



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 investigational BD® Stem Cell Enumeration Assay, a single tube assay, along with the BD® Stem Cell Enumeration Kit on the 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 leukapheresis
- 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 the BD FACSLyric™ Flow Cytometer:

- Method comparison of viable dual-positive CD45+/CD34+ cells between BD FACSLyric™ and BD FACSCanto™ II
- Repeatability of quantification for viable dual-positive CD45+/CD34+ cells using three BD FACSLyric™ systems, and
- Evaluation of linear range for measurement of viable dual-positive CD45+/CD34+ cells on the 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 eight sample types tested were procured from a qualified external vendor. Absolute count (cells/μL) of cell populations were used for analysis. The samples were stained as per manufacturer specifications and were placed in wet ice as soon as they were stained. The samples were acquired within one hour of being stained.

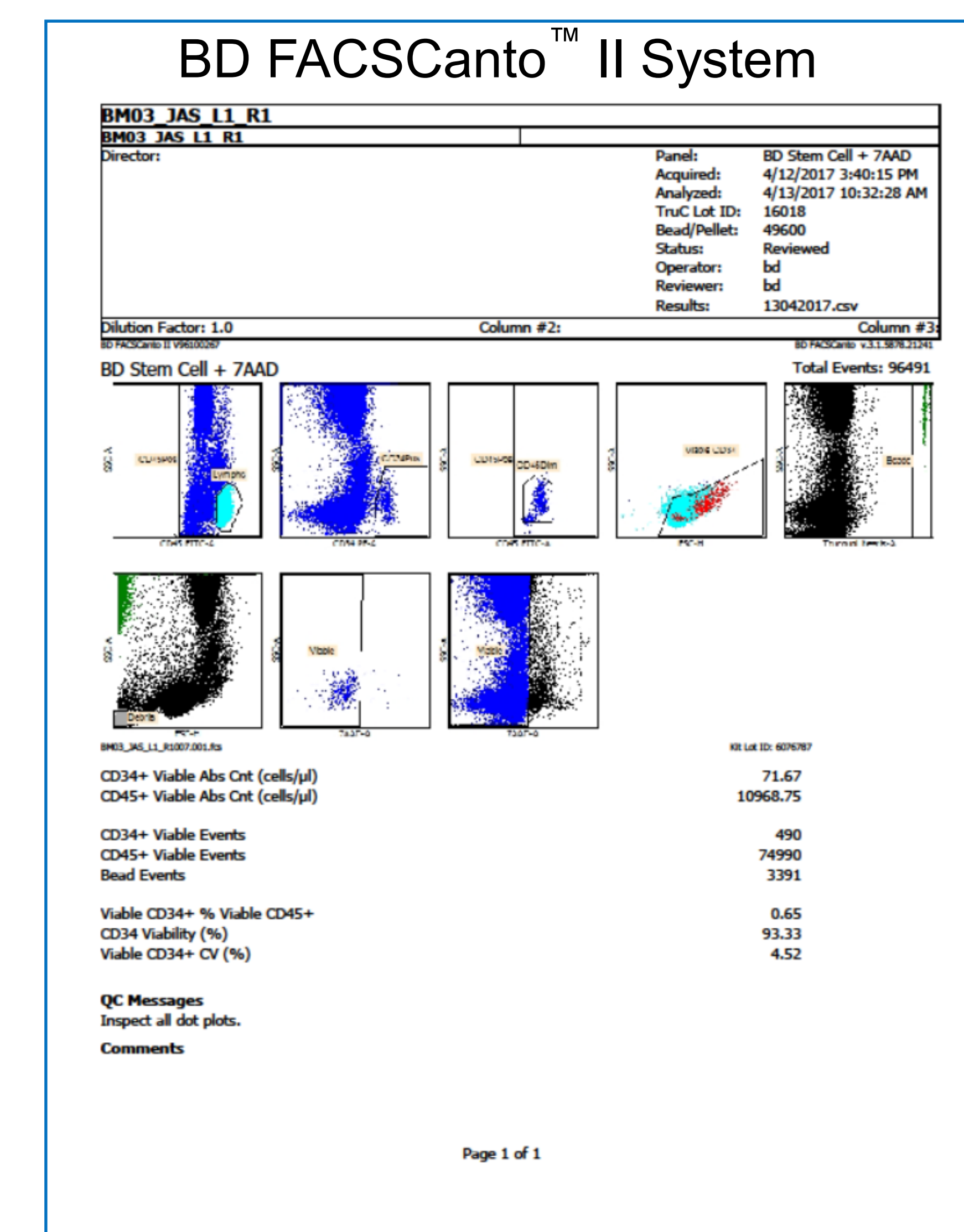
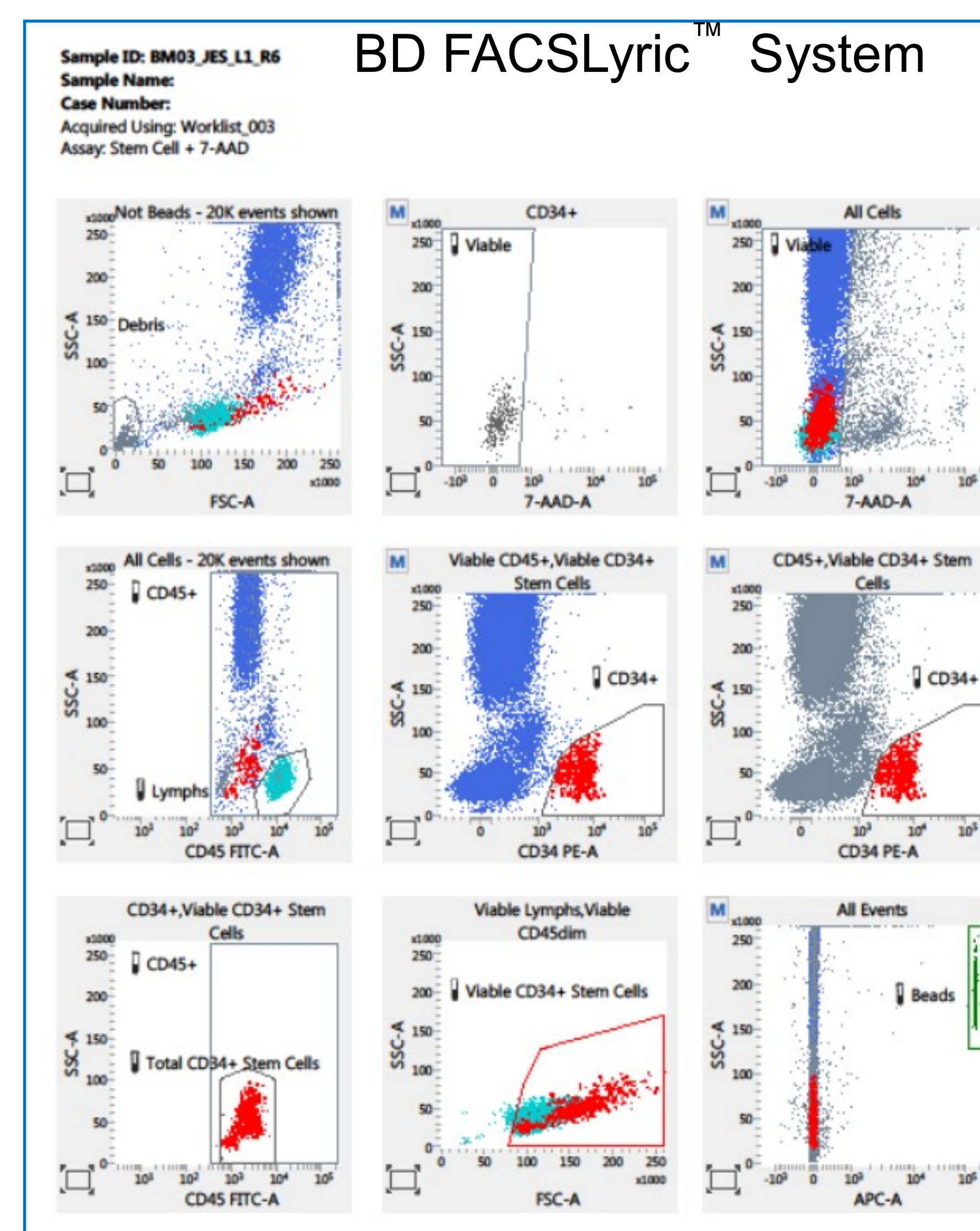
Deming regression was performed between the test (BD FACSLyric™) and predicate (BD FACSCanto™ II) systems to compare the two platforms.

For evaluation of repeatability, four replicates of each donor were run on each of the three FACSLyric systems. Variance component analysis and total precision was evaluated to determine 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 Leukapheresis	Thawed Leukapheresis
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 – 1,000 cells/μL. There were nine dilutions created across the range and three replicates of each dilution level were each run on three FACSLyric systems.

