STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Acquisition and Analysis of Lyse-No Wash Samples by Flow Cytometry

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FLOW-GEN-009
ACQUISITION AND ANALYSIS OF LYSE-NO WASH SAMPLES BY FLOW CYTOMETRY

1 PURPOSE

1.1 This procedure is intended to enable the operator to use the BD FACSCalibur flow cytometry or equivalent for the acquisition of samples prepared by the lyse/no wash staining method. This procedure also provides instruction and examples for performing analysis using various software programs.

2 INTRODUCTION

2.1 Monoclonal antibodies conjugated to fluorescent molecules, are used to stain cell surface antigens from sources including fresh bone marrow, peripheral blood, fresh and thawed peripheral blood stem cells, and fresh and thawed umbilical cord blood products. Samples are then acquired and analyzed using the FACSCalibur flow cytometer equipped to perform 4-color measurements or equivalent.

3 SCOPE AND RESPONSIBILITIES

3.1 This procedure must be used when transplant products or peripheral blood test specimens are to be evaluated by the lyse/no wash testing method.

3.2 It is the responsibility of the medical director, the flow cytometry supervisor, and applicable STCL staff to ensure that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1 MAB-Monoclonal Antibody

4.2 PB-Peripheral blood

4.3 PBSC-peripheral blood stem cells

4.4 UCB-Umbilical cord blood

4.5 BM-Bone marrow

4.6 CQ-CellQuest

4.7 MT – Multitest

5 MATERIALS

5.1 Worksheet

5.2 CaliBRITE Beads, Becton Dickinson

5.3 FACSComp Software, Becton Dickinson

6 EQUIPMENT

6.1 Flow Cytometer(s) equipped with a minimum capability to perform 4-color acquisition

6.2 Vortex mixer

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, goggles, lab coats, sleeve covers, disposable gowns, disposable aprons, etc.

8 PROCEDURE

8.1 ACQUISITION- FACSCalibur/CellQuest Pro software

8.1.1 Click to open the Routine Test template folder on the cytometer computer desktop.

8.1.2 Refer to Table 1 in order to determine the correct acquisition template.

8.1.3 Go to the Acquire heading and scroll to Connect to cytometer. A browser window will appear. Perform the following steps:

8.1.3.1 At Directory: click on the change button and enter the location that the data file will be stored. Example: Navigate to the data storage location and create a new folder by clicking on the new folder button at bottom left. Name the new folder by instrument initial and the date. For example: C1 08-25-00 (for FACSCalibur 1), or C2 08-25-00 (for FACSCalibur 2).

8.1.3.2 At File: click on the change button and enter the data file name using the naming conventions listed below:

8.1.3.2.1 For patient name use: first letter of first name, first 3 letters of last name

8.1.3.2.2 Umbilical cord blood = UCBA, UCBB, UCBC, etc.

8.1.3.3 Include one of the following for the for sample type followed by the date of draw (mmddyy):

8.1.3.3.1 Peripheral Blood = PB

8.1.3.3.2 Peripheral Blood immune reconstitution= PBIR (insert time point)

8.1.3.3.3 Leukapheresis (Peripheral blood stem cells) = L1, L2, L3 etc. (add A, B, or C as needed if the test sample is from a selection step)

8.1.3.3.4 Peripheral blood stem cell reinfusion = LR

8.1.3.3.5 Umbilical cord blood reinfusion = CBR

8.1.3.3.6 Donor lymphocyte infusion = DLI

8.1.3.3.7 Bone Marrow (OR bag or Post Processed)- BM, BMOR, BMPO

8.1.3.4 At Operator ID: enter distinctive operator name or initials.

8.1.3.5 Go to the Acquire heading, scroll to Custom Keywords and enter the required information as listed, when applicable. Leave the custom keyword box open until all samples are acquired.

8.1.4 Go to the cytometer heading and scroll to instrument settings. A window will open displaying the current settings.

8.1.4.1 Click on the OPEN button and go to the designated file location: FACStation/BD files/instrument settings.

8.1.4.2 Refer to Table 1 to determine the appropriate settings. Click on the SET then DONE buttons at the bottom of window.

8.1.5 If an adjustment to the amplifier gain setting is required:

8.1.5.1 Go to the Cytometer heading and scroll to Detector/Amps.

8.1.5.2 When the window opens find the amp gain and change the settings to the current setting, posted on the side of each instrument, using the radio dial.

8.1.6 Go to the cytometer heading and scroll to Threshold:

8.1.6.1 Reference Table 1 and choose the required threshold for the acquisition by clicking in the circle on the left of the parameter and using the radio dial to adjust the threshold value to the setting in current use. The current threshold values will be posted at the cytometer however it may be necessary to adjust these settings depending on sample condition or stain variability.

8.1.7 Check to make sure there is adequate volume of sheath fluid in the sheath tank and that the waste tank has been emptied and replenished with 400 mls of Clorox bleach prior to re-connecting it to the cytometer.

DO <u>NOT</u> OVERFILL SHEATH TANK. LEAVE APPROXIMATELY <u>ONE INCH</u> OF SPACE AT THE TOP.

8.1.8 Verify that the sample that is about to be acquired is the sample corresponding to the information entered into the parameter description and Keyword windows by observing the tubes on the loader or by checking each tube as it is placed on the sip manually.

8.1.8.1 Use the manual key pad Up, Down, Left, Right and Mix functions to load tubes onto the CAL 1 instrument. This keypad is located beside the instrument on the right.

8.1.9 For the first tube of the panel, in the acquisition window, make sure the setup box is checked and click acquire. Observe the acquisition windows and place the acquisition region in the correct position according to the acquisition stopping rule for the template. (It is ok to use run and abort instead of setup at this step.)

8.1.10 Once the regions have been properly placed, return to the acquisition window and proceed as follows:

8.1.10.1 Click **STOP**, then click **ABORT** the setup run.

8.1.10.2 Remove the check from the setup box.

8.1.10.3 Click **ACQUIRE**.

8.1.11 Follow the acquisition stopping rules relating to time or event count as you complete the panel acquisition.

NOTE: Always observe the first few seconds of each tube acquisition to be sure that the tube is not missing an antibody, or that a clog or air bubble is not interfering with proper sample aspiration. If a missing antibody is discovered at acquisition, the sample must be re-stained using the markers in the tube with the missing reagent.

8.1.12 Fill out the section of the flow worksheet relating to data collection and storage

8.1.13 Depending on urgency for result, proceed to analysis using the analysis template in the Routine Test Template folder, for the sample type to be analyzed or place the sample worksheet in the tray labeled "To Be Analyzed" if the analysis is not priority.

