BD Stem Cell Enumeration Kit

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CONTENTS

1. INTENDED USE

The BD® Stem Cell Enumeration Kit is intended for enumeration of viable dual-positive $CD45^+/CD34^+$ hematopoietic stem cell populations to determine absolute counts (cells/µL) of viable $CD34^+$ and the percentages of viable $CD45^+/CD34^+$ hematopoietic stem cells (%CD34) to aid in quality assessment of the following cellular-based products used in hematopoietic stem cell transplantation process, which includes preparation and transplantation of cellular-based products (specimens).

- Normal and mobilized peripheral blood
- Fresh and thawed leukapheresis products
- Fresh and thawed bone marrow
- Fresh and thawed cord blood

The kit is intended for use on any of the following flow cytometer systems:

- BD FACSLyric™ flow cytometer using BD FACSuite™ Clinical application
- BD FACSCanto™ II flow cytometer using BD FACSCanto™ clinical software
- BD FACSCalibur™ flow cytometer using BD CellQuest™ or BD CellQuest™ Pro software.

The BD® Stem Cell Enumeration Kit is intended for in vitro diagnostic use by laboratory professionals.

2. SUMMARY AND EXPLANATION

Transplantation of hematopoietic progenitor cells is used increasingly in the treatment of blood disorders, malignancies, and genetic abnormalities.[1-3] Progenitor cells are rare and are found primarily in the bone marrow, with extremely low frequencies in peripheral blood. However, with the arrival of mobilization regimens (G-CSF, GM-CSF, and chemotherapy), mobilized peripheral blood has become a preferred source for stem cells.[1-3]

The CD34 antigen is present on immature hematopoietic precursor cells and hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitor cells.[4]

An accurate measure of the $CD34^+$ cell count is necessary for dose requirement protocols in stem cell transplantation.[2] An incorrectly high result could lead to an infusate with less than the recommended threshold dose of $CD34^+$ cells. Quantitating the $CD34^+$ cell population can also be useful to monitor mobilization.

Fluorochrome-conjugated monoclonal antibodies directed against the CD34 molecule can be used to identify $CD34^+$ cells by flow cytometry. Flow cytometric applications for $CD34^+$ cell identification and enumeration provide a rapid, quantitative, and reproducible method to evaluate the progenitor cell population.

Significant site-to-site variation has been reported with flow cytometric methods for determining the percentages and absolute numbers of $CD34^+$ cells.[5] Single-platform flow cytometric absolute cell counting protocols have been shown to provide increased robustness of CD34 enumeration by limiting potential sources of imprecision.[6] The BD® Stem Cell Enumeration Kit includes BD Trucount™ Tubes to determine the absolute cell count, thereby eliminating variability associated with hematology-derived absolute counts.[5] Enumeration of

the cell populations in this assay is obtained using either an automated or a manual method for gating and analysis. See the *BD® Stem Cell Enumeration Application Guide* for your flow cytometer for more information.

3. PRINCIPLES OF THE PROCEDURE

The single-tube assay is performed by staining the sample with the reagent in individual BD Trucount™ Tubes for absolute counts.[6] When a sample is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to the cell surface. Additionally, the lyophilized pellet in the BD Trucount™ Tube dissolves, releasing a known number of fluorescent beads. BD FACSuite™ Clinical application determines the absolute counts (cells/µL) of gated cells in the sample by comparing cellular events to bead events.

The dye 7-AAD is added to assess viability of the cells. Cells that are 7-AAD$^+$ are not viable. Ammonium chloride is added to lyse erythrocytes before the sample is acquired on a flow cytometer.

During analysis of the sample, the concentration of viable CD34$^+$ cells and viable CD45$^+$ cells, and the percentage of viable CD34$^+$ cells in the viable CD45$^+$ cell population, are calculated.

4. REAGENTS

Reagent Composition

The BD® Stem Cell Reagent contains the following conjugated antibodies:

Table 1 BD® Stem Cell Reagent composition

Antibody	Fluorochrome	Clone	Isotype	Concentration (µg/mL)
CD45	FITC	2D1[7,8]	IgG_1, kappa	12.5

Table 1 BD® Stem Cell Reagent composition

Antibody	Fluorochrome	Clone	Isotype	Concentration (µg/mL)
CD34	PE	8G12[9]	IgG_1, kappa	10.0

CD45 (2D1) recognizes a 180- to 220-kilodalton (kDa) human leucocyte antigen that is a member of the leucocyte common antigen (LCA) family.[7,10] The CD45 antigen is present on all human leucocytes and is weakly expressed on hematopoietic progenitor cells.

CD34 (8G12) recognizes the class III human progenitor cell antigen (HPCA). The CD34 antigen is present on immature hematopoietic precursor cells and all hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitor cells.[10-14]

Precautions

- The reagent should be clear. Do not use the reagent if you observe any change in appearance. Precipitation, cloudiness, or change in color indicates instability or deterioration.
- The antibody reagent contains sodium azide as a preservative; however, take care to avoid microbial contamination, which can cause erroneous results.
- Do not decontaminate ammonium chloride lysed samples with bleach.
- To achieve an accurate result, it is critical to add a precise volume of specimen to BD Trucount™ Tubes. Calibrate pipettes to deliver 100 µL. Use reverse pipetting or a positive displacement pipette to deliver samples. See the pipette manufacturer's instructions for more information.

- Bead count varies by lot of BD Trucount™ Tubes. It is critical to use the bead count shown on the current lot of BD Trucount™ Tubes when entering this value in the software or when manually calculating absolute counts. Do not mix multiple lots of tubes in the same run.
- BD Trucount™ Tubes are designed for use with a specific lyse/no-wash procedure. Do not threshold on forward scatter (FSC) for data collection.
- Go to regdocs.bd.com to download the Safety Data Sheet.

