

Evaluation of Viable Dual-positive CD45+/CD34+ Stem Cells on BD FACSLyric™ System using BD® Stem Cell Enumeration Kit



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INTRODUCTION

The BD® Stem Cell Enumeration Assay, a single tube assay, along with BD® Stem Cell Enumeration kit on BD FACSLyric™ System is designed to provide simultaneous enumeration of total and viable CD45+ cells, and total and viable dual-positive CD45+/CD34+ hematopoietic stem cells (CD34+).

The following specimens were analyzed:

- Normal and mobilized peripheral blood
- Fresh and thawed leucopheresis
- Fresh and thawed bone marrow
- Fresh and thawed cord blood

We conducted three studies to assess performance of the BD® Stem Cell Enumeration Assay using BD® Stem Cell Enumeration kit on BD FACSLyric™ flow cytometer

- Method Comparison of viable dual-positive CD45+/CD34+ cells between BD FACSLyric™ and BD FACSCanto™ II Flow Cytometers
- Repeatability of quantification for viable dual-positive CD45+/CD34+ cells using three BD FACSLyric™ Systems
- Evaluation of linear range for measurement of viable dual-positive CD45+/CD34+ cells on BD FACSLyric™ System.

MATERIALS AND METHODS

For Method Comparison and for the Evaluation of Repeatability, a total of 61 donors were acquired using BD Trucount™ Tubes. All 8 sample types tested were procured from a qualified external vendor. Absolute count (cells/μL) of cell populations were used for analysis. All the samples were stained as per manufacturer specifications and were placed in wet ice as soon as they were stained. All the samples were acquired within one hour of being stained.

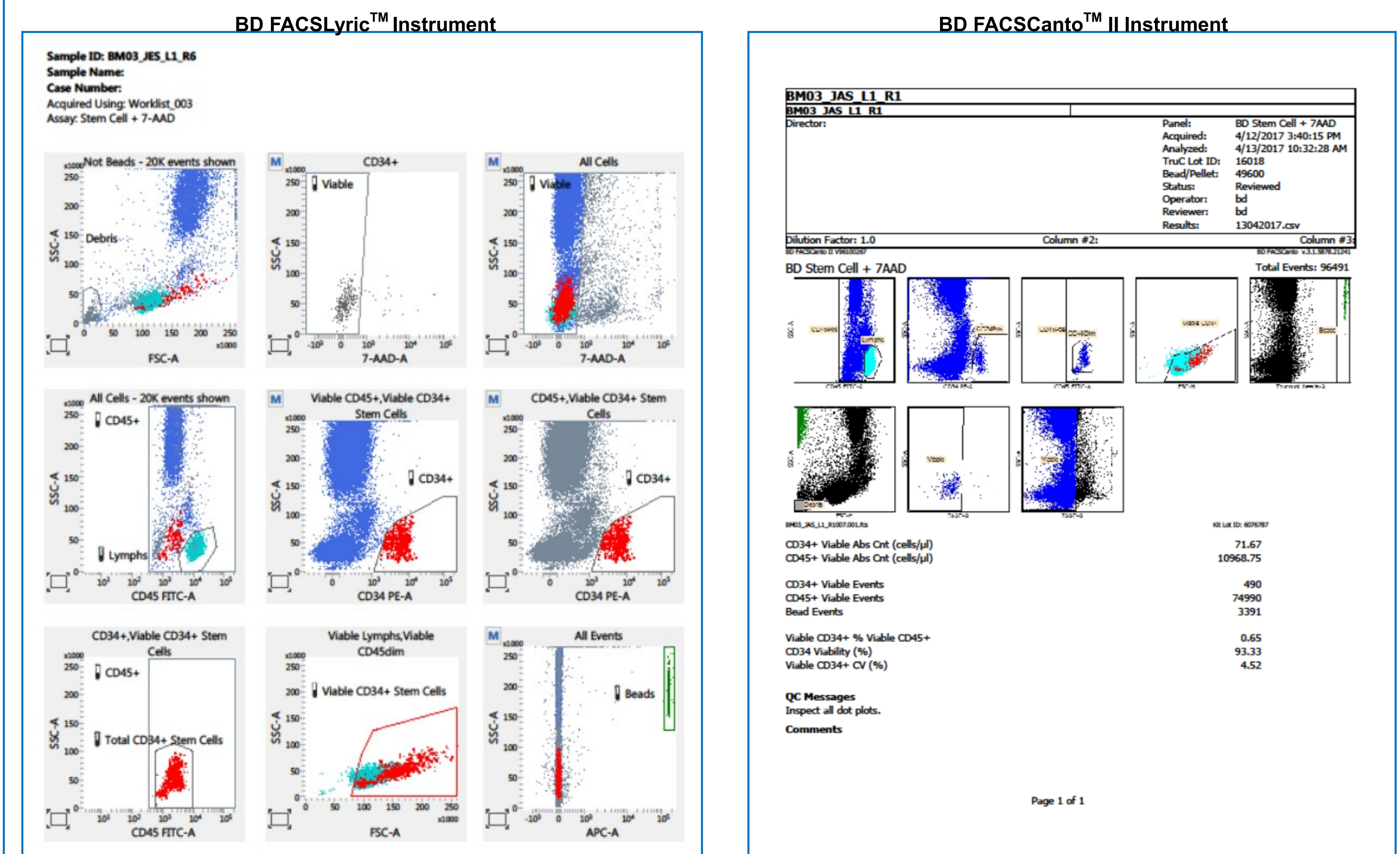
Deming regression was performed between the test (BD FACSLyric™) and predicate (BD FACSCanto™ II) Instruments to compare two platforms.