8.2 ANALYSIS- CellQuest Pro

8.2.1 See the corresponding analysis example in this procedure as a guide for placing regions and statistical markers using the applicable CQ Pro analysis software template found in the Routine Test templates folder of each FACSCalibur workstation.

8.2.2 For tests using BD Trucount counting beads, the following formula is in place within the BD CellQuest Pro software templates to determine the number of events of interest per microliter (µl) in the stained sample:

8.2.2.1 The # of events of interest (i.e. CD3 or other) / the # of beads acquired x #beads in tube (found on foil pouch, varies lot to lot) /test volume x dilution factor = cells/µl. The values required for this calculation are supplied by the statistical information obtained through region and gate placement during analysis.

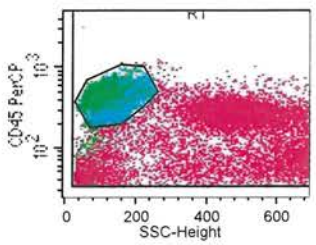
8.2.3 Results are recorded using the Flow Cytometry Worksheet or the appropriate EXEL form. All results and calculations must have a second review prior to final result entry.

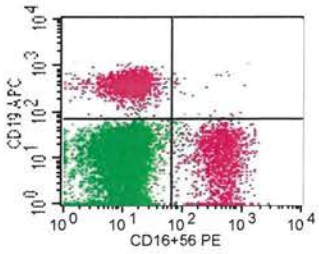
8.2.4 CellQuest Pro analysis examples:

UCB 4-color analysis template for tubes 3 and 4

R1 defines the lymphocyte population for both tubes (exploded view in tube 3). Quadrants define the CD19 and CD16+CD56+ populations in tube 3.

TUBE 3





% CD19: 18.05

% CD16+56: 16.19

Tube: tube #3
Panel: Lymphocyte markers
Acquisition Date: 27-Jul-04
Gate: G2
Gated Events: 60187
Total Events: 61776

Region	Events	% Gated
R1	11206	18.62
R2	60187	100.00
R3	7492	12.45

File: UCBA072704.003
Gate: G1
Gated Events: 11206
X Parameter: CD16+56 PE (Log)
Y Parameter: CD19 APC (Log)
Quad Location: 64, 68

Quad	Events	% Gated
UL	2023	18.05
UR	30	0.27
LL	7339	65.49
LR	1814	16.19

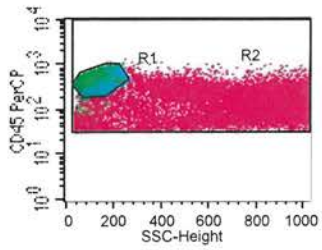
% Lymphs: 18.54

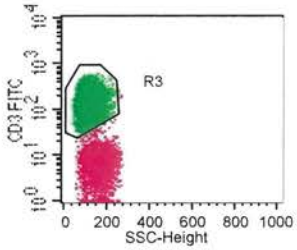
% CD3: 64.84

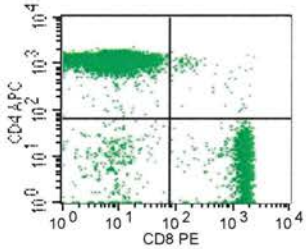
% CD4: 77.10

% CD8: 20.40

TUBE 4







File: UCBA072704.004
Gate: G2
Gated Events: 60528

Region	Events	% Gated
R1	11220	18.54
R2	60528	100.00
R3	7461	12.33

File: UCBA072704.004
Gate: G1
Gated Events: 11220

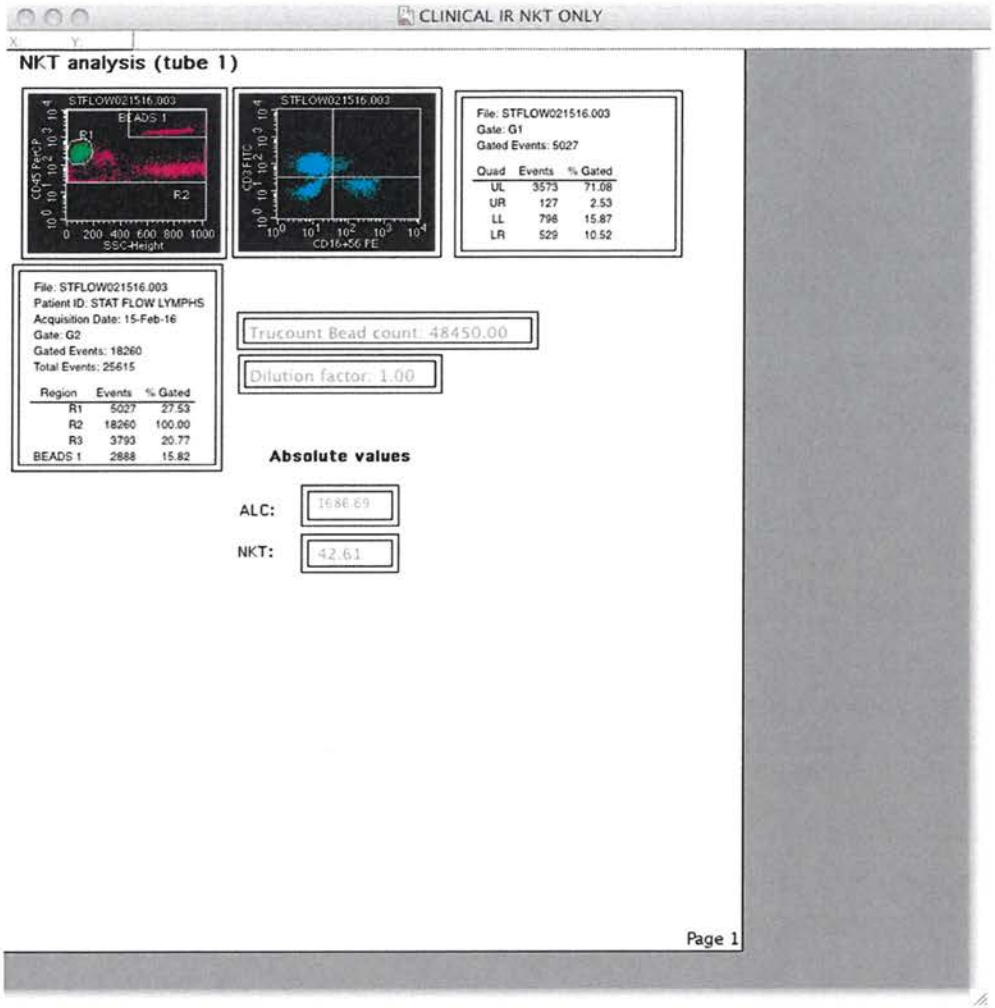
Region	Events	% Gated
R1	11220	100.00
R2	11220	100.00
R3	7275	64.84