Storage and Handling

- Store the BD® Stem Cell Reagent (CD45/CD34) at 2°C–8°C. Do not use after the expiration date shown on the label. Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the reagent vial dry.
- Store 7-AAD at 2°C–8°C. Protect from light.
- Store 10X ammonium chloride at 2°C–8°C.
- For each of the three liquid reagents, close the vial immediately after dispensing the reagent and return it to storage at 2°C–8°C.
- Store BD Trucount™ Tubes in their original foil pouch at 2°C–25°C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the pouch immediately after removing a tube. An unopened pouch is stable until the expiration date shown on the packaging. Use tubes within 1 hour after removal from the foil pouch. Use remaining tubes within 1 month after opening the pouch. Do not use tubes after the expiration date.

5. INSTRUMENTS

The BD® Stem Cell Enumeration Kit is designed for use on the following BD systems. See the corresponding reagent, cytometer, or software user documentation for details.

Table 2 Recommended BD systems

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™	BD® CS&T Beads[a] BD® FC Beads 7-Color Kit[b]	BD FACSuite™ Clinical application v1.4 or later	BD FACSuite™ Clinical application v1.4 or later
BD FACSCanto™ II	BD FACS™ 7-Color Setup Beads[c]	BD FACSCanto™ clinical software v2.4 or later	BD FACSCanto™ clinical software v2.4 or later
BD FACSCalibur™	BD Calibrite™ Beads 3-Color Kit	BD FACSComp™ software v4.2 through v6.0	BD CellQuest™ software v3.3 or BD CellQuest™ Pro software v4.0.2, v5.2.1, or v6.0

a. To perform daily cytometer quality control.
b. To calculate compensation.
c. To set photomultiplier tube (PMT) voltages and fluorescence compensation and check instrument sensitivity before use.

6. SPECIMEN COLLECTION AND PREPARATION

General considerations:

- Follow the Clinical and Laboratory Standards Institute (CLSI) (H42-A2) guidelines for specimen storage and handling.[15] Labs must validate any specimen storage or handling conditions other than those described here.
- Optimally, keep undiluted specimens stored at 2°C–8°C.[15,16]
- Stain fresh specimens within 24 hours of collection.[17] Stain fresh cord blood within 48 hours of collection. Store stained samples on wet ice* and acquire within 1 hour of lysing.
- Stain frozen specimens immediately after thawing. Acquire stained samples immediately post-lysis.

* Wet ice: Ice in a small amount of water. Allows better contact with the tube so that the contents chill quickly.

- The following anticoagulants have been verified for use with this assay:
  - EDTA, ACD-A, and heparin for peripheral blood (normal and mobilized), cord blood (fresh and thawed), bone marrow (fresh and thawed), and leukapheresis products (fresh and thawed).
  - CPD for peripheral blood (normal and mobilized) and cord blood (fresh and thawed).
  - For leukapheresis products, a mixture of ACD-A, heparin, and EDTA can also be used with this assay.
- A minimum of 100 µL of neat/diluted specimen is required per test.
- Perform a white blood cell (WBC) count on all specimens to be evaluated. If the WBC count is greater than 40×10^3 cells/µL, dilute the specimen according to standard laboratory procedures, using phosphate-buffered saline (PBS) with 0.5% BSA.[5] Lipemic specimens or specimens containing particulates might have to be diluted even if the concentration is less than 40×10^3 cells/µL. Use reverse pipetting to make dilutions.
- Record and enter the dilution factor for the calculation of the final CD34 result into the software:
  - Dilution Factor column of the worklist for BD FACSuite™ Clinical application with the Stem Cell + 7-AAD assay or BD FACSCanto™ clinical software with the BD® Stem Cell Enumeration module
  - Template for BD CellQuest™ or BD CellQuest™ Pro software

  See the appropriate *BD® Stem Cell Enumeration Application Guide* for your instrument for acquisition and analysis instructions.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[18,19] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Interfering Conditions

Do not use previously fixed and stored specimens. Reject hemolyzed, clotted, or clumped specimens.

The table lists the substances that were tested for interference with the BD® Stem Cell Enumeration Kit. Testing for interference was performed using leukapheresis products, bone marrow, and cord blood in accordance with CLSI guidelines.[20,21] There was no detectable interference at the following concentrations.

Table 3 Interferents tested

Analyte	Maximum concentration
Albumin	60 mg/mL (6 g/dL)
Bilirubin	400 µg/mL (40 mg/dL)
Cyclophosphamide	550 µg/mL (55 mg/dL)
Hemoglobin	10 mg/mL (1 g/dL)
Doxorubicin	1.932 µg/mL
G-CSF	60 ng/mL
Intralipid	57 µL/mL (1,140 mg/dL)
Paclitaxel	10.8 µg/mL

7. PROCEDURE

Reagents and Materials

Reagents and materials provided

The BD® Stem Cell Enumeration Kit is sufficient for 50 tests when used as directed. The kit contains the following components:

- BD® Stem Cell Reagent

The reagent contains CD45 FITC and CD34 PE. It is provided in PBS containing bovine serum albumin (BSA) and 0.1% sodium azide.

- 7-AAD

  7-AAD (45–65 µg/mL) is a nucleic-acid dye used to assess cell viability.

- Ammonium Chloride Lysing Solution 10X

  The ammonium chloride solution is a fixative-free solution for red blood cell lysis.

- BD Trucount™ Tubes

  Two pouches are provided, each containing 25 single-use tubes. Each tube contains a freeze-dried pellet of fluorescent beads. By adding the reagent and the sample directly to the BD Trucount™ Tube, the absolute count of the cell population of interest can be directly determined.

