- 8.3.21. At the Save Setup Results dialog, click “Yes” to continue. Click “Next: to proceed with the application setup.
- 8.3.22. Select “BD Stem Cell” from the Panel Types menu in the Setup Optimization wizard and click “Next”.
- 8.3.23. Click “Start” button at the BD Stem Cell Enumeration setup view.
- 8.3.24. Load “OPT” tube on the cytometer when prompted and click OK to begin acquiring data.
- 8.3.25. Check the status message under the Calculating Spillover checkbox.

- 8.3.25.1. If spillover was determined successfully proceed with next step.
 - 8.3.25.2. If spillover was not determined successfully, click “Cancel” button and repeat step 21-24.
 - 8.3.26. Click “View Report” button. Confirm that the Overall Results read “PASS”.
 - 8.3.27. Print the “Application Setup Report”.
 - 8.3.28. Close the Report and click save button.
 - 8.3.29. Remove the tube when prompted and click “Finish” button.
- 8.4. Running Process Controls

NOTE:

- Using an incorrect bead count will result in inaccurate absolute counts, so it is important to update Lot IDs and Trucount values when lots change.
- Due to the temperature requirements of this assay, the BD FACS Loader cannot be used to acquire controls or samples.



- 8.4.1. If not already performed, select Tools>Lot IDs. The lot IDs dialog appears.
- 8.4.2. Click the “BD Stem Cell” tab and enter (or verify) the lot ID from the BD Stem Cell Enumeration Kit.
- 8.4.3. Click the “Absolute count Beads” tab, enter (or verify) the Trucount beads lot ID from the BD Trucount beads pouch, and enter (or verify) the beads per pellet value.
- 8.4.4. Click OK to save the information.
- 8.4.5. At the “Name” column in the acquisition worklist type “Control”.
- 8.4.6. At the “ID” column in the acquisition worklist enter the control lot ID including H or L for the high or low reagent.
- 8.4.7. Enter High or Low in the Case column corresponding to the correct control reagent.
- 8.4.8. Select “BD Stem Cell” from the Panel drop down menu.
- 8.4.9. Click Run  to start the run.
 - 8.4.9.1. A prompt appears asking to save the worklist; Choose No.
 - 8.4.9.2. A prompt appears asking if manual run is OK; Choose OK.

- 8.4.9.3. At the next prompt choose Ignore.
- 8.4.10. Vortex the stained controls on medium speed 2-4 second (vortex dial should be placed where arrow is drawn) before acquisition.
- 8.4.11. When prompted, manually install tube on the cytometer by doing the following:
 - 8.4.11.1. Push the aspirator arm to the left.
 - 8.4.11.2. Place the tube on the SIT, ensure that the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.
 - 8.4.11.3. Center the aspirator arm under the tube so that the bottom of the tube sits centered above the three sensor pins on the aspirator arm.
 - 8.4.11.4. Click **OK**.
- 8.4.12. After acquisition is complete the lab report appears on the screen.
- 8.4.13. See below the gating guidelines and lab report example taken from the BD Stem Cell Enumeration Application Guide for BD FACS Canto II Flow Cytometer.

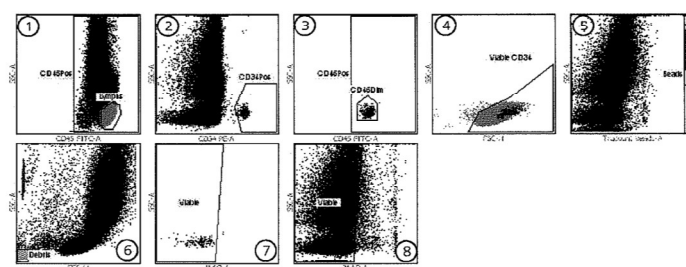
Gating guideline

About this topic	This topic shows an example of how BD FACSCanto clinical software processes a sample stained using the BD Stem Cell Enumeration kit and analyzed on a BD FACSCanto II flow cytometer. Gates shown are auto-gates set by the software. Manual gate adjustment was not necessary. The stained sample in this example is fresh mobilized peripheral blood.
How to use this information	Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust gates as necessary. Adjust gates <i>only</i> if necessary.
Key terms	Key terms in the gating guideline table correspond to the following information: Step No. The order in which the gating algorithm processes the data. Plot No. The order in which the plot appears in the Lab Report. Plot Type. The plot axis labels. Software Action. How the gating algorithm processes the data. Gate Name. The name of the gate as it appears on the Lab Report. User Action. What to look for on the plot and how to manually adjust the gate (if necessary).

