Accurate Enumeration of CD34+ Cells with the BD® Stem Cell Enumeration Kit on the BD FACSLyric™ Flow Cytometer

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Introduction

Hematopoietic stem cells (HSCs) are CD34+ and are responsible for engraftment in the bone marrow transplant setting. Enumerating CD34+ HSCs in peripheral blood, leukapheresis and cord blood samples provides critical information to the transplant physicians. The number of viable CD34+ cells present in the peripheral blood after mobilization and/or chemotherapy predicts the yield of CD34+ cells in the leukapheresis product. Additionally, the number of CD34+ cells collected predicts time to engraftment after transplantation. The infusion of a minimum of two million viable CD34+ cells per kilogram patient weight generally enables rapid (10–12 days to 500 neutrophils/μL) and sustained engraftment in the auto-transplant setting. The International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol for CD34+ cell enumeration is the most widely used flow cytometric method in clinical laboratories. BD Biosciences has developed an algorithm for the BD FACSLyric™ Flow Cytometer that closely follows the ISHAGE protocol. This study was performed to demonstrate the accuracy of the new BD Stem Cell Enumeration Assay algorithm used to enumerate CD34+ HSCs.

Materials and Samples

BD® Stem Cell Enumeration Kit
BD® Stem Cell Control
BD® CS&T RUO Beads
BD® FC Beads 7-Color Kit
BD FACSLyric™ Flow Cytometer
BD FACSuite™ Clinical Application
BD Stem Cell Enumeration Assay module
BD FACSDiva™ Software
BD CellQuest™ Pro Software

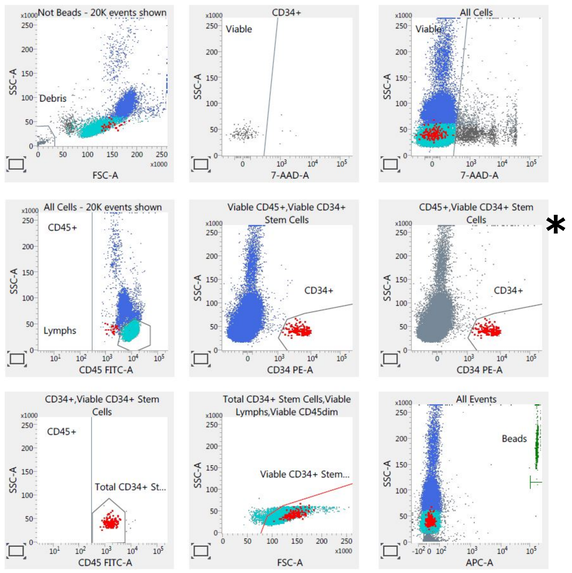
Samples (50 total):
- 15 leukapheresis
- 15 mobilized leukapheresis
- 15 cord blood
- 2 bone marrow
- 3 normal peripheral blood

Method

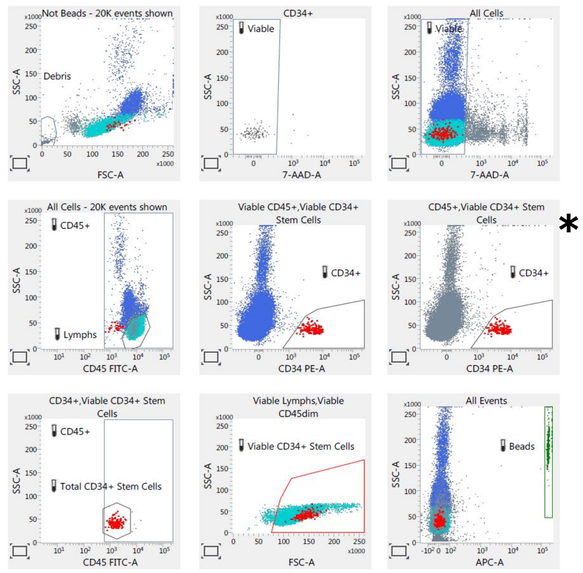
The samples were stained using the BD® Stem Cell Enumeration Kit. The stained samples were acquired on the BD FACSLyric™ Flow Cytometer using BD FACSuite™ Clinical Application. The cytometer was set up using BD® CS&T RUO Beads for daily performance quality control, BD® FC Beads and stained BD® Stem Cell Control for automated compensation.