Reagents and materials required but not provided

- For BD FACSLyric™ flow cytometers:
  - BD® CS&T Beads (Catalog Nos. 656504, 656505)
  - BD® FC Beads 7-Color Kit (Catalog No. 656867)
- For BD FACSCanto™ II flow cytometers:
  - BD FACS™ 7-Color Setup Beads (Catalog No. 335775)
- For BD FACSCalibur™ flow cytometers:
  - BD Calibrite™ Beads 3-Color Kit (Catalog No. 340486)
- Reagent-grade deionized water
- BD Vacutainer® blood collection tubes or equivalent
- Vortex mixer
- Timer
- Ice-water bath

- Falcon® disposable 12 × 75-mm polystyrene test tubes or equivalent
- Calibrated micropipettor with tips capable of delivering 20 µL
- Calibrated micropipettor with tips capable of delivering 100 µL
- Bulk dispenser or pipettor for dispensing 2 mL of 1X ammonium chloride lysing solution
- 1X PBS (Dulbecco's modified, pH 7.2 ±0.2) with 0.5% BSA[5], if sample dilution is necessary
- BD® Stem Cell Control (Catalog No. 340991)

  See the *BD® Stem Cell Control* IFU for storage, handling, and other information.

NOTE Labs must validate any deviations from the following procedures.

Diluting the 10X Ammonium Chloride Lysing Solution

Each day, prepare enough 1X ammonium chloride lysing solution for use. To make, dilute 1 part of 10X ammonium chloride lysing solution with 9 parts of deionized water. Store and use at room temperature (20°C–25°C).

Reverse Pipetting

Accurate pipetting is critical when using a BD Trucount™ Tube. Use the reverse pipetting technique, or a positive displacement pipettor, to pipette specimen onto the side of the tube just above the retainer.

For reverse pipetting, depress the button to the second stop. Insert the pipettor into the specimen and release the button. When you release the button, excess specimen is drawn up into the tip. When dispensing, press the button to the first stop to expel a precise volume of specimen. This leaves excess specimen in the tip.

Performing Quality Control

Run low and high process controls with known assayed CD34+ absolute count values to confirm staining and system integrity. We recommend using the BD® Stem Cell Control for this purpose, stained without the addition of the 7-AAD reagent. BD® Stem Cell Control contains fixed cells and therefore stain positively for 7-AAD. Other process controls must be validated by the lab.

Staining the Specimens

Use the volumes in the following table to prepare the specimens for staining.

Task	Sample Type	Tube Type	Reagent (µL)		Control (µL)		Specimen (µL)
			SCE[a]	7-AAD	CD34+ High	CD34+ Low	
7-AAD setup	Optimization[b]	Polystyrene	20	20	100 of either		
	7-AAD– Stained[c]	Polystyrene	–	20	100 of either		–
	7-AAD Unstained[c]	Polystyrene	–	–	100 of either		–
Stain process controls	High control	BD Trucount™	20	–	100	–	–
	Low control	BD Trucount™	20	–	–	100	–
Stain specimen	Specimen	BD Trucount™	20	20	–	–	100

a. SCE = Stem Cell Enumeration
b. For acquisition on BD FACSCanto™ II flow cytometer only.
c. For acquisition on BD FACSLyric™ flow cytometer only.

1. For each specimen, remove a tube and label it with the appropriate sample identification.

   We recommend that you stain the process controls, acquire them, and verify that the results are within the values reported in the

Assay Values sheet, provided with the controls, before you start staining the specimens.

NOTE For samples stained in BD Trucount™ Tubes, verify that the BD Trucount™ bead pellet is under the metal retainer at the bottom of the tube. If this is not the case, discard the BD Trucount™ Tube and replace it with another. Do not transfer beads to another tube.

2. Pipette 20 µL of the BD® Stem Cell Reagent into the bottom of the tube.

   Pipette just above the stainless steel retainer of the BD Trucount™ Tube. Do not touch the bead pellet.

   NOTE Always change to a new tip between tubes. Discard tips in an appropriate biohazard container.

   NOTE Close the vial immediately after dispensing the reagent and return it to storage at 2°C–8°C.

3. Pipette 20 µL of 7-AAD into the tube.

   NOTE Do not add 7-AAD to the control tubes.

4. Pipette 100 µL of a well-mixed control or specimen onto the side of the tube just above the retainer.

   NOTE Make sure to thoroughly mix the controls before pipetting them. See the *BD® Stem Cell Control* IFU for information.

   NOTE Use the reverse pipetting technique to pipette specimen onto the side of the tube just above the retainer. See Reverse Pipetting on page 14. Avoid smearing the specimen down the side of the tube. If any specimen remains on the side of the tube, it will not be stained with the reagent but can be resuspended by the lysing solution and therefore can affect results.

5. Cap each tube and vortex gently to mix.

6. Incubate for 20 minutes in the dark at room temperature (20°C–25°C).
7. Add 2 mL of 1X ammonium chloride lysing solution to each tube to lyse red blood cells.
8. Cap each tube and vortex gently to mix.
9. Incubate for 10 minutes in the dark at room temperature (20°C–25°C).
10. Immediately place tubes on wet ice in the dark until ready to acquire samples.

    Acquire samples within 1 hour of lysing. For specimens stained post-thaw, acquire samples immediately after lysing them. Highly manipulated or processed samples can be more susceptible to increased cell death after preparation.[16]

    For information on setup, acquisition, and data analysis, see the *BD® Stem Cell Enumeration Application Guide* for your flow cytometer.

Running the Assay on BD FACSLyric™ Flow Cytometers

Before you begin:

1. Verify that the BD® Stem Cell Reagent, 7-AAD, BD® CS&T Beads, and BD® FC Beads have not expired.
2. Add reagent and bead lots to library, if needed.
3. Verify that Characterization QC (CQC) and lyse/wash reference settings have not expired.

    See the *BD FACSLyric™ Clinical Reference System* and the *BD® Stem Cell Enumeration Application Guide for BD FACSLyric™ Flow Cytometers* for more information.
4. Perform CQC and update lyse/wash reference settings, if needed.

To run the assay:

1. Perform daily Performance QC (PQC) using BD® CS&T Beads.
2. Add 7-AAD to the reference settings, as needed.

   This needs to be done before you run the assay for the first time, whenever a new lot of BD® CS&T Beads is used without performing a Bead Lot Transfer, or when recommended by BD Service.