Example Lab Report

The plots are displayed on the Lab Report in the following order.

Specimen
ALP16-BD1



Plot no.	Gate(s)	Plot no.	Gate(s)
①	CD45Pos, Lymphs	⑤	Beads
②	CD34Pos	⑥	Debris
③	CD45Pos, CD45Dim	⑦	Viable
④	Viable CD34	⑧	Viable

- 8.4.14. Refer to the FACSCanto II BD Stem Cell Enumeration Application Guide for examples of analysis troubleshooting to help determine if region placements are correct.
 - 8.4.15. To make adjustments to regions, click on the plot to be changed (this action activates the plot and allows the user to modify gates) and click on the region to make adjustments as needed.
 - 8.4.16. Once the analysis is complete, click on the Review button>select User ID >click OK. Print report.
 - 8.4.17. Verify that the results (CD34+ absolute counts and CD34+ % of CD45+ events) are within the manufacturer's assayed range for the process control lot you are using.
 - 8.4.18. If the results are not acceptable then product testing cannot begin.
 - 8.4.19. Notify the flow cytometry supervisor or lab manager.
 - 8.4.20. Use the guidelines provided in Flow-Gen-020 Section (8.6.1.3 New Lot Testing and Process Validation) to troubleshoot and resolve the issue.
- 8.5. Running test samples:
- 8.5.1. It is critical to verify the identity of each test sample at the staining step and throughout the acquisition and analysis by comparing the test sample ID to the any accompanying worksheets and labels.
 - 8.5.2. Testing should be performed in groups of 3 or less since samples must be stored on ice and acquired within 1 hour of the final staining step (erythrocyte lysis).
 - 8.5.3. The performing tech must do the following:
NOTE: Refer to the work list pictured above in section 8.4 and substitute the information below in the designated columns.
 - 8.5.3.1. At the "Name" column in the acquisition worklist type "Specimen".
 - 8.5.3.2. At the "ID" column in the acquisition worklist enter the Sample lab ID.
 - 8.5.3.3. At the case number column in the acquisition worklist enter (use bar code reader) the bar code associated with the sample (if applicable).
 - 8.5.3.4. Click in the panel field and click on the drop down menu (arrow) to select "BD Stem Cell + 7AAD".
 - 8.5.3.5. Follow the steps outlined above in 8.4.9 through 8.4.15 in order to acquire and analyzed the test samples.

NOTE: If the performing technologist feels there is need for further review of a particular test result prior to completion of the analysis, the flow supervisor or the designated supervisory tech should be consulted. The supervisor would then sign off as the reviewer.
 - 8.5.4. Enter the CD34 results of testing into the designated fields on the Graft Characterization Form.

- 8.5.5. Input the required information relating to test acquisition on the Flow Cytometry Worksheet.
- 8.5.6. Attach the printed results to the appropriate worksheet(s) and file in the designated location.

9. RELATED DOCUMENTS/FORMS

- 9.1. FLOW-GEN-012 FRM5 Stem Cell laboratory Flow Cytometry Worksheet
- 9.2. FLOW-FORM-012 Graft Characterization
- 9.3. FLOW-GEN-020 Stem Cell Laboratory Quality Management-Quality Control Policies for Flow Cytometry.

10. REFERENCES

- 10.1. BD Stem Cell Enumeration Kit product insert: catalog # 344563.
- 10.2. BD Stem Cell Enumeration Application Guide For BD FACSCanto II Flow Cytometer. *Electronic version stored on the cytometer workstation desktop and on the shared network drive.*

11. REVISION HISTORY

Revision No.	Author	Description of Change(s)
03	M. Reese	Updated Graft Characterization form number in Related Documents.

Signature Manifest

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All dates and times are in Eastern Time.

FLOW-GEN-039 Enumeration of Viable CD34+ Stem Cells in Fresh Umbilical Cord Blood Using

Author

Name/Signature	Title	Date	Meaning/Reason
Melissa Reese (REESE008)		08 Jan 2021, 04:03:09 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
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Medical Director

Name/Signature	Title	Date	Meaning/Reason
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Quality

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Name/Signature	Title	Date	Meaning/Reason
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Author

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Medical Director

Name/Signature	Title	Date	Meaning/Reason
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