Gating with BD FACSuite™ Clinical Application

Manual, BD Experts (A)

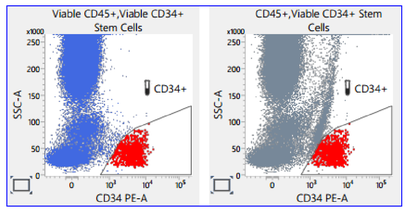


New Algorithm (B)



The BD template includes additional **SSC-A vs. CD34 PE-A** plots (*) that show the total rather than viable CD45+ cells. The total plot displays the same CD34+ gate, linked to the gate in the viable plot.

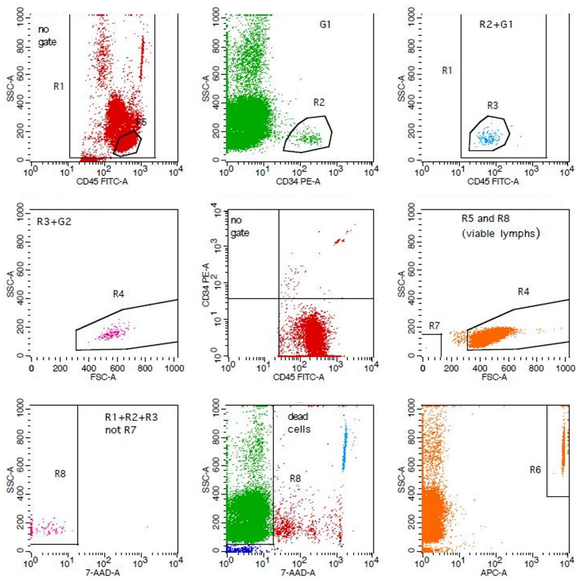
The additional plot for total CD45+ helps to exclude non-stem cell particles (e.g., platelets), which can appear as a larger population in the CD45+ total plot than is represented in the viable CD45+ —see the example (right). This provides a means to ensure that results for total stem cell count and for stem cell viability are more robust, especially for bone marrow samples.



Some of the algorithmic gates have a different construct from the traditional manual gates, but these produce results that are statistically equivalent to the manual results, as demonstrated by the regression analysis.

Gating with BD CellQuest™ Pro

Manual, Academic Expert (C)



Correspondence between "R" regions and populations

Label	Definition
Viable CD45	R1 and R8
G2 (viable CD34+)	R2 and "viable CD45"
G3 (viable CD34+ and CD45dim)	R3 and G2
Viable stem cells	R4 and G3
Beads	R6
Total stem cells	R1 and R2 and R3
Viable lymph	R5 and R8
Total CD45	R1 and not beads
Debris	R7

Analysis and Results

Acquired samples were analyzed in parallel using three different methods. They were gated:

A. Manually by BD experts, using BD gating templates (BD internal use) in BD FACSuite™ Clinical Application
B. Automatically by the new BD Stem Cell Enumeration Assay algorithm used in BD FACSuite™ Clinical Application
C. Manually by an academic expert, using well-established sequential gating criteria and BD CellQuest™ Pro Software

Each analysis produced cell counts for:

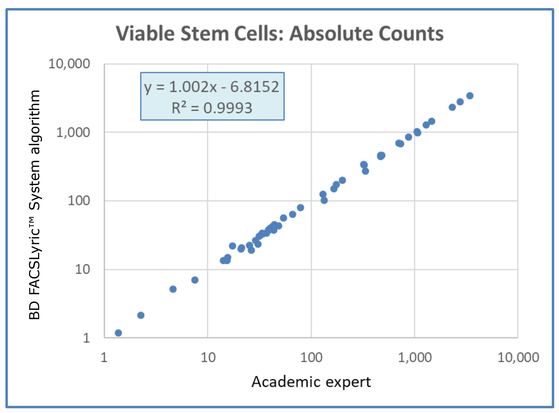
- Viable stem cells (CD34+)
- Total stem cells (CD34+)
- Viable CD45+ Cells
- Total CD45+ Cells