File: UCBA072704.004
Gate: T- CELLS
Gated Events: 7275
Quad Location: 81, 63

Quad	Events	% Gated
UL	5535	76.08
UR	74	1.02
LL	256	3.52
LR	1410	19.38

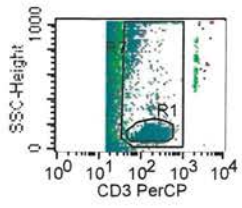
R3 defines the CD3 population and subsets CD4 and CD8 are gated off of CD3+ lymphocytes. CD4 and CD8 are separated using quadrants. **If the sum of CD3+, CD19+ NK+ expressed as % of lymphs (lymphosum) is <95% of all lymphs, then the lymphocyte gate must be reviewed for accuracy.**

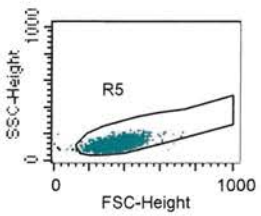
Clinical IR NKT analysis- Tube 2 (Acquired in Multiset software)

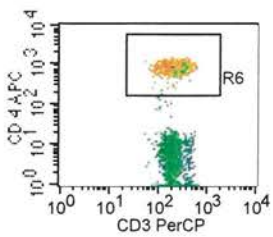


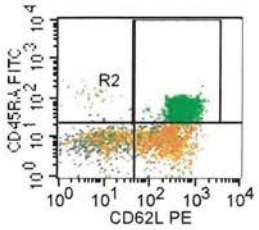
Clinical IR analysis- Tube 3 and 6

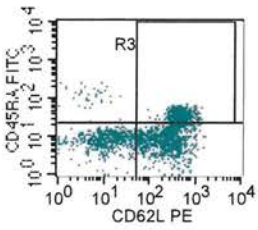
Tube 3 "RTE"











Results expressed as % CD3 cells

% CD45RA+/CD62L+ RTE cells=

Gate: G5
Gated Events: 2881

Region	Events	% Gated
R2	1042	36.17
R3	1048	36.38
R1	2881	100.00
R5	2881	100.00
R7	2881	100.00
R6	1861	64.60

Gate: G9
Gated Events: 1861

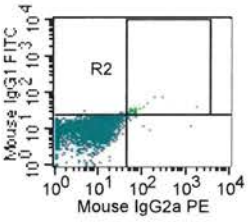
Region	Events	% Gated
R2	459	24.66
R3	463	24.88
R1	1861	100.00
R5	1861	100.00
R7	1861	100.00
R6	1861	100.00

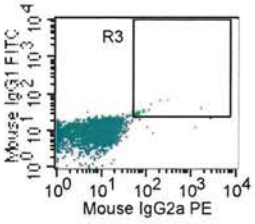
Results expressed as % CD4+ cells

% CD45RA+/CD62L+ RTE cells=

TUBE 3 - "RTE"

Isotype controls- tube 6





G5= R1 and R5
G9= R1 and R5 and R6

Gate: G5
Gated Events: 2716
X Parameter: Mouse IgG2a PE
Y Parameter: Mouse IgG1 FIT

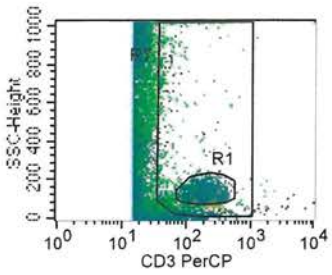
Region	Events	% Gated
R2	20	0.74
R3	18	0.66
R1	2716	100.00
R5	2716	100.00
R7	2716	100.00
R6	0	0.00

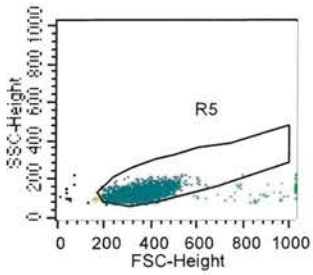
Gate: G5
Gated Events: 2716
X Parameter: Mouse IgG2a PE
Y Parameter: Mouse IgG1 FITC

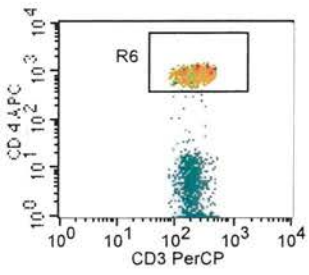
Region	Events	% Gated
R2	20	0.74
R3	18	0.66
R1	2716	100.00
R5	2716	100.00
R7	2716	100.00
R6	0	0.00

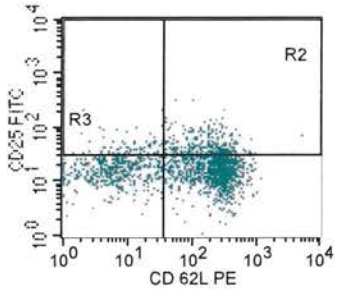
Clinical IR analysis- Tube 4 and 6

TUBE 4 - T REGS

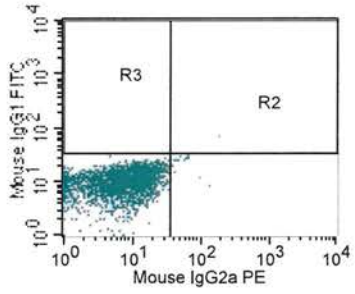








Isotype control- tube 6

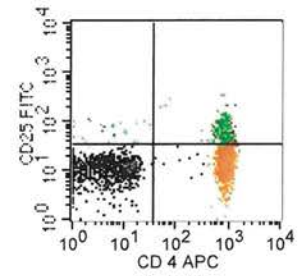


Gate: G9
Gated Events: 1810
X Parameter: CD 62L PE (Log)
Y Parameter: CD25 FITC (Log)

Region	Events	% Gated	Y
R2	243	13.43	
R3	326	18.01	
R1	1810	100.00	
R6	1810	100.00	
R7	1810	100.00	
R5	1810	100.00	

Gate: G5
Gated Events: 2527
X Parameter: Mouse IgG2a P
Y Parameter: Mouse IgG1 FI

Region	Events	% Gated
R2	1	0.04
R3	1	0.04
R1	2527	100.00
R6	0	0.00
R7	2527	100.00
R5	2527	100.00



Use the quadrants for this plot to align the R2 and R3 on the CD25/ CD62L plot to the left.

The double negative (CD8) cells should be negative for CD25 and therefore be a good background control for that marker in addition to the isotype control.

