Multi-site evaluation of the BD® Stem Cell Enumeration Kit for CD34 cell enumeration on BD FACSLyric™ and BD FACSCanto™ II Flow Cytometers.

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Abstract

Hematopoietic stem cells (HSCs) are CD34+ cells and can differentiate in different mature blood cells (erythrocytes cells, platelets, and leucocytes blood cells). Transplantation of HSCs is an established procedure to treat malignant and non-malignant diseases by replacing or rebuilding a patient's hematopoietic system. Flow cytometric enumeration of CD34+ HSCs has been employed as a means to assess HSC transplantation products. CD34+ cell viability correlates with HSC engraftment.

This study quantitatively determined the CD34+ HSCs in absolute counts and as a percentage of leukocytes in fresh (whole blood, mobilized blood) and fresh and thawed specimens (cord blood (CB) and leukapheresis (LP), bone marrow) using the BD® Stem Cell Enumeration (SCE) Kit on the BD FACSLyric™ Flow Cytometer (FC) with BD FACSuite™ Clinical Application utilizing the modified International Society of Hematotherapy and Graft Engineering (ISHAGE) gating strategy for acquisition and analysis.

Four sites enrolled and tested different types of specimens. Samples that provided compliant results (n = 501) were included in the analysis.

Methods

Instrument
BD FACSLyric™ Flow Cytometer

Assay
BD® Stem Cell Enumeration Kit with BD Trucount™ Tubes

Table 1. Enrolled Specimens

Specimen Type	Target	Current
Normal Peripheral Blood (NPB)	40	48
Mobilized Peripheral Blood (MPB)	60	63
Fresh Cord Blood (FCB)	56	61
Thawed Cord Blood (TCB)	56	71
Fresh Bone Marrow (FBM)	52	60
Thawed Bone Marrow (TBM)	52	61
Fresh Leukapheresis Product (FLP)	60	68
Thawed Leukapheresis Product (TLP)	60	69
Total	436	501

Table 2. Viable CD34+ Bin Target and Current Enrollment

Viable $CD34^+$ Absolute Count (cells/µL)	Target	Current
0 - 10	60	100
>10 and ≤100	130	242
>100 and ≤500	85	134
>500 and ≤1000	25	25
Total	300	501

Table 3. Anticoagulant Type

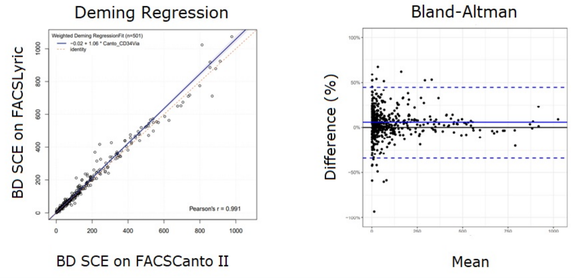
Anticoagulant Type	Total
EDTA	202
Heparin	21
ACD-A	87
CP2D	60
CPD	49
CPDA	5
Mix (Heparin + ACD-A)	77
Total	501

Results – Viable CD34+ and CD45+ Absolute Counts

Table 4. Deming Regression Summary

Parameter	R^2	Intercept (95% CI)	Slope (95% CI)
Viable CD34+ Absolute Count	0.983	-0.02 (-0.14, 0.10)	1.06 (1.04, 1.08)
Viable CD45+ Absolute Count	0.989	1.2 (-27.43, 29.84)	1.00 (0.99 1.01)
Total CD34+ Absolute Count	0.977	-0.03 (-0.23, 0.16)	1.03 (1.01 1.05)
Total CD45+ Absolute Count	0.987	6.72 (-30.34, 43.78)	1.01 (1.00 1.02)

