STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: FLOW-GEN-012 FRM5

DOCUMENT TITLE:

Stem Cell Laboratory Flow Cytometry Worksheet FRM5

DOCUMENT NOTES:

Document Information

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Control Information

Author: MGREESE	**Owner:** MGREESE
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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 and/or Recipient Demographic Info) NA	**Bar Code label NA** ***(Attach bar code label HERE)*** **UCB LAB ID:_____**
If NMDP Product: ☐ **OUTGOING** ☐ **INCOMING** ☐ **NA**	**Recipient Weight NA** _____**kg**

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_____@_____ **NA**

Date / Time Product Sampled for tests_____@_____By_____ **NA**

Cell Concentration _____x10^6/mL Product volume _____ mLs

To be completed by STCL flow cytometry staff for stem cell product collections:

Date/Time stained_____@_____BY_____ Dil. Factor X _____
For Banked UCB: Sample stained within 4hr of sampling time √ **tech/date:**_____/_____ **NA**

√ if used	Product used or flow test request	Vendor / reagent name	Lot #	Expiration date (Original)		√ QC Pass?
	RBC Lysing solution	BD/ FACS Lyse Solution				
	RBC Lysing solution	BD/Pharm Lyse				
	SCE	BD/Stem Cell Enumeration kit			SCE TC Bead #	
	SCE Trucount tube	BD	Included in SCE kit			
	CD3/CD16+56/CD19/CD45	BD / Multitest				
	CD3/CD4/CD8/CD45	BD / Multitest				
	Immune Reconstitution ☐ Full ☐ Tube 1 only	BD/Listed in staining procedure	Record the BD Multitest (T, B, NK, and T subset) lot info in spaces above.			
	Adult allo additional markers	BD/Listed in staining procedure	Found on reagent QC form completed on the day of testing			

Additional info or comments:_____

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BD Trucount tube information (if used): NA
Multitest
BD Trucount lot #_____Exp. Date_____

Bead count _____

DATA COLLECTION: Enter only once if the data file name is the same for all tests within the panel.

Test Request	Instrument used / Date Acquired	DATA FILE NAME	Acquired. by	DATE ANALYZED (if different)	Analyzed by

BD Trucount tube QC check: Use the automated analyzer WBC vs. the SCE assay viable CD45+ cells/µl ($x10^3$) to perform the following calculation on testing of fresh blood sources. Not required for thawed product testing. Low value/ High value
_____/_____ = _____ **IF the result is < 0.85 (<0.75 for UCB/BM) repeat testing. NA**

(Report only Viable CD34+cells/µl from mobilized peripheral blood testing)

BD SCE assay calculation: NA

___ ___ ___ ___ . ___ ___ x _____ / 1000 = ___ ___ ___ ___ . ___ ___ ($x10^6$)
Viable CD34+cells/µl **volume (mls)** **total viable CD34+ cells**

/ _____ Kgs. = ___ ___ ___ . ___ ___ **x 10^6 viable CD34+cells/kg** **Tech**_____
Recipient Wt

Round the final values to at least two decimal places.
T-cell and T-cell subset calculations: Total WBC calc. = Cell conc. x volume (mLs) / 1000 = Total WBC ($X10^9$) NA

___ ___ ___ . ___ ___ $X10^9$ x_____x_____=_____$x10^6$ / _____kgs = _____$x10^6$
Total WBC % Lymphs %CD3 Total CD3+ Recipient Wt **CD3+/kg**

_____$x10^6$ / _____$x10^6$ x 100 =_____**%CD3**
Total CD3 Total WBC

_____x _____$x10^6$=_____**x10^6**
%CD3+CD4+ Total CD3+ **Total CD3+4+**
_____x _____$x10^6$=_____**x10^6** **Tech**_____
%CD3+CD8+ Total CD3+ **Total CD3+8+**

Complete if calculation is performed or if result of testing is verbally reported: NA

Calculation Verified by_____**Date**_____**@**_____
Result reported to_____**By**_____**Date**_____**@**_____

Note any unusual result or lab error that could affect patient management:
_____
_____
_____

EPIC BEAKER (*or equivalent*) data entry by_____**Date**_____**@**_____ NA

Work Reviewed by: _____**Date**_____**@**_____

Instructions for Filling out Stem Cell Laboratory Flow Cytometry Worksheet

One worksheet should be completed for each flow cytometry sample being tested.