Results expressed as %CD4 cells

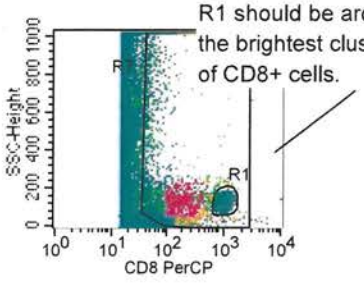
G5= R1 and R5
G9= R1 and R5 and R6

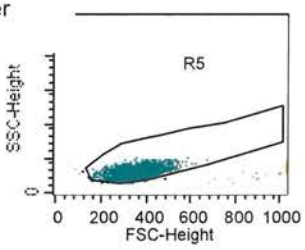
%CD25+CD62+T-Reg cells= 13.39

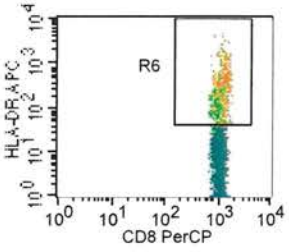
Total %CD25+ cells= 17.97

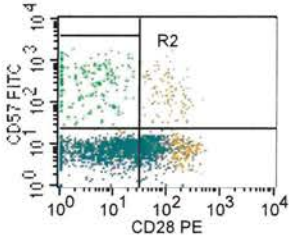
Clinical IR analysis- Tube 5 and 6

TUBE 5- "CTL"









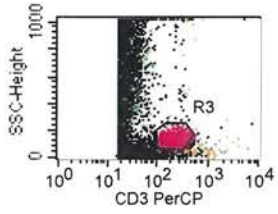
Gate: G5
Gated Events: 2183
X Parameter: CD28 PE (Log)
Y Parameter: CD57 FITC (Log)

Region	Events	% Gated	Y
R1	2183	100.00	
R2	147	6.73	4
R6	406	18.60	1
R7	2183	100.00	
R3	0	0.00	
R5	2183	100.00	

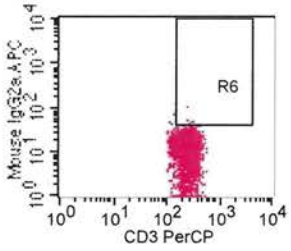
Gate: G5
Gated Events: 2183

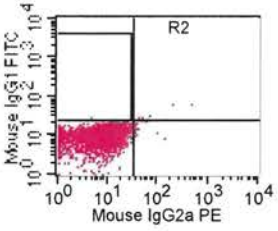
Region	Events	% Gated
R1	2183	100.00
R2	147	6.73
R6	406	18.60
R7	2183	100.00
R3	0	0.00
R5	2183	100.00

Isotype control-tube 6



Isotype control- tube 6





Gate: G6
Gated Events: 2553

Region	Events	% Gated	Y
R1	0	0.00	
R2	15	0.59	
R6	7	0.27	
R7	2553	100.00	
R3	2553	100.00	
R5	2553	100.00	

Gate: G6
Gated Events: 2553

Region	Events	% Gated
R1	0	0.00
R2	15	0.59
R6	7	0.27
R7	2553	100.00
R3	2553	100.00
R5	2553	100.00

Results expressed as % CD8 cells

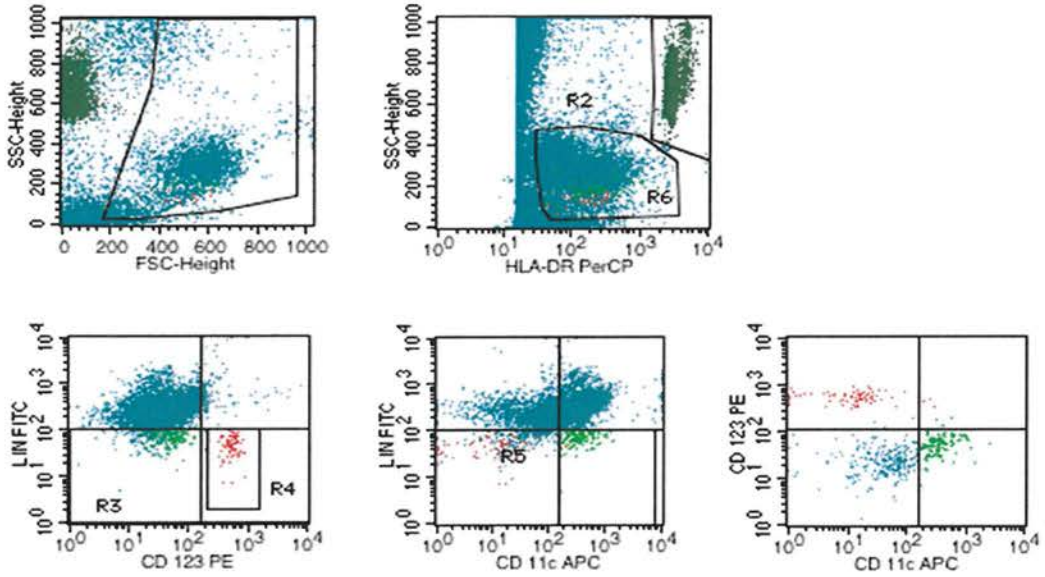
%CD57+/CD28- "CTL" cells= 6.14

%HLA-DR+ "Activated" cells= 18.33

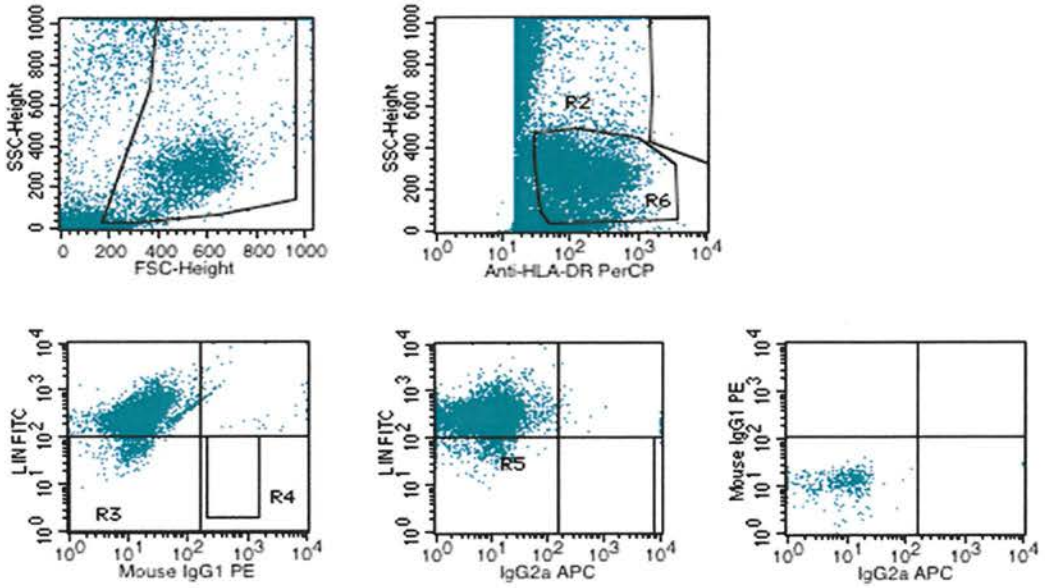
G5= R1 and R5
G6= R3 and R5

Clinical IR analysis- tube 7 and 8 Dendritic cells= Hla-DR+ Lin cocktail -

Use the scatter gate to eliminate debris. R6 defines the Hla-Dr+ population.
R4 defines the Lymphoid DC population. R5 defines the myeloid DC population.



