YALE-NEW HAVEN HOSPITAL	TITLE: STEM CELL ENUMERATION KIT ANALYSIS		DEPT OF LAB MEDICINE – Flow Cytometry Policy and Procedure Manual
			DOCUMENT # FLW-51
			Page 1 -6
WRITTEN BY: Patricia Lenz, MT(ASCP)	EFFECTIVE DATE: December 4, 2012	REVISION: N/A	SUPERCEDES: FLW-41

PRINCIPLE:

Transplantation of hematopoietic progenitor cells obtained from the bone marrow of a patient or HLA-matched donor is used increasingly in the treatment of blood disorders, malignancies and genetic abnormalities. When used in treating hematologic malignancies, stem cell transplants enable physicians to destroy diseased bone marrow using high doses of chemo/radiotherapy and replace it with healthy progenitor cells that repopulate the marrow and produce normal blood cells.

With the arrival of mobilization regimes (GCSF, GM-CSF, and chemotherapy) peripheral blood has become the preferred source of stem cells. Several publications show that peripheral blood stem cell (PBSC) transplants are less traumatic to the patient, and they shorten the time to engraftment and provide adequate numbers of stem cells for either autologous an allogeneic transplants.

Another source of hemopoietic progenitor cells for transplantation comes from bloods that remain in the umbilical cord when mother and newborn are separated. This blood can be collected, washed and frozen for the newborn in case they need it later in their life for transplantation, or can be stored in banks for the general population to use. These stem cells are more versatile than those from the bone marrow or peripheral blood simply because the infant donor's immune system is immature. The stem cells are relatively "uneducated" compared to cells from an older child or an adult. There is less likelihood of an adverse interaction between the transplanted cells and the recipient's cells. This means that in order to be useful, stem cells from cord bloods do not have to match the recipient's tissue as closely as bone marrow or peripheral blood stem cells do.

The BD Stem Cell Enumeration (SCE) kit provides simultaneous enumeration of viable dual-positive CD45+/CD34+ hemapoietic stem cell populations in CD34+ absolute counts (cells/uL) as well as the percentage of the total viable leucocytes counts that is CD34+ (%CD34+).
During analysis of the sample on the FACSCanto II, the concentration of viable CD34+ cells and viable CD45+ cells, and the percentage of viable CD34+ cells in the viable CD45+ cell population are calculated. The software uses an automatic gating algorithm to identify and isolate stemcells. Software acquisition and analysis methods are based on Standards Institute H42-A2 approved guideline. Apart from calculations for apheresis bag products, there is not need for external calculations – all results can be used directly from the instrument.

Procedure:

- Verify that Stem Cell Enumeration kit lot numbers are correct in the FACSCanto II Canto app under Lot ID. The 7-Color Bead lot # is found under Setup-Standard Setup-Cytometer Setup Wizard-Setup Information.

2. Perform FACSCanto II setup and 7-Color Bead calibration:

- Immediately after a successful 7-Color setup, instead of saving and clicking on **Finish**, click on **Next**- a popup message appears asking if you want to save the setup and continue the **Optimization**.
- Click **Next** to continue to BDStem Cell
- Click **Next** to click on **Start**. A popup will direct you to put the Optimization tube on manually.
- Vortex gently. Make sure tube is up as far as it can go and is secure.
- Once the optimization is completed, a message will popup that it has passed. Note the PE-%7-AAD spectral overlap value. A value of -0.5% to 10.5% indicates a successful setup optimization.

- Click **Save**, another popup will tell you it is time to take the tube off.
- Click **Finish** to end the program. If spillover was not determined successfully
- Click **Cancel** and repeat the Optimization process. See **Troubleshooting** if necessary. Make sure the tube guide is in place.

3. Setup for Controls on Canto:

- Enter "Control" in the **Name** column. NB: If you do not enter "Control" in the **Name** column, the BD Stem Cell panel will be disabled.
- Enter lot ID in the **ID** column.
- Enter the control type (High or Low) in the **Case Number** column.
- Select the **BD Stem Cell** panel.
- Save the worklist under StemCellDate (StemCell month day year)

.