   See the *BD® Stem Cell Enumeration Application Guide for BD FACSLyric™ Flow Cytometers* for more information.
3. Perform Assay/Tube Settings Setup for the BD® Stem Cell assay.

   We recommend selecting the Run Setup and Generate Reports checkboxes.
4. Create a worklist.
   - Create a task and select Stem Cell Control for each process control you are running.
   - Create a task and select Stem Cell + 7-AAD for each specimen you are running.
5. Run the Stem Cell Control tasks on the worklist.

   Vortex each tube thoroughly at low speed immediately before acquiring it.
6. Review the BD® Stem Cell Control Lab Report and confirm that the values are within the ranges shown on the BD® Stem Cell Control Assay Values sheet, provided with the process controls.
7. Run the Stem Cell + 7-AAD tasks on the worklist.

   Vortex tube for each sample thoroughly at low speed immediately before acquiring it.

   See the *BD® Stem Cell Enumeration Application Guide for BD FACSLyric™ Flow Cytometers* for more information.

Running the Panel on BD FACSCanto™ II Flow Cytometers

1. Run Setup using BD FACS™ 7-Color Setup Beads.
2. Select the BD Stem Cell panel.
3. Vortex each tube thoroughly at low speed immediately before acquiring it.

   It is important to reduce aggregation before running samples on the flow cytometer.[22]
4. When prompted by the software, load the control stained with 7-AAD.

   After the sample is acquired, the instrument is ready to acquire the stained process controls and specimens.
5. Add a BD Stem Cell panel entry for each process control to the worklist.

   NOTE The word "Control" must appear in the sample name.
6. Add a BD Stem Cell + 7-AAD entry for each sample to the worklist.
7. Follow the prompts in the software to acquire the stained process controls and samples.

   Verify that the process control values are within the ranges shown on the BD® Stem Cell Control Assay Values sheet.

Running the Assay on BD FACSCalibur™ Flow Cytometers

1. Set up the flow cytometer using BD Calibrite™ Beads and BD FACSComp™ software with 3-color or 4-color lyse/no-wash (LNW) settings.

   See the *BD® Stem Cell Enumeration Application Guide for BD FACSCalibur™ Flow Cytometers* for more information.

2. Vortex each tube thoroughly at low speed immediately before acquiring it.
3. Acquire the controls on a BD FACSCalibur™ flow cytometer immediately after staining.
4. After acquisition, add 20 µL of the 7-AAD reagent to one of the tubes. Cap the tube and vortex gently.
5. Incubate for 10 minutes in the dark at room temperature (20°C–25°C).
6. Immediately place the tube on wet ice in the dark until ready to acquire it.
7. Acquire the control stained with 7-AAD reagent within 1 hour of lysing.

   This ensures that samples stained with 7-AAD are correctly compensated.
8. Acquire the stained specimens within 1 hour of lysing.

Analyzing the Data

Review the laboratory report for the assay. See the appropriate instrument or software user documentation, or the *BD® Stem Cell Enumeration Application Guide* for your instrument for more information.

NOTE A platelet streak might appear in the CD34-PE vs SSC dot plot when running samples collected in heparin anticoagulant. If this occurs, see the *BD® Stem Cell Enumeration Application Guide* for your instrument for troubleshooting information.

8. LIMITATIONS

- Due to the temperature requirements of this assay, BD FACSDuet™ system cannot be used to prepare the samples and the BD FACS™ Universal Loader and the BD FACS™ Loader cannot be used to acquire samples.
- Laboratories must establish their own $CD34^+$ viability requirements for each specimen type.

9. EXPECTED VALUES

Validation of the reference interval using the BD® Stem Cell Enumeration Kit was performed at BD Biosciences laboratories in San Jose, CA.

Reference Intervals

Reference intervals for the BD® Stem Cell Enumeration Kit were validated with normal peripheral blood specimens. Hematologically normal adult subjects between the ages of 18 and 77 years were enrolled in a study to determine reference intervals for the BD FACSLyric™ flow cytometer. Laboratories must establish reference intervals using the BD® Stem Cell Enumeration Kit for their own donor populations to reflect potential sources of variability. Age, gender, clinical characteristics, race, and ethnicity of patients should be known when a reference interval is determined.[23] The provided reference intervals are for information only. See Table 4.

Table 4 Representative adult reference intervals

Measure reported	N	Mean	SD	95% Reference Interval	
				Lower (90% conf. bounds)	Upper (90% conf. bounds)
Viable CD34 absolute counts (cells/µL)	130	2.35	1.98	0 (0, 1)	7 (6, 13)

Table 4 Representative adult reference intervals

Measure reported	N	Mean	SD	95% Reference Interval	
				Lower (90% conf. bounds)	Upper (90% conf. bounds)
%CD34 cells	130	0.04	0.03	0.01 (0, 0.01)	0.1 (0.09, 0.18)

10. PERFORMANCE CHARACTERISTICS

BD FACSLyric™ Flow Cytometers

Specimen handling and collection (AOB/AOS) (BD FACSLyric™ flow cytometers)

A study was performed to assess the Age of Blood (AOB) and Age of Stain (AOS) using the BD® Stem Cell Enumeration Kit. The stability of fresh leukapheresis products, cord blood, and bone marrow specimens was evaluated by assessing the combined effect of:

- AOB: Time duration between specimen draw and staining
- AOS: Time duration between staining specimen (end of lysis) and acquiring the stained sample.

All specimens were maintained at 2°C–8°C before staining. Based on the results of this study, we recommend staining fresh specimens within 24 hours of collection. Fresh cord blood can be stained within 48 hours of collection. We recommend keeping stained samples on wet ice in the dark and analyzing stained samples within 1 hour of lysing.