A **Viable CD34+ Absolute Counts**



B **Viable CD45+ Absolute Counts**

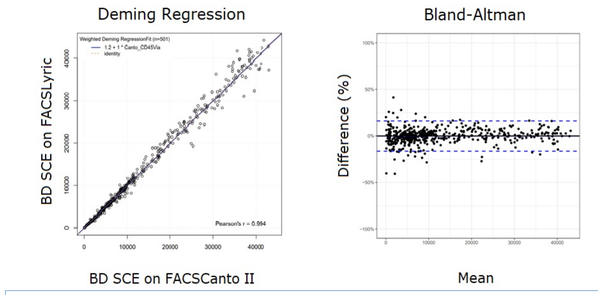


Table 5. Predicted Bias

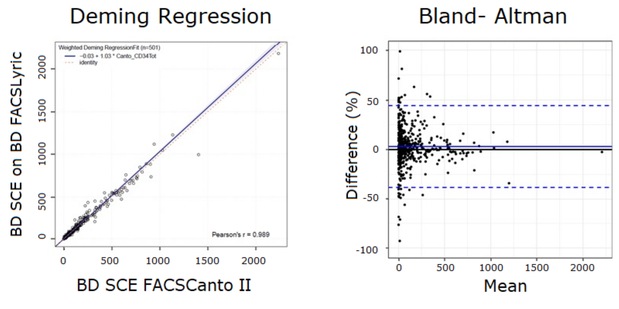
Viable CD34+ Absolute Count at 10 Cells/µL Threshold		
Bias Type	CD34 Threshold	Bias (95% CI)
Proportional %Bias	10 cells/µL	5.67 (3.83, 7.52)
Absolute Bias	10 cells/µL	0.57 (0.38, 0.75)

- **C** shows the Total CD34+ Absolute Counts Deming regression (**Table 4**) and bias results in Bland-Altman plots with percent mean bias of 3.03% and 95% limits of agreement (LOA) between -38.21% to 44.28%.
- **D** demonstrates the Total CD45+ Absolute Counts Deming regression (**Table 4**) and bias results in Bland-Altman plots with 1.19% percent mean bias and 95% LOA between -12.97% and 15.35%.

- Predicted bias was calculated for viable CD34 counts at 10 cells/µL threshold showing a proportional percent bias of 5.67 (3.83, 7.52) and absolute bias of 0.57 (0.38, 075); for details see **Table 5**.
- Agreement analysis exhibited overall percent agreement of 97.4%, 93% PPA and 98.5% NPA (**Table 6**). Thirteen samples showed discrepant results, eight samples had one or two cell differences; two showed three cell differences and the last three sample showed between 9 and 19 cell differences.

Results – Total CD34+ and CD45+ Absolute Counts

C **Total CD34+ Absolute Counts**



D **Total CD45+ Absolute Counts**

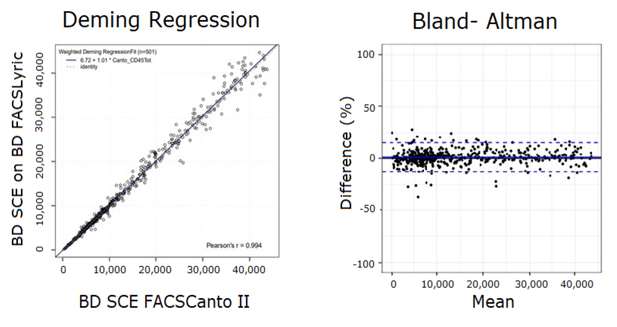


Table 6. Agreement Analysis

Method		SCE Kit on BDFACSCanto™ II FC		
		Positive (≤10 cells/µL)	Negative (>10 cells/µL)	Total
SCE Kit on BD FACSLyric™ FC	Positive (≤10 cells/µL)	93	6	99
	Negative (>10 cells/µL)	7	395	402
	Total	100	401	501
Agreement 95% CL (LCL, UCL)		PPA[$]	NPA[&]	Overall
		93% (86.3, 96.6)	98.5% (96.8, 99.3)	97.4% (95.6, 98.5)

$= positive percent agreement (PPA); & negative percent agreement (NPA)

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- **D** demonstrates the Total CD45+ Absolute Counts Deming regression (**Table 4**) and bias results in Bland-Altman plots with 1.19% percent mean bias and 95% LOA between -12.97% and 15.35%.

- Predicted bias was calculated for viable CD34 counts at 10 cells/µL threshold showing a proportional percent bias of 5.67 (3.83, 7.52) and absolute bias of 0.57 (0.38, 075); for details see **Table 5**.
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Conclusions

- Different types of fresh and thawed specimens were collected in a variety of anticoagulants and successfully tested using the BD® SCE Kit by collecting and analyzing stained samples on both BD flow cytometry systems, BD FACSCanto™ II with BD FACSCanto™ Clinical Software and test BD FACSLyric™ Flow Cytometer with BD FACSuite™ Clinical Application.
- Equivalent performance of the BD® SCE Kit between the BD FACSLyric™ and BD FACSCanto™ II Flow Cytometers was demonstrated by the Deming regression results and predicted bias of viable CD34+ absolute counts at 10 cells/µL, by enumeration of viable CD45+, total CD34+ and CD45+ absolute counts.