GATING STRATEGY



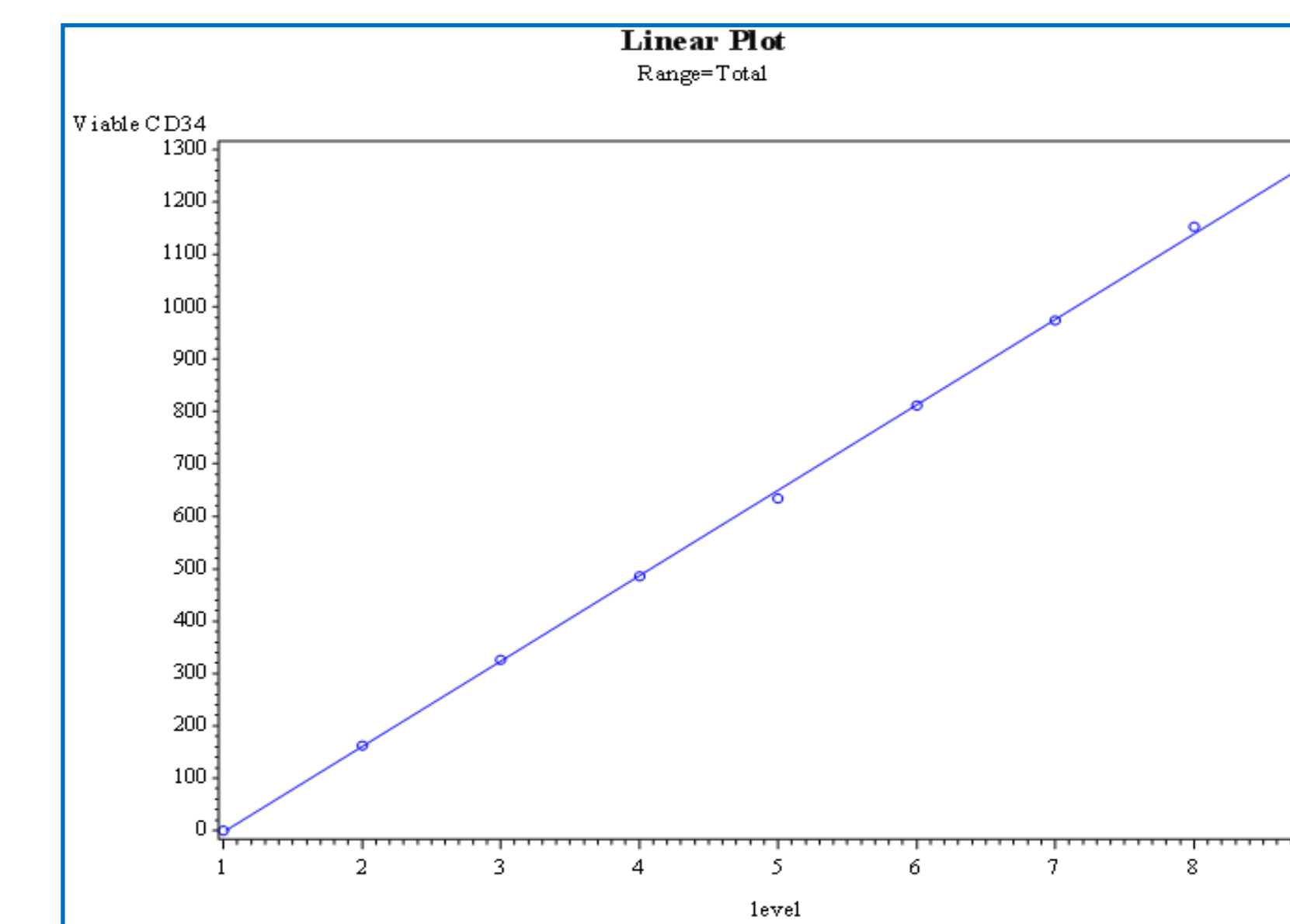
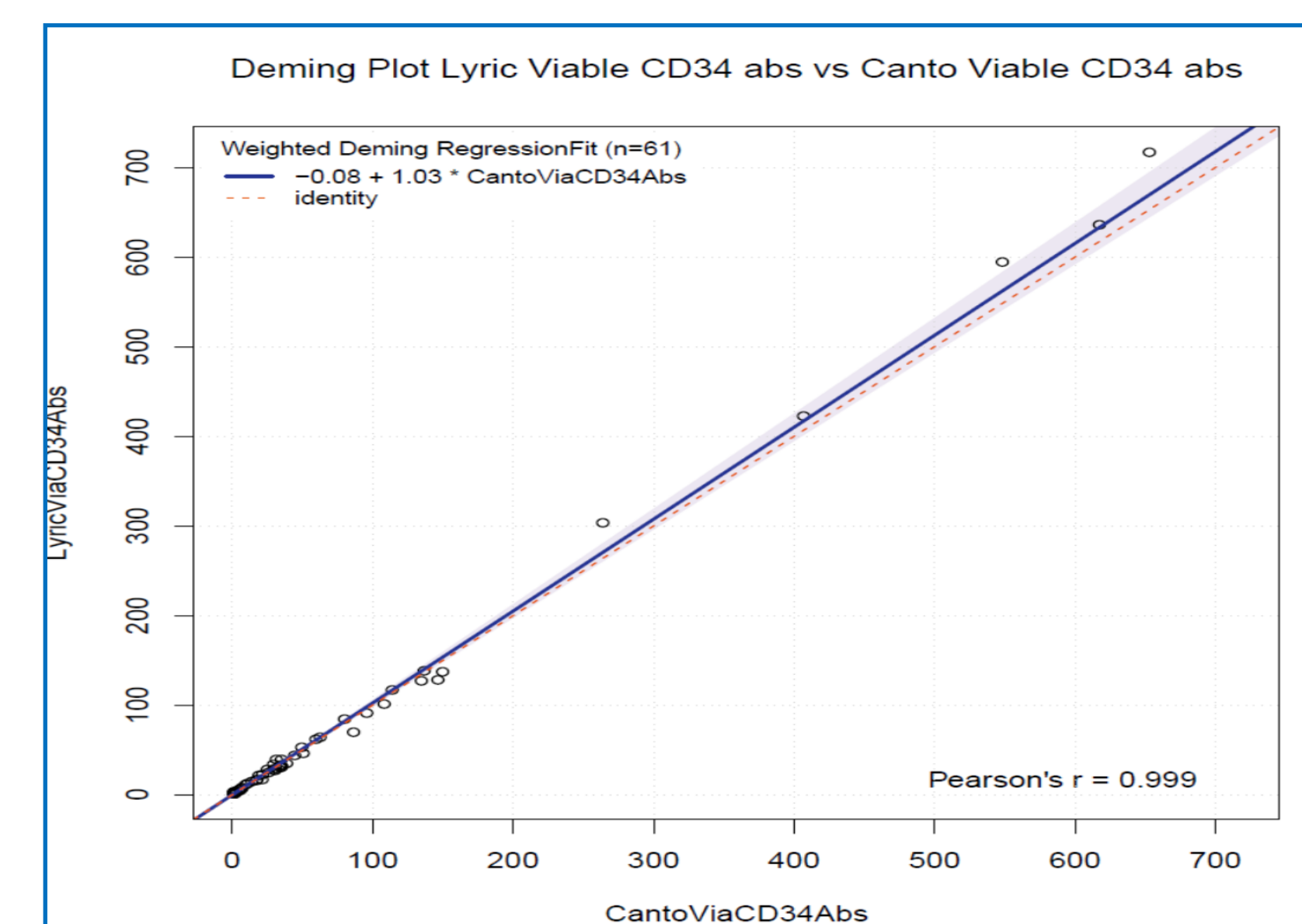
Gating strategy used for the BD FACSLyric™ system (left) in the BD FACSuite™ Clinical application for comparison against gating strategy for the BD Stem Cell Enumeration assay on BD FACSCanto™ Clinical software (right).

RESULTS

We demonstrated correlation between the BD FACSLyric™ and BD FACSCanto™ II 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 – 1,085 cells/μL.

Linear range of 1 – 1,272 cells/μL was established for CD34+ absolute counts with an R² value of 0.99



Representative Deming Regression Plot for Viable CD34+ (FACSLyric vs FACSCanto II) (Left) Linearity graph (Right)

Disclaimer: BD® Stem Cell Enumeration Kit and BD® Stem Cell Controls are CE marked in compliance with the European In Vitro Diagnostic Medical Device Directive 98/79/EC. The BD FACSLyric™ Flow Cytometer is CE marked in compliance with the European In Vitro Diagnostic Medical Device Directive 98/79/EC. The BD FACSCanto™ II Flow Cytometer is CE marked in compliance with the European In Vitro Diagnostic Medical Device Directive 98/79/EC. The BD FACSLyric™ and BD FACSCanto™ II Flow Cytometers are a Class 1 Laser Product. BD® Stem Cell Enumeration Kit is not available for use with BD FACSLyric™ Flow Cytometer in the United States.