Method comparison (BD FACSLyric™ flow cytometers)

A method comparison study between the investigational BD FACSLyric™ system and the predicate BD FACSCanto™ II system using the BD® Stem Cell Enumeration Kit and the BD® Stem Cell Enumeration assay module was conducted at five clinical sites. The BD FACSLyric™ system comprises either a 10-color (4-3-3 configuration) or a 12-color (4-3-5 configuration) BD FACSLyric™ flow cytometer with BD FACSuite™ Clinical application, BD® CS&T

Beads, and BD® FC Beads 7-Color Kit. The BD FACSCanto™ II system comprises the BD FACSCanto™ II flow cytometer with BD FACSCanto™ clinical software and BD® 7-Color Setup Beads. Normal and mobilized peripheral blood, fresh and thawed leukapheresis products, cord blood, and bone marrow specimens were collected from donors from five clinical laboratories.

Regression statistics for viable $CD34^+$ absolute counts and the difference (bias) for $\%CD34^+$ in viable $CD45^+$ between the values of the investigational and predicate systems are summarized. See Table 5 and Table 6, respectively. The results were pooled to produce mean difference of $\%CD34^+$ for each bin, along with 95% confidence interval (CI). The lab report provides results for other parameters for information only.

Table 5 Regression statistics for viable $CD34^+$ absolute counts of the BD® Stem Cell Enumeration Kit on the BD FACSLyric™ system compared with the BD FACSCanto™ II system

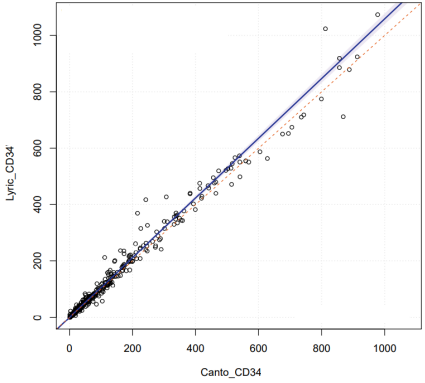
Variable	N	R^2	Slope (95% CI)	Intercept (95% CI)
Viable $CD34^+$ absolute counts (cells/µL)	501	0.983	1.06 (1.04, 1.08)	–0.02 (–0.14, 0.10)

Table 6 Difference statistics for % viable $CD34^+$ in viable $CD45^+$ of the BD® Stem Cell Enumeration Kit on the BD FACSLyric™ system compared with the BD FACSCanto™ II system

Variable	Bin	N	Mean of Predicate	Mean Absolute Difference (95% CI)	Mean Relative Difference (95% CI)
% Viable $CD34^+$ in viable $CD45^+$	≤0.5	247	0.19	0.02 (0.01, 0.03)	NA
	>0.5	254	2.35	NA	6.36% (4.57%, 8.14%)

The regression plot for viable CD34+ absolute counts on the BD FACSLyric™ flow cytometer vs the BD FACSCanto™ II is shown. See Figure 1. The solid line is the fitted line. The dotted line is the line where the results from the predicate system are equal to the results from the investigational system.

Figure 1 Regression plot of viable CD34+ absolute counts (cells/µL) on BD FACSCanto™ II vs viable CD34+ absolute counts on BD FACSLyric™ flow cytometers for all specimen types



Regression statistics of the assay on the BD FACSLyric™ flow cytometer compared to the BD FACSCanto™ II flow cytometer are summarized by specimen type. See Table 7.

Table 7 Regression statistics by specimen type

Specimen Type	Variable	N	R^2	Slope (95% CI)	Intercept (95% CI)
Peripheral blood	Viable CD34+ (cells/μL)	111 (normal: 48, mobilized: 63)	0.99	1.06 (1.02, 1.10)	0.00 (–0.15, 0.16)
	% Viable CD34+ in viable CD45+		0.99	1.04 (0.90, 1.18)	0.00 (–0.03, 0.03)
Leukapheresis products	Viable CD34+ (cells/μL)	137 (fresh: 68, frozen: 69)	0.98	1.03 (0.97, 1.10)	0.56 (–2.56, 3.67)
	% Viable CD34+ in viable CD45+		0.99	1.09 (1.03, 1.14)	–0.02 (–0.08, 0.04)
Cord blood	Viable CD34+ (cells/μL)	132 (fresh: 61, frozen: 71)	0.98	1.08 (1.04, 1.11)	–0.20 (–0.56, 0.15)
	% Viable CD34+ in viable CD45+		0.98	1.07 (0.98, 1.15)	0.00 (–0.04, 0.05)
Bone marrow	Viable CD34+ (cells/μL)	121 (fresh: 60, frozen: 61)	0.96	1.07 (1.03, 1.11)	–0.08 (–0.44, 0.28)
	% Viable CD34+ in viable CD45+		0.98	1.15 (1.01, 1.29)	–0.17 (–0.42, 0.08)

Precision (repeatability), control material (BD FACSLyric™ flow cytometers)

A 21-day single-site precision study was performed to assess repeatability and within-site precision using control material.[24] Estimates of precision were determined across three BD FACSLyric™ flow cytometers and three operators by acquiring four concentrations of analyte, CD-Chex Plus®, CD-Chex CD34® Level 1, CD-Chex CD34® Level 2, and CD-Chex CD34® Level 3, stained in duplicate using three lots of BD® Stem Cell Enumeration Kit. Two separate runs were analyzed during each of the 21 tested days.

Precision was estimated for the following subsets:

- Total $CD34^+$ absolute counts (cells/µL)
- Total $CD34^+$ stem cells as a percentage of total $CD45^+$ (%)
- Total $CD45^+$ absolute counts (cells/µL)

Standard deviations (SD) or coefficients of variation (%CV) for within-run and total precision are shown in the following tables.