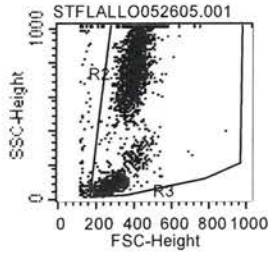
Lymphoid DC (red) = Dim Lin /123+ / DR+
Myeloid DC (green) = Dim Lin/ CD 11c+/DR+



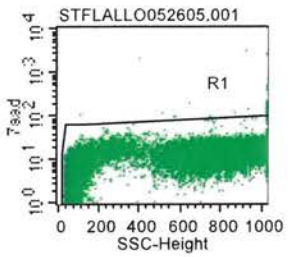
CD34 Selection (all fractions) analysis Tubes 1-5 (Lymphocyte marker analysis)
(CD34 analysis is performed using the BD SCE analysis template)

Tube 1 Isotype control. Quadrants are left in place after analysis of positive markers in tubes 2 and 3.

PLOT 1



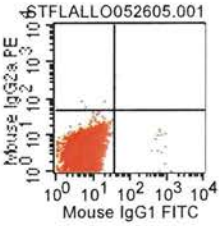
PLOT 2



File: STFLALLO052605.001
Sample ID: LOT FC 055 5/26/0
Patient ID: ST FL ALLO PANEL
Gate: G2
Gated Events: 71030
Total Events: 73245

Region	Events	% Gated	Px,Py
R1	70921	99.85	2, 5
R2	71030	100.00	1, 2
R3	21083	29.68	1, 2

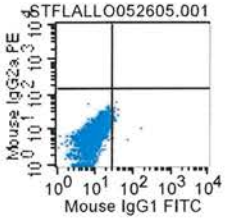
PLOT 3



Gate: G5
Gated Events: 21080
Total Events: 73245

Quad	Events	% Gated
UL	4	0.02
UR	0	0.00
LL	21063	99.92
LR	13	0.06

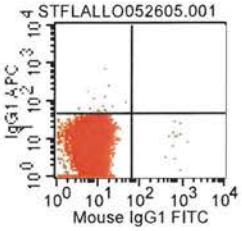
PLOT 4



Gate: G4
Gated Events: 70921
Total Events: 73245

Quad	Events	% Gated
UL	4	0.01
UR	4	0.01
LL	70218	99.01
LR	695	0.98

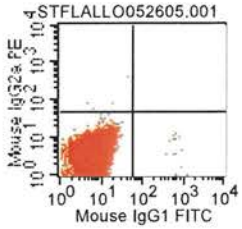
PLOT 5



Gate: G5
Gated Events: 21080
Total Events: 73245

Quad	Events	% Gated
UL	26	0.12
UR	0	0.00
LL	21041	99.81
LR	13	0.06

PLOT 6



Gate: G5
Gated Events: 21080
Total Events: 73245

Quad	Events	% Gated
UL	4	0.02
UR	0	0.00
LL	21063	99.92
LR	13	0.06

% Lymphocytes: 29.72

% CD3+CD56- : 0.06

% CD14 of lymphocytes: 0.00

% CD56+CD3-: 0.02

% CD14 of viable cells: 0.02

%CD19: 0.12

%CD45= 0.99

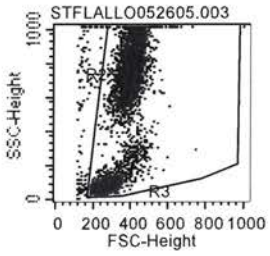
ISOTYPE CONTROL

CD34 selection Tube 2-3 Lymphocyte analysis

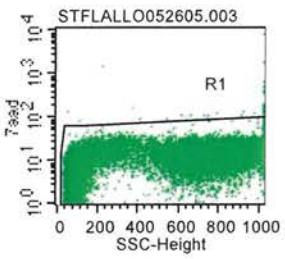
Plot 1 is ungated and shows a debris exclusion region (R2) and a lymphocyte region (R3 not clearly seen).

Plot 2 is gated off of the R2 and shows a viable cell region (R1). Plots 3, 5, and 6 are gated from viable lymphocytes. Plot 4 is gated from viable, debris–free events. Plot 3 is shown to demonstrate the level of monocyte contamination in the lymph gate (Lymph gate must be <u>≥95% pure)</u>. Plot 4 is shown to determine the % of monocytes in the sample. Plots 5 and 6 are shown to display the lymphocyte subsets % by use of quadrants placed around the negative population in the lower left quadrant.

PLOT 1



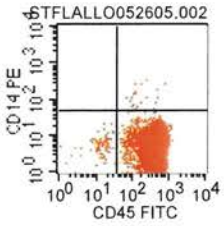
PLOT 2



File: STFLALLO052605.003
Sample ID: LOT FC 055 5/26/0
Patient ID: ST FL ALLO PANEL
Gate: G2
Gated Events: 101081
Total Events: 104356

Region	Events	% Gated	Px,Py
R1	100933	99.85	2, 5
R2	101081	100.00	1, 2
R3	29498	29.18	1, 2