For Evaluation of Repeatability, 4 replicates of each donor were run on each of the three BD FACSLyric™ Instruments. Variance component analysis and total precision was evaluated for determining the Repeatability of quantification.

Sample Type	Normal Peripheral blood	Mobilized Peripheral Blood	Fresh Bone Marrow	Thawed Bone Marrow	Fresh Cord Blood	Thawed Cord Blood	Fresh Leucopheresis	Thawed Leucopheresis
Samples Tested	12	8	8	6	6	6	6	9

Linearity of the assay was evaluated using purified CD45+/CD34+ stem cells spiked into peripheral whole blood. Linearity was tested across the dynamic measuring range of 0-1000 cells/μL. There were 9 dilutions created across the range and 3 replicates of each dilution level were each run on 3 BD FACSLyric™ Instruments.

GATING STRATEGY



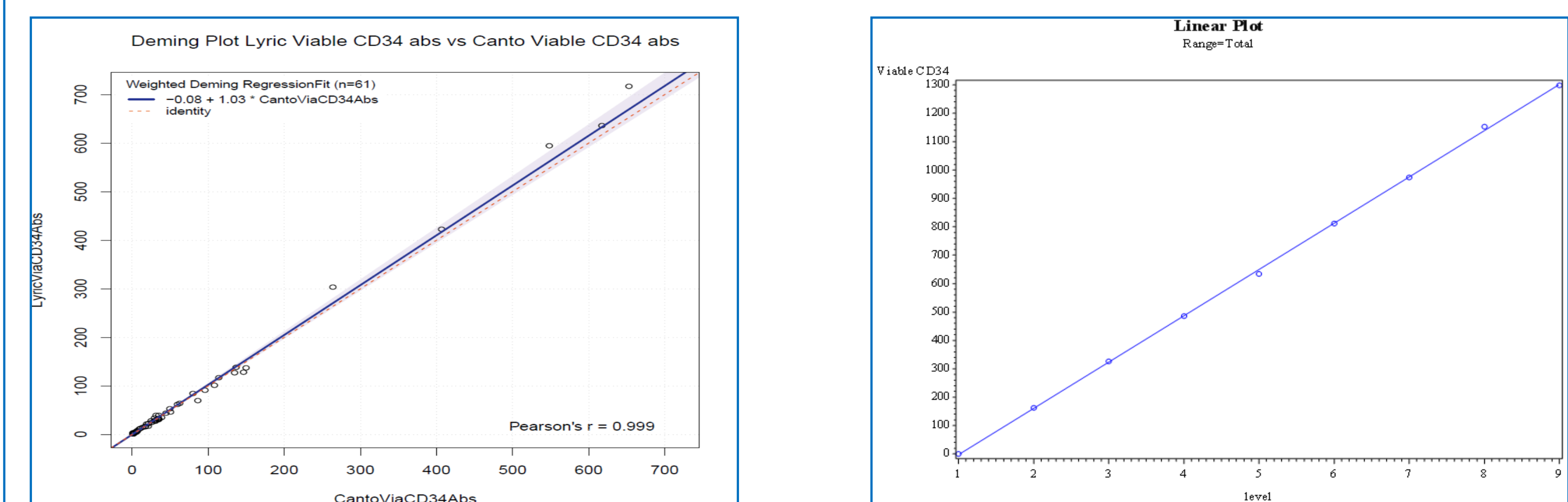
Gating strategy used in the BD FACSLyric™ Instrument (left) using BD FACSuite™ Software for comparison against gating strategy for the BD® Stem Cell Enumeration Assay on BD FACSCanto™ Clinical Software (right).

RESULTS

We demonstrated correlation between BD FACSLyric™ and BD FACSCanto™ II Systems with an R² value of 0.99 and a bias estimate of 1.03 (95% CI, 0.99 to 1.06) for CD34+ absolute counts.

We showed repeatability of 15.2% CV (one-sided 97.5% CI, 16.3%) for CD34+ absolute counts on the BD FACSLyric™ System. The absolute count for Viable CD34+ cells in the 61 donor samples acquired ranged from 0-1085 cells/μL.

Linear range of 1-1272 cells/μL was established for CD34+ absolute counts with an R² value of 0.99.



Representative Deming Plot for Viable CD34 (BD FACSLyric™ vs BD FACSCanto™ II Systems) (Left) Linearity graph (Right)

BD Flow Cytometers are Class 1 Laser Products. The BD® Stem Cell Enumeration Kit is for In Vitro Diagnostic Use with the BD FACSLyric™ Flow Cytometer, BD FACSCanto™ II Flow Cytometer and the BD FACSCalibur™ Flow Cytometer. The BD FACSLyric™ Flow Cytometer is for In Vitro Diagnostic Use with BD FACSuite™ Clinical Application for up to six colors. The BD FACSLyric™ Flow Cytometer is for Research Use Only with BD FACSuite™ Application for up to 12 colors. Not for use in diagnostic or therapeutic procedures. The BD FACSCanto™ II Flow Cytometer is for In Vitro Diagnostic Use for up to six colors. Seven and eight colors are for Research Use Only. The BD FACSCalibur™ Flow Cytometer is discontinued.