Table 8 Repeatability and within-site precision for total $CD34^+$ absolute counts

Sample type	Precision	Mean (cells/µL)	SD	%CV
CD-Chex Plus	Within run	3.53	0.56	NA
	Total		0.62	NA
CD-Chex CD34 Level 1	Within run	13.23	1.55	NA
	Total		1.59	NA
CD-Chex CD34 Level 2	Within run	36.47	NA	8.50
	Total		NA	8.93
CD-Chex CD34 Level 3	Within run	120	NA	5.59
	Total		NA	6.35

Table 9 Repeatability and within-site precision for total CD34+ stem cells as a percentage of total CD45+

Sample type	Precision	Mean (%)	SD	%CV
CD-Chex Plus	Within run	0.05	0.01	NA
	Total		0.01	NA
CD-Chex CD34 Level 1	Within run	0.21	0.02	NA
	Total		0.02	NA
CD-Chex CD34 Level 2	Within run	0.56	NA	6.63
	Total		NA	6.94
CD-Chex CD34 Level 3	Within run	1.8	NA	3.03
	Total		NA	3.12

Table 10 Repeatability and within-site precision for total CD45+ absolute counts

Sample type	Precision	Mean (cells/µL)	%CV
CD-Chex Plus	Within run	6,764.71	6.14
	Total		8.11
CD-Chex CD34 Level 1	Within run	6,403.86	4.67
	Total		5.35
CD-Chex CD34 Level 2	Within run	6,465.39	5.13
	Total		6.09
CD-Chex CD34 Level 3	Within run	6,597.5	4.68
	Total		5.48

Precision (repeatability), clinical specimens (BD FACSLyric™ flow cytometers)

A single-site precision study was performed to evaluate system repeatability and within-site precision using clinical specimens. The following specimen types were assessed:

- Normal and mobilized peripheral blood
- Fresh and thawed cord blood
- Fresh and thawed leukapheresis products
- Fresh and thawed bone marrow.

For each sample type, four replicates were stained per instrument using three lots of BD® Stem Cell Reagent, and acquired. Three BD FACSLyric™ flow cytometers and multiple operators were used in the study.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for repeatability and within-site precision of $CD34^+$ absolute counts, $CD45^+$ absolute counts, percent viable $CD34^+$ (as a percentage of viable $CD45^+$), and the percent total $CD34^+$ (as a percentage of total $CD45^+$).

Table 11 Repeatability and within-site precision of $CD34^+$ absolute counts

Subset	Mean (cells/µL)	Repeatability		Within-site precision	
		SD	%CV	SD	%CV
Viable $CD34^+$	3.30	0.59	17.54	0.58	17.60
	119.97	18.72	15.60	19.15	15.96
Total $CD34^+$	3.70	0.73	19.85	0.75	20.33
	123.36	21.79	17.6	22.28	18.06

Table 12 Repeatability and within-site precision of CD45+ absolute counts

Subset	Mean (cells/µL)	%CV	
		Repeatability	Within-site precision
Viable CD45+	15,501.5	6.6	6.6
Total CD45+	16,695.1	6.2	6.3

Table 13 Repeatability and within-site precision of %CD34+ (as % of CD45+)

Subset	Mean (%)	Repeatability		Within-site precision	
		SD	%CV	SD	%CV
% Viable CD34+	1.03	0.11	10.8	0.11	10.8
% Total CD34+	0.83	0.08	9.4	0.08	9.5

Precision (reproducibility), control material (BD FACSLyric™ flow cytometers)

A study was performed at three sites to assess the reproducibility of the system. A single lot of each control material, CD-Chex CD34® (Level 1, Level 2, and Level 3) and CD-Chex Plus®, and three lots of BD® Stem Cell Enumeration Kit were provided to each site. For each type of control material, three replicates were stained using the BD® Stem Cell Reagent. Testing was performed twice per day for 10 non-consecutive days, with one operator and one BD FACSLyric™ flow cytometer at each site.

The following tables present the standard deviations (SDs) or coefficients of variation (%CVs) for reproducibility of the following:

- Total CD34+ absolute counts (cells/µL)
- Total CD34+ as a percentage of total CD45+
- Total CD45+ absolute counts (cells/µL)

Table 14 Reproducibility for total CD34+ absolute counts (cells/µL)

Sample type	Mean (cells/µL)	Repeatability		Reproducibility	
		SD	%CV	SD	%CV
CD-Chex Plus (very low)	2.30	0.44	NA	0.47	NA
Level 1 (low)	12.19	1.35	NA	1.38	NA
Level 2 (normal)	34.70	NA	6.40	NA	6.69
Level 3 (high)	118.33	NA	4.88	NA	5.37

Table 15 Reproducibility for total CD34+ percentage (%)

Sample type	Mean (%)	Repeatability		Reproducibility	
		SD	%CV	SD	%CV
CD-Chex Plus (very low)	0.04	0.01	NA	0.01	NA
Level 1 (low)	0.18	0.02	NA	0.02	NA
Level 2 (normal)	0.51	NA	5.63	NA	5.80
Level 3 (high)	1.72	NA	3.77	NA	3.87

Table 16 Reproducibility for total CD45+ absolute counts (cells/µL)

Sample type	Mean (cells/µL)	Repeatability (%CV)	Reproducibility (%CV)
CD-Chex Plus (very low)	6,699.30	3.20	3.31
Level 1 (low)	6,778.64	3.88	4.48
Level 2 (normal)	6,752.52	3.23	3.35
Level 3 (high)	6,894.76	2.99	3.37

Limit of blank (BD FACSLyric™ flow cytometers)

A study evaluated the Limit of Blank (LOB) detection capability of the BD® Stem Cell Enumeration Kit with the BD FACSLyric™ flow cytometer for viable CD34+ absolute counts. A 10-day study across three BD FACSLyric™ flow cytometers and three operators used cell-free plasma extracted from normal peripheral blood (one donor per day). For each donor, six replicates were stained with each of three lots of the BD® Stem Cell Enumeration Kit. The LOB is 0 cells/µL.

Analytical sensitivity (BD FACSLyric™ flow cytometers)

Studies were performed to evaluate the analytical sensitivity of the BD® Stem Cell Enumeration Kit on the BD FACSLyric™ flow cytometer at the low end of the measuring range (≤5 cells/µL) for viable CD34+ cells. Accuracy and repeatability at the low end of the range were assessed.