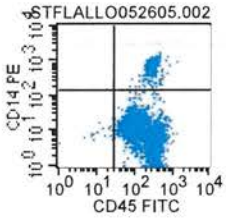
PLOT 3



Gate: G5
Gated Events: 29800
Total Events: 104212

Quad	Events	% Gated
UL	0	0.00
UR	34	0.11
LL	90	0.30
LR	29676	99.58

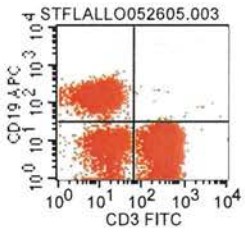
PLOT 4



Gate: G4
Gated Events: 100625
Total Events: 104212

Quad	Events	% Gated
UL	0	0.00
UR	4108	4.08
LL	490	0.49
LR	96027	95.43

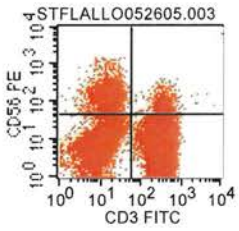
PLOT 5



Gate: G5
Gated Events: 29494
Total Events: 104356

Quad	Events	% Gated
UL	4318	14.64
UR	31	0.11
LL	2773	9.40
LR	22372	75.85

PLOT 6



Gate: G5
Gated Events: 29494
Total Events: 104356

Quad	Events	% Gated
UL	1958	6.64
UR	537	1.82
LL	5122	17.37
LR	21877	74.17

% Lymphocytes: 29.58

% CD3+CD56- : 74.17

% CD14 of lymphocytes: 0.11

% CD56+CD3-: 6.64

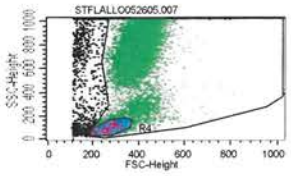
% CD14 of viable cells: 4.08

%CD19: 14.75

%CD45= 99.51

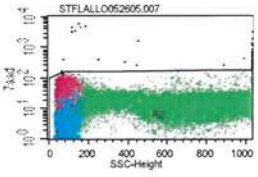
CD 34 selection CD4 CD25 Analysis of tubes 4 and 5: T-Regulatory cell analysis

The CD25 FITC/SSC plot is gated from the CD4+ (R3) viable lymphocytes (R2 and R4). The %CD25 is then corrected to reflect the %lymphocytes by dividing the number of CD25+ events in this gated plot by the number of viable lymphocyte events. CD 3+4+ events are defined in the upper right plot and CD3+8+ events are defined in the lower left plot. As described in the template, an irrelevant population in tube 3 (B-cells) are used to establish a background level for CD25+ events.



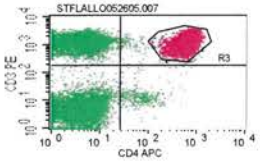
File: STFLALLO052605.007
Sample ID: LOT FC 055 5/26/05
Patient ID: ST FL ALLO PANEL
Gate: DEBRI FREE
Gated Events: 100431

Gate	Events	% Gated
CD4+ T-CELLS	15270	15.20
LYMPHS	30168	30.04
VIABLE	100355	99.92
DEBRI FREE	100431	100.00
G5	100355	99.92



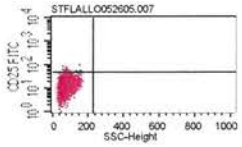
File: STFLALLO052605.007
Gate: LYMPHS
Gated Events: 30237

Region	Events	% Gated
R3	15315	50.65
R1	30168	99.77
R2	30237	100.00
R4	30237	100.00



File: STFLALLO052605.007
Gate: LYMPHS
Gated Events: 30237

Quad	Events	% Gated
UL	7243	23.95
UR	15444	51.08
LL	7256	24.00
LR	294	0.97



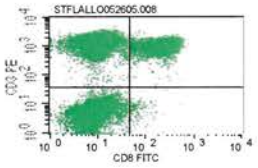
File: STFLALLO052605.007
Sample ID: LOT FC 055 5/26/05
Patient ID: ST FL ALLO PANEL
Gate: CD4+ T-CELLS
Gated Events: 15315
Quad Location: 226, 50

Quad	Events	% Gated
UL	121	0.79
UR	0	0.00
LL	15194	99.21
LR	0	0.00

CD3+CD4+= 51.08

CD3+CD8+= 20.14

CD4+CD25+= 0.40



File: STFLALLO052605.008
Gate: LYMPHS
Gated Events: 30741

Quad	Events	% Gated
UL	17076	55.55
UR	6192	20.14
LL	7342	23.88
LR	131	0.43

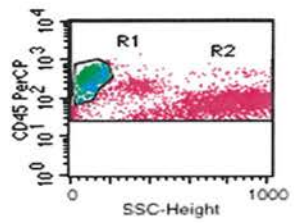
viability= 99.92

To set background for Fl-1 vs SS plot, change the data file to tube 3 and place R3 around the CD19+ populaton. Set the quadrants for the Fl-1 background to <1% in UL quadrant based on this gate.

DLI or T-Cell subset analysis (CD3,4,8 analysis template)

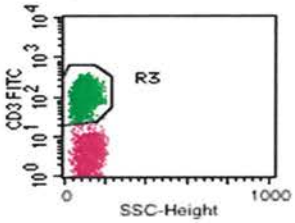
Adjust regions as shown. Use the % lymphs, CD3, CD4, and CD8 results obtained to calculate total values in the calculation section of the flow worksheet.

T-CELL AND T-CELL SUBSETS



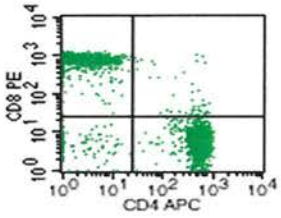
File: UCBA
Sample ID:
Patient ID:
Gate: G2
Gated Events: 24884