In the field...	Information
Accession #:	Enter Accession # (*if applicable*). Fresh UCB units from the CCBB Program do NOT require an accession #.
Sample ID	Attach printed label or write in the ID.
Bar Code label	
Recipient wt.	Weight in kilograms of recipient *if applicable*.
NMDP Outgoing or Incoming	Put a check in the appropriate box *if applicable*.
Sample classification:	Check the product classification that applies for the sample to be tested or fill in description in "other".
Sample source/condition	Check the source and circle the condition that applies. Use "Other" to record collection number or other sample source.
Date/Time PB or Product collected:	Enter the date/time of collection (*if applicable*).
Date/Time Sampled/by	Enter the date/time the flow sample was drawn from the original product and tech initial *(if applicable)*
For Banked UCB 4hr. time check	Staining tech must verify that the time between sampling and staining is within 4hrs of one another by tech initial and date.
Cell concentration:	Enter the SYSMEX (*or equivalent*) derived cell concentration for the sample to be tested at per million/mL concentration (x 10^6/ml).
Vol. of product:	Enter the volume in milliliters (mLs) of the product to be tested (*if applicable*).
Date/Time Stained	Enter the date and time of flow staining.
Dil. Fac.	Enter a dilution factor if the sample needs to be diluted or write 1 if no dilution is needed. Some samples may arrive diluted from the count tube; if further dilution is required, only enter the final staining dilution.
Flow testing and Reagent used box	Check all that apply and enter the lot # information and QC Check result
Additional info or comments:	Note any reagents used to stain that are not listed above or comments related to test preparation, etc.
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Multitest Trucount tube: If applicable.	
Trucount (TC) lot#:	Enter the TC bead lot information found on the bag currently in-use.
Trucount (TC) bead count:	Enter the bead count from the corresponding TC bead tube bag in use.
Exp. date	Enter the date of expiration found on the TC bead bag.
Data Collection:	To be entered at the time of acquisition and analysis.
Test Request	Enter the test panel name (i.e. UCB, IR, CD34)
Instrument used/date of acquisition	Enter the designation for the instrument used to acquire the sample and the date of acquisition
Data file name	Enter the data file name
Acquired by	Enter the initials of the technologist performing Acquisition
Date analyzed if different	If the data analysis date is different from the date of acquisition, enter that date.
Analyzed by	Enter the initials of the technologist performing analysis of the acquired data.
Trucount tube QC:	Determine the difference between the white blood count obtained from the automated hematology analyzer (x10^3µl) and the absolute viable CD45+ cells (x10^3µl)

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In the field...	Information
	obtained from the SCE Trucount tube analysis. If the result of the calculation (low/high values) is **< 0.85 for fresh PB or PBSC products or < 0.75 for fresh UCB or bone marrow, the testing must be reviewed and testing must be repeated if discrepancy is deemed to be due to staining error. This calculation is not required for thawed test samples due to potentially low WBC viability.**
Calculations: If applicable	Some calculations in this section may not be applicable for the sample type. Flow software or Excel forms are used to perform some flow result calculations. In these cases, circle *NA*.
BD SCE Calculation:	
Viable CD34+cells/µl	Enter the viable CD34+ cells/ µl result from the SCE assay analysis.
Volume (mls)	Enter the number of milliliters (mls) in the product.
Total viable CD34+cells	Calculate this value from previous info and record as $x10^6$.
Kgs.	Enter the recipient's weight in kilograms (kgs).
Total CD34+cells/kg	Calculate and report viable CD34+cells/kg $x10^6$
Tech:	Initials of the technologist performing the calculation.
T-cell and T-cell subset calculations	**Use for UCB reinfusion and DLI products.**
Total WBC:	Result obtained by taking the "cell concentration x volume" expressed as " $x10^9$ "
% Lymphs:	Enter % lymph value obtained from the T-lymphocyte analysis Tube 4.
% CD3	Enter %CD3 value obtained from T-lymphocyte analysis Tube 4.
Total CD3+:	Calculate by using the information provided above along with the formula on the form expressed as $x10^6$
Wt. (kg):	Enter the recipient's weight expressed as kilograms (kgs).
CD3/kg	Calculate using the information provided expressed as $x10^6$/ kilogram.
Total CD3:	Obtained from previous calculation.
Total WBC:	Obtained from previous calculation but expressed $x10^6$
% CD3	Calculated as a % of the total white cells.
%CD3+CD4+:	Enter the % of CD3+CD4+ cells from Multitest tube data analysis.
Total CD3+:	Obtained from calculated value above.
Total CD3+4+:	Calculated from previous information expressed as $x10^6$
%CD3+8+:	Enter the %CD3+CD8+ cells from Multitest tube data analysis.
Total CD3+8+:	Calculated from previous information expressed as $x10^6$
Calculation Verified by/Date/Time (if applicable)	Verifier should record initials, the date, and time information was verified.
Results reported To/By/Date/Time:	Enter the initials or other identifier of the person receiving the verbal report or note that a message was left (include to whom along with the date and time the message was left).
Note...:	Record any pertinent flow-related comments or information regarding on this line.

In the field...	Information
EPIC BEAKER data entry By/Date/Time	If these results will be entered in EPIC BEAKER or equivalent LIS (Laboratory Information System), the technologist who performs the data entry must initial and record the date and time of the entry. Data entry may not be required depending on the specimen being tested. If data entry is NOT required, circle ***NA***.
Work Reviewed by / Date/ Time	The reviewer must ensure that there are no omissions and that the information on the worksheet is complete and accurate. The reviewer must also ensure the results are complete and accurate and that the final results are accurately recorded as required by the type of testing. The reviewer should include initials or name, the date, and the time of review. This review should be performed after EPIC BEAKER data entry is complete, if required, so this serves as the FINAL document review. The review of worksheets completed for fresh umbilical cord blood samples must be performed by a second technologist who did not complete the worksheet initially. The final reviewer is responsible for gathering any the missing information whenever possible. If the reviewer is unsuccessful in gathering the missing information, notify the Flow Cytometry Supervisor or Laboratory Manager so they can assist you in this effort.

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Signature Manifest

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