Accuracy was assessed using the BD® Stem Cell Enumeration Kit to stain normal peripheral blood from 36 donors. Specimens containing ≤5 cells/µL were stained in two replicates using one of three lots of BD® Stem Cell Reagent in BD Trucount™ Tubes. One replicate was acquired on one of three BD FACSLyric™ flow cytometers and the other was acquired on a BD FACSCanto™ II flow cytometer.

The mean absolute difference with the 95% confidence interval (CI) is summarized.

Table 17 Bias analysis for BD FACSLyric™ vs BD FACSCanto™ II flow cytometers

Mean absolute difference (95% CI)
0.15 (–0.06, 0.37)

Repeatability of low count specimens, containing ≤5 cells/µL, was evaluated using the BD® Stem Cell Enumeration Kit to stain normal peripheral blood from 19 donors. Specimens were stained in five replicates with three lots of BD® Stem Cell Reagent in BD Trucount™ Tubes. For each donor, 15 replicates were acquired across three BD FACSLyric™ flow cytometers.

The standard deviation (SD) and the upper 97.5% confidence level (CL) of the SD are presented.

Table 18 Repeatability of viable CD34+ absolute counts in low count specimens

Source	SD	Upper 97.5% CL of SD
Instrument	0.10	0.60
Within run	0.60	0.66
Total precision	0.61	0.67

Linearity (BD FACSLyric™ flow cytometers)

Thawed purified CD34+ cells were diluted in normal peripheral blood across the reportable range of the assay for viable CD34+ absolute counts. Nine dilutions were prepared to cover the low end of the range (0–100 cells/µL) and nine dilutions were prepared to cover the total range (0–1,000 cells/µL). Each diluted sample was stained by three operators using three lots of the BD® Stem Cell Enumeration Kit (one lot per operator), and acquired on one of three BD FACSLyric™ flow cytometers. Viable CD34+ absolute counts were observed to be linear across the full range of 1–1,000 cells/µL.

Analytical measuring range (AMR) (BD FACSLyric™ flow cytometers)

Data from the analytical sensitivity and linearity studies and from the method comparison study was used to establish the AMR for viable CD34+ absolute counts using the BD® Stem Cell Enumeration Kit. The lower end of the AMR was defined by the linearity study and confirmed by the analytical sensitivity studies. The upper end of the AMR was supported by data from the method comparison study and the linearity study. The AMR for viable CD34+ absolute counts is 1–1,000 cells/µL.

BD FACSCanto™ II Flow Cytometers

Method comparison (BD FACSCanto™ II flow cytometers)

CD34+ absolute counts were enumerated and percentages of CD34+ cells were determined with the BD® Stem Cell Enumeration Kit on BD FACSCanto™ II flow cytometers and compared with results from the predicate reagent† on a BD FACSCanto™ II flow cytometer.

Normal and mobilized peripheral blood, leukapheresis products, cord blood, and bone marrow specimens were collected from donors from four clinical laboratories.

Table 19 summarizes the results of the BD® Stem Cell Enumeration Kit accuracy study on the BD FACSCanto™ II flow cytometer. For each evaluable specimen, the differences (bias) between the values of the investigational and predicate systems for absolute viable CD34 and % viable CD34 in CD45 were calculated. The results were pooled to produce mean biases of CD34 and %CD34 for each bin, along with the 95% confidence interval (CI).

† Beckman Coulter Stem-Kit™ Reagents

Table 19 Accuracy study results of the BD® Stem Cell Enumeration Kit on the BD FACSCanto™ II flow cytometer compared with the predicate method

Variables	Bin	N	Absolute Difference		Relative Difference to Predicate	
			Mean Absolute Bias	95% CI	Mean Relative Bias	95% CI
Viable CD34	Low	167	–0.1	(–0.5, 0.3)	NA	NA
	Mid	496	–0.7	(–1.4, –0.04)	–1.6	(–3.1, –0.2)
	High	255	–1.4	(–4.3, 1.6)	–1.0	(–2.4, 0.4)
%CD34 in CD45	Low	512	–0.004	(–0.01, 0.0002)	–2.2	(–4.4, –0.1)
	High	406	0.08	(0.04, 0.1)	2.1	(–0.1, 4.3)

Regression plots and statistics for viable CD34 counts and %CD34 in CD45 on the BD FACSCanto™ II flow cytometer are shown in Figure 2 and Figure 3. The solid line in each plot is the fitted line. The dotted line in each plot is the identity line where the predicate results are equal to the BD® Stem Cell Enumeration Kit results.

Figure 2 Regression plot of all specimen types combined (BD FACSCanto™ II flow cytometer)

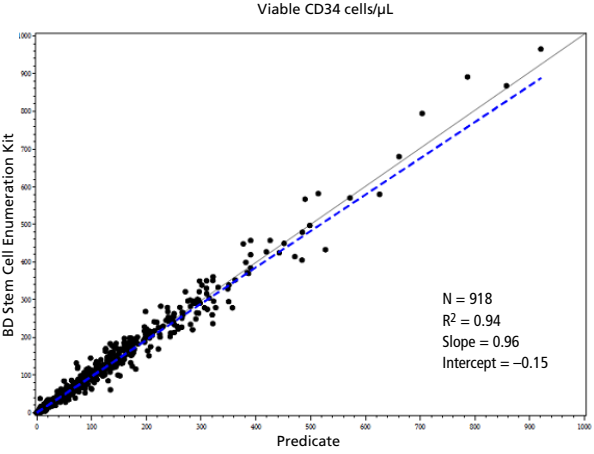


Figure 3 Regression plot of all specimen types combined (BD FACSCanto™ II flow cytometer)

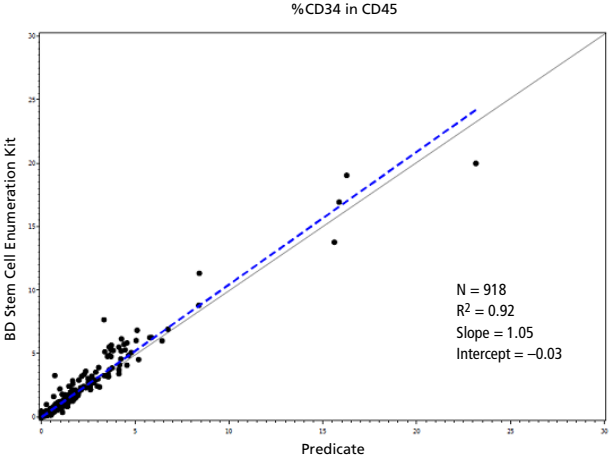


Table 20 summarizes the regression statistics of the assay for each specimen type on BD FACSCanto™ II flow cytometers.