Region	Events	% Gated
R1	10220	41.07
R2	24884	100.00
R3	6356	25.54



File:
Gate: G1
Gated Events: 10220

Region	Events	% Gated
R1	10220	100.00
R2	10220	100.00
R3	6309	61.73



File:
Gate: T- CELLS
Gated Events: 6309
Quad Location: 25, 24

Quad	Events	% Gated
UL	2187	34.66
UR	104	1.65
LL	174	2.76
LR	3844	60.93

% Lymphs: 41.07

% CD4: 62.58

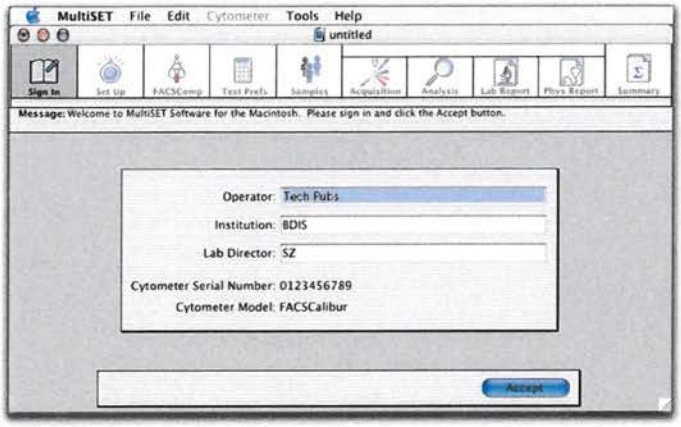
% CD3: 61.73

% CD8: 36.31

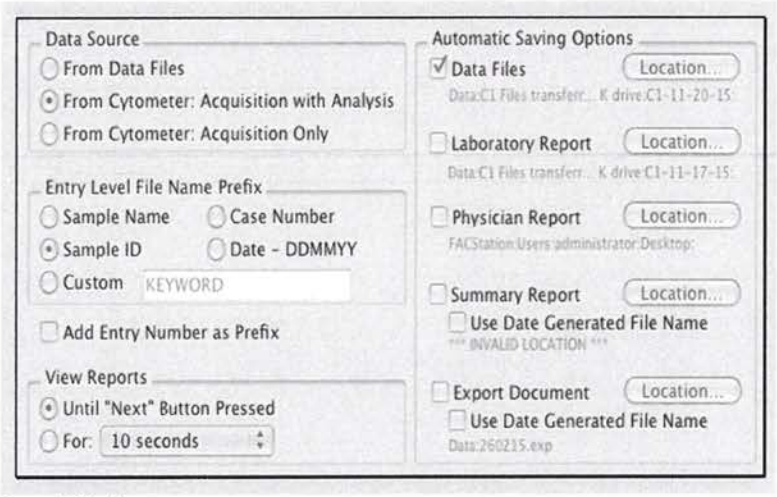
8.3 IR Panel Acquisition/Analysis of Tube 1 (CD3/CD4/CD45/CD8) and Tube 2 (CD3/NK/CD45/CD19) using BD Multiset Software:

8.3.1 Launch multiset from dock

8.3.2 At Sign in enter operator name or initial then click accept.



Sign in Window



Setup Window

8.3.3 Data source set to: From Cytometer Acq. with Analysis

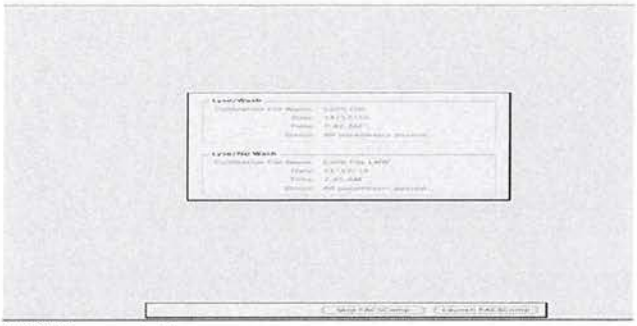
8.3.4 If performing an analysis from previously acquired data then the choice would be "From Data files".

8.3.5 Entry level file name prefix-Sample ID

8.3.6 View reports Until "Next" Button Pressed

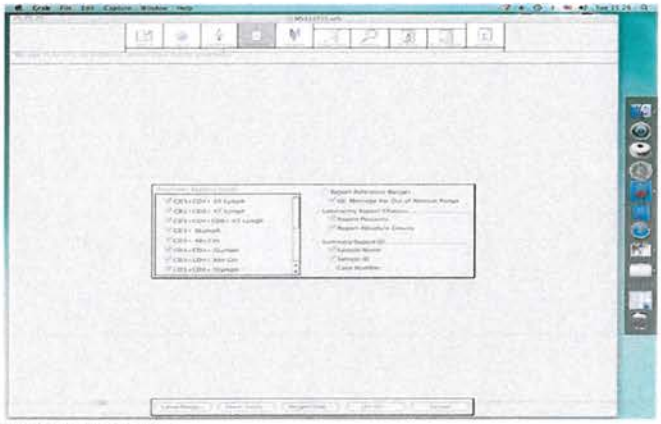
8.3.7 At Data Files, Click on Location and browse to storage local (i.e. Data drive).

8.3.8 Leave all other boxes unchecked and click "ACCEPT".



FACSComp window

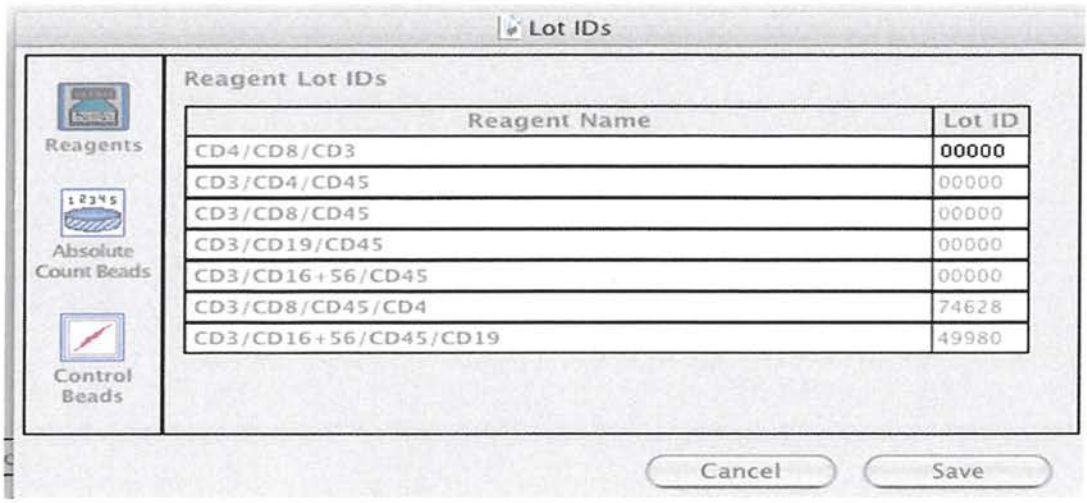
8.3.9 Skip FACSComp if already performed.



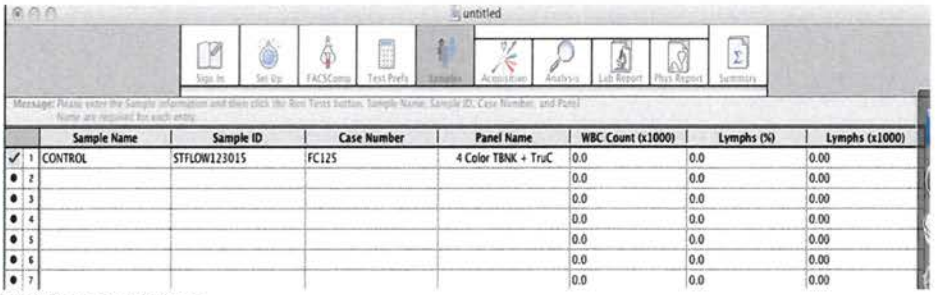
Test Prefs window

8.3.10 Check lot ids by clicking on the lot ID tab at bottom of window.

8.3.11 Choose either the Reagent or absolute count bead icons (located on left side of window) to enter/or check that the correct lot info is entered.



8.3.12 Click SAVE then ACCEPT.



Test Sample window

8.3.13 Prior to entering sample information, go to the cytometer menu and scroll to instrument settings to verify that the appropriate LNW settings are installed in cytometer.

(If running test from stored data, click Add Samples and browse to file to be run, click add, and run test.)