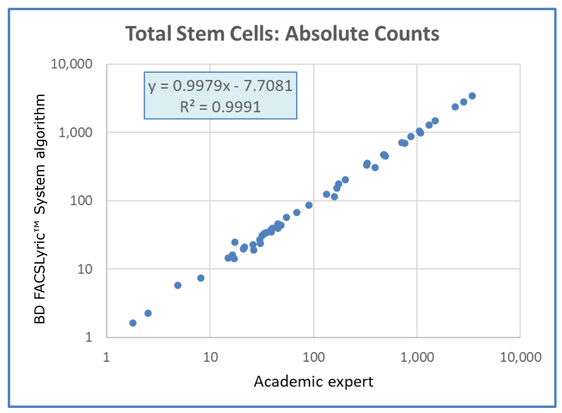
The cell counts derived by the three methods were compared using regression analysis.

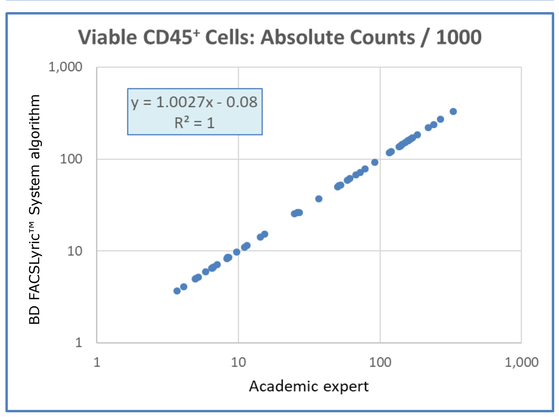
	Method A/B vs. Method C (Academic Expert)			
	Method A BD Manual Results		**Method B** BD Stem Cell Enumeration Assay algorithm used in BD FACSuite™ Clinical Application	
	Av. Bias	St. Dev.	Av. Bias	St. Dev.
Viable SCE	-2.1%	6.3%	-3.6%	7.8%
Total SCE	-1.8%	8.4%	-3.9%	9.7%
Viable CD45+	0.5%	0.9%	0.5%	0.8%
Total CD45+	-0.1%	0.1%	0.1%	0.2%

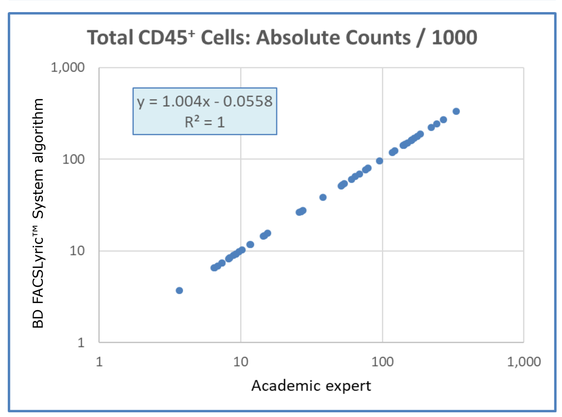
The matched data show excellent concordance between all three methods (**R^2 = 0.99**). Data derived from the algorithm generated slightly lower stem cell counts than BD experts, who in turn generated slightly lower counts than the academic expert. However, the differences were all below 5% for viable and total stem cells and less than 1% for viable and total CD45+ cells.

Regression plots: BD® Stem Cell Enumeration Assay vs. academic expert









Conclusion

The results demonstrate that the BD® Stem Cell Enumeration Assay algorithm used in BD FACSuite™ Clinical Application on the BD FACSLyric™ System accurately enumerates CD34+ cells.