4. **Acquisition and Analysis:**

- Controls should be placed on the carousel in the order they were listed on the worklist.
- Click **Run (test tube with >)** to acquire**.** A message may pop up saying "Canto has not been washed within the last 24 hours, do you want to run?" Click **Yes** if instrument had fluidic start-up done. Another pop up will come up with the message that usually samples are run manually, do you want to continue? Click **Yes.**
- Click **Ignore** if the instrument confirms that the carousal is not carousel 1 and did you want to continue?
- When samples are processed, the worklist reappears. Check **Status** to see if controls were OK or "Needs Review". If necessary, click on Needs Review and review gates. Manually move the gates if needed. See **Gating Guidelines** later in this procedure if needed. Check gating and confirm that the high and low control results meet the expected values for absolute CD34+ and %CD34+. Controls should be run within 1 hour of lysing and prior to running specimens. Verify that the results are within the established range for the process control lots you are using. Results should be entered on the "L" drive in the Flow folder.

5. Setup for Patients on Canto:

- Enter the Patient's name or scan in the name from the demographic label (label has the name, medical record number, and flow number.
- Enter the medical record number under **ID**.
- Enter or scan in the flow number from the flow test label under **Case Number**
- If this is the first patient, drop down the Test column to test name **BD Stem Cell + 7-AAD** panel. Test will automatically appear in the Test column for the rest of the patients.
- Enter the dilution factor. 1 will pop up automatically.
- Click on **Run** (test tube>). A message will come up, choose to save the changes to the Worklist.
- Due to the temperature requirements of this essay, the Loader cannot be used to acquire samples.
- Vortex each tube just prior to acquiring manually.
- The same messages that pop up during the Controls run might pop up again during the Patients' run. Follow the same responses as above.

Biohazard Warning! Always wear gloves when manually loading samples on the cytometer. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples and might contain biohazardous waste.

When prompted, install the tube containing the first patient in the worklist on the cytometer and click **OK**. To load the tube onto the cytometer, push the aspirator arm to the left. The black Tube Guide will also be moved to the left. Place the tube on the SIT, ensure that the tube is straight, and firmly push up until the tube come to a complete stop and is fully seated. The aspirator arm will swing back into place. The bottom of the tube should sit centered above the three sensor pins that are on the arm. The Acquisition tab appears and events appear in the plots. Once the acquisition targets are met, the Lab Report appears. The Lab Report will automatically print out. A prompt will appear to take the sample from the SIT. You could hear a hiss of air and the aspirator arm will lower. Hold the tube while pushing the aspirator arm to the left. When you release the aspirator arm, the arm will swing back into position. SIT cleaning occurs when aspirator arm comes back to the center position. If you do not remove the sample when prompted, another message will come up to remind you to remove the tube from the SIT. Click **Okay** to remove the tube at this point.

Biohazard Warning! If you do not hold the tube when you move the aspirator arm, the tube could fall off the SIT and expose you to potentially biohazardous sample.

Inspect all plots to verify that all gates include the appropriate populations. See Gating Guidelines to manually regate if necessary.

You can enter a Patient list all at once as long as the samples are run in the order listed or you can enter a Patient and then run the sample. Repeat the process for the next patient.

Gating Guidelines

Adjust gates only if necessary. If the **Status** is "OK", no adjustments are needed. However if the **Status** is "Needs Review", the gates will need to be adjusted.

Click on any plot to adjust its gate. The Debris plot should be the first plot to look at. If the cells/debris on the lower left hand side of the plot looks bigger than the gated area, enlarge the gate to include much of the material.

The selected plot appears in an enlarged view.
Adjust gates as necessary. Click **OK** if saving adjustments or Cancel if plot looks okay.
To set the gates back to the original positions, click **Autogate**.
If you enlarge the Debris gate, the WBC and the viability will change. If you move the gates on the CD34+ gate or on the Dim CD45+ gate, the CD34+ value will also change.

Trouble Shooting

QC message	Possible cause	Recommended solution
Spillover failure	• Did not add the 7-AAD to the tube • Did not use BD Stem • Cell controls to perform setup • did not add the CD45/CD 34 reagents to the tube	Re-stain and run the setup and optimization tubes. Check the cytometer fluidics.
Worklist will not run	7-Color Beads were run after Application Setup	• Re-stain the Setup and Optimization tubes. • Re-run 7-Color Beads followed by application setup.