8.3.14 SN= control or Patient name

8.3.15 SI=Data file name according to standard practice (i.e. MREEPBIR

8.3.16 Case number: Lot number for control/ Hist. # for pts or bar code for product.

8.3.17 Panel name= 4-color-TBNK+ Trucount.

8.3.18 Do not enter anything into the last 3 columns.

8.3.19 Click RUN TESTS.

8.3.20 Begin sample acquisition by installing tube with CD4 and CD8.

8.3.21 Insure sample is running appropriately with no fluidics troubleshooting required.

8.3.22 Click on "Acquire" (bottom right button).

8.3.23 After first tube is acquired, choose manual gate if needed to adjust regions. (See MS Help under "Setting Manual Gate")

8.3.24 Select the Analyze button at bottom to see result of manual gating.

8.3.25 When the tube is analyzed to user satisfaction, select Continue.

8.4 Load next tube (contains CD19 and NK) and select Acquire.

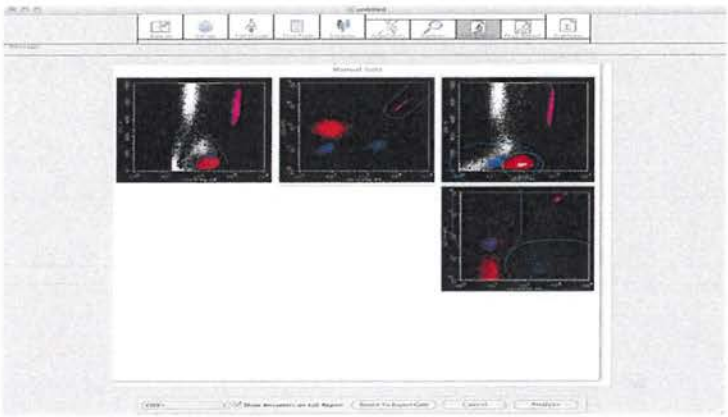
8.4.1 Repeat analysis steps as above.

8.4.2 Continue to print result.

8.5 If the sample was diluted prior to staining, the resulting cells/µl must be multiplied by the dilution factor (DF) on final report. **It is critical that the DF be recorded properly on the Flow Cytometry Worksheet.**

8.6 Multiset examples screenshots:

MS acquisition screen



MS analysis screen

NOTE: If the sum of CD3+, CD19+ NK+ expressed as % of lymphs (lymphosum) is <95% of all lymphs, then the lymphocyte gate must be reviewed for accuracy.

Table 1 LNW Test acquisition templates and settings

Test Sample	Acquisition template(s)	Instrument Settings	Threshold Parameter Setting posted at cytometer
Pediatric PBSC CD34 Peripheral blood CD34 Auto Bone Marrow CD34 Auto UCB CD34	*BD SCE CQ Pro	BD SCE	FL-1
UCB infusion Allo Bone Marrow Adult Allo PBSC	*BD SCE CQ Pro /4-color Acq.(skip tube 1)	BD SCE / Calib File.LNW**	FL-1 / FL-3
Immune reconstitution	**BD Multiset** TBNK + Truc for MT tube 1-2 **BD CellQuest Pro** Clinical IR Acq. MT tube 3 Clinical IR ACQ. Tube 4-8	Calib File.LNW** (MT reagent tubes) LNW settings** Ins. Settings-IR Panel 4-8 (tube 4-8)	FL-3
CD34 selection	CD 34 Sel. Acq. /Adult Allo Acq	Ins. Settings-7aad-No Wash	Forward Scatter
NK cell selection	NK sel. Acq	Ins. Settings- No Wash -7aad	Forward Scatter
Fresh umbilical cord blood	*BD SCE CQ Pro / 4-color Acq	BD SCE / Calib File.LNW**	FL-1 / FL-3
Donor Lymphocyte infusion (DLI)	4-color acq. (skip tube 1)	Calib File.LNW**	FL-3
Other studies	Designated study template	Designated settings	Designated TH

*Refer to SOP FLOW-GEN to use the BD SCE CQ Pro acquisition/analysis template. It is listed in this table for reference only as CD34 testing is often combined with other testing performed using Lyse/No Wash methods.
** If Calib file (LNW) FACSComp settings are optimized on a given day, the OPT setting should be used for test acquisitions when applicable.

Legend:

PBSC Peripheral Blood Stem Cells; **UCB** Umbilical Cord Blood ; **NK** Natural Killer
IR Immune Reconstitution; **Acq** Acquisition; **Allo** Allogeneic

Acquisition stopping rules are built into the templates. Generally 300,000 events are acquired, when possible, for CD34 testing depending on sample type. 5-10,000 lymphocyte events should be acquired. The immune reconstitution panel has stopping rules defined for each tube based on number of events or time limit. For acquisitions that do not have a designated acquisition end time and the event count stopping rule is not met, the acquisition may be stopped after 5 min and saved, unless the user has been specifically directed to continue until tube volume is depleted.

9 RELATED DOCUMENTS/FORMS

9.1 FLOW-GEN-007 Lyse No Wash Staining For Flow Cytometry

9.2 FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet

9.3 EXCEL Reporting Forms

9.3.1 FLOW-FORM-001 Adult Allogeneic Donor PSC Lymphocyte Subset Results

9.3.2 FLOW-FORM-003 Flow Cytometry Results

9.3.3 FLOW-FORM-004 Adult PSC CD34 Selection Product Lymphocyte Subset Results

9.3.4 FLOW-FORM-005 Immune Reconstitution

9.4 BD MultiSET Help menu

10 REFERENCES

10.1 Becton Dickinson CellQuest Pro Software User's Manual, v. 5.1

10.2 D. Robert Sutherland, et. al. The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. Journal of Hematotherapy 5:213-226(1996)

10.3 Szabolcs, Paul, et.al. Absolute Values of Dendritic Cell Subsets in Bone Marrow, Cord Blood and Peripheral Blood enumerated by a Novel Method. STEM CELLS 2003; 21:296-303 www.StemCells.com

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
09	M. Reese	Made corrections to SOP titles in section 9. Changed SOP title

Signature Manifest

Document Number: FLOW-GEN-009 **Revision:** 09
Title: Acquisition and Analysis of Lyse-No Wash Samples by Flow Cytometry
Effective Date: 19 Oct 2020

All dates and times are in Eastern Time.

FLOW-GEN-009 Acquisition and Analysis of Lyse-No Wash Samples by Flow Cytometry

Author

Name/Signature	Title	Date	Meaning/Reason
Melissa Reese (REESE008)		29 Sep 2020, 03:43:53 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		29 Sep 2020, 05:26:19 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		29 Sep 2020, 06:34:58 PM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
Isabel Storch (IMS19)		30 Sep 2020, 12:43:15 PM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		09 Oct 2020, 07:13:36 PM	Approved

Quick Approval

Approve Now

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		09 Oct 2020, 08:16:06 PM	Approved