No Results Reported

Lymph gate failure	• Insufficient number of lymphs in sample. • Unresolved lymphocyte population	Manually regate the sample
CD45 gate failure	Insufficient number of CD45 cells in the sample.	Manually regate the sample
CD34+ gate failure	Insufficient number of CD34+ cells in the sample.	Manually regate the sample
Viable CD34 gate failure	Insufficient number of Viable CD34 cells in the sample	Manually regate the sample
CD34 gate failure	Insufficient number of CD34 cells in the sample.	Manually regate the sample

Viability gate failure	Insufficient number of viable cells in the sample because all cells are dead.	Repeat staining, then acquire the newly stained sample.
Incomplete Results Reported		
No beads detected	• A BD Trucount tube was not used. • The pellet was missing from the tube.	Repeat staining using a BD Trucount tube, then acquire the newly stained sample.
Less than 1,000 beads collected	A BD Trucount tube was not used.	Repeat staining using a BD Trucount tube, then acquire the newly stained sample.
Concentration of white blood cells is too high	WBC concentration is > 45,000 cells /uL	Dilute the sample, then re-stain and acquire the newly stained sample.
Gate location is suspect, but all results are reported		
Viability gate suspect	Unusual gate location	Manually re-gate the sample.
Beads gate suspect	• Unusual gate location • A BD Trucount tube was not used. • Pellet missing from tube.	• Manually re-gate the sample. • Repeat staining using a BD Trucount tube, then acquire the newly stained sample.
CD34+ gate suspect	Unusual gate location	Manually re-gate the sample.
CD45 gate suspect	Unusual gate location	Manually re-gate the sample.
CD45dim gate suspect	Unusual gate location	Manually re-gate the sample.
Lymphs gate suspect	Unusual gate location	• Manually re-gate the sample. • Manually adjust the CD34 gate.
Insufficient number of events collected, but all results are reported.		
Less than 100 viable CD34 Events are collected.	• Low CD34 count • Acquisition timed out after 15 min.	• Review plots, report results • Restain sample, verify correct amount of sample is used(100ul)
Insufficient viable CD45 events collected. Insufficient Viable CD45(cont)	• Insufficient viable cells in sample. • Acquisition timed out after 15 min • Sample was too dilute.	• Review plots, report results • Verify sample <24hrs old • Use smaller dilution factor
General Warnings		
Cytometer settings were generated from a failed setup result.	Cytometer setup failed	Re-run cytometer setup and verify that setup is successful. Re-run the samples.
Setup optimization failed	• 7-AAD was not added to the	• Add 7-AAD to the optimization tube and

	tube. • BD Stem Cell reagent was • not added to the tube • An incorrect control sample was used.	re-run setup • Re-stain the sample and acquire the newly stained sample. • Re-stain and acquire controls.
One or more results are outside the alarm range.	• The WBC concentration is >45,000 cells/uL	• Dilute the sample, then stain and acquire the newly stained sample.
Stem cell controls are out of range.	• Cytometer setup failed • Pipetting error • Controls were not well mixed.	• Re-run cytometer setup and verify that setup passes, then restain/rerun Controls. • Open new vials of controls, if fails again.
Research Use Only- Not for Use in diagnostics or therapeutic procedures.	A BD FACSCanto cytometer and/or loader was used to acquire samples.	Load tubes manually and acquire samples using a BD FACSCanto II flow cytometer.
Inspect all dot plots	Dot plots must be reviewed to verify that gates were set correctly and cell Populations appear as expected.	NA
Heparin anticoagulant/interfering substances		
Platelet streak	A sample was collected in Heparin anticoagulant.	• In Lymph plot, move the left boundary of the CD45+ gate to the right to exclude the platelets. • By adjusting the Lymph gate in the lymph plot the platelet streak in the CD34+ plot is partially removed.

Reference:

"Enumeration of Immunologically Defined Cell Populations by Flow Cytometry: Approved Guideline- Second Edition." Wayne, PA:Clinical and Laboratory Standards Institute; 2007. CLSI document H42-A2.

"BD Stem Cell Enumeration Application Guide for BD FACSCanto II Flow Cytometers"
Becton, Dickinson and Company. 23-11196-00 Rev.A . 4/2010.

"Viability Staining". eBioscience Inc. Copyright 2000-2010. Revised 10-5-2010.

"Stem Cell Innovations" Seattle Care Alliance. www.seattlecca.org/diseases/bmt-stem-cell -innovations.cfm

Document Author
Patricia Lenz
November 2012

Signature Approval for Annual Review

Title: STEM CELL ENUMERATION KIT ANALYSIS
Document #: FLW-51

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
BRIAN SMITH	Chairman Laboratory Medicine		12/4/12		NA	December 4, 2012
TEODORICO LEE	LAB MANAGER	Teodorico Lee	12/4/12		NA	December 4, 2012
Richard Torres	LAB DIRECTOR		12/11/12		NA	December 4, 2012