Table 20 Regression statistics on BD FACSCanto™ II by specimen type

Specimen Type	Variable	N	R^2	Slope	Intercept
Peripheral blood	Viable CD34	188 (normal: 57, mobilized: 131)	0.94	0.96 (0.93, 0.99)	–0.07 (–0.21, 0.07)
	%CD34 in CD45	188 (normal: 57, mobilized: 131)	0.98	0.99 (0.93, 1.06)	0.00 (–0.01, 0.00)
Leukapheresis products	Viable CD34	341 (fresh: 232, frozen: 109)	0.97	0.96 (0.94, 0.98)	–0.02 (–0.2, 0.17)
	%CD34 in CD45	341 (fresh: 232, frozen: 109)	0.95	0.96 (0.94, 0.99)	–0.02 (–0.03, 0.00)
Cord blood	Viable CD34	241 (fresh: 124, frozen: 117)	0.88	0.97 (0.93, 1.02)	–0.52 (–0.92, –0.11)
	%CD34 in CD45	241 (fresh: 124, frozen: 117)	0.87	1.02 (0.94, 1.09)	–0.02 (–0.04, 0.01)
Bone marrow	Viable CD34	148 (fresh: 75, frozen: 73)	0.95	1.00 (0.96, 1.03)	–0.02 (–0.13, 0.10)
	%CD34 in CD45	148 (fresh: 75, frozen: 73)	0.89	1.21 (1.13, 1.28)	–0.05 (–0.10, 0.00)

Precision (BD FACSCanto™ II flow cytometers)

Estimates of assay precision were determined at BD Biosciences using BD® SCE Control High (with a range of >10 to ≤100 cells/µL) and BD® SCE Control Custom Low (with a range of ≤10 cells/µL) process controls stained in duplicate with the BD® Stem Cell Enumeration Kit and run on the BD FACSCanto™ II flow cytometer. Two separate runs were analyzed during each of the 21 days. Means, standard deviations (SDs), and coefficients of variation (CVs) are provided for within-run and total system precision in Table 21 through Table 24.

Table 21 Within-run precision of CD34+ absolute counts (cells/µL)

Control	Mean (cells/µL)	SD	%CV
Control High	35.3	1.83	5.2
Control Custom Low	8.8	0.90	10.3

Table 22 Within-run precision of %CD34+

Control	Mean (%)	SD	%CV
Control High	0.58	0.03	4.7
Control Custom Low	0.15	0.01	9.6

Table 23 Total system precision of CD34+ absolute counts (cells/µL)

Control	Mean (cells/µL)	SD	%CV
Control High	35.3	1.83	5.2
Control Custom Low	8.8	0.95	10.8

Table 24 Total system precision of %CD34+

Control	Mean (%)	SD	%CV
Control High	0.58	0.03	4.7
Control Custom Low	0.15	0.01	10.2

Linearity (BD FACSCanto™ II flow cytometers)

Linearity was assessed at BD Biosciences. Triplicate measurements of multiple concentrations of frozen purified CD34+ cells spiked into normal blood were analyzed across the reportable range of the assay for CD34+ absolute counts on the BD FACSCanto™ II and BD FACSCalibur™ instruments. Low range = 0–100 cells/µL and total range = 0–1,000 cells/µL. Results were observed to be linear within the CD34+ absolute count range.

Specimen handling and collection (AOB/AOS) (BD FACSCanto™ II flow cytometer)

A study was performed to assess the Age of Blood (AOB) and Age of Stain (AOS) using the BD® Stem Cell Enumeration Kit. The stability of fresh leukapheresis products was evaluated by assessing the combined effect of:

- AOB: Time duration between specimen draw and staining
- AOS: Time duration between staining specimen (end of lysis) and acquiring the stained sample.

All specimens were maintained at 2°C–8°C before staining. Based on the results of this study using leukapheresis products, we recommend staining specimens within 24 hours of collection, keeping stained samples on wet ice, and analyzing stained samples within 1 hour of lysing.

BD FACSCalibur™ Flow Cytometers

Agreement (BD FACSCalibur™ flow cytometers)

$CD34^+$ absolute counts were enumerated and percentages of $CD34^+$ cells were determined with the BD® Stem Cell Enumeration Kit on both BD FACSCanto™ II and BD FACSCalibur™ flow cytometers and compared with results from the predicate reagent‡ on a BD FACSCanto™ II flow cytometer.

Normal and mobilized peripheral blood, leukapheresis products, cord blood, and bone marrow specimens were collected from donors from four clinical laboratories.

Table 25 summarizes the results of the BD® Stem Cell Enumeration Kit accuracy study on the BD FACSCalibur™ flow cytometer. For each evaluable specimen, the differences (bias) between the values of the investigational and predicate systems for absolute viable CD34 and % viable CD34 in CD45 were calculated. The results were pooled to produce mean biases of CD34 and %CD34 for each bin, along with the 95% confidence interval (CI).

‡ Beckman Coulter Stem-Kit™ Reagents

Table 25 Accuracy study results of the BD® Stem Cell Enumeration Kit on the BD FACSCalibur™ flow cytometer compared with the predicate method

Variables	Bin	N	Absolute Difference		Relative Difference to Predicate	
			Mean Absolute Bias	95% CI	Mean Relative Bias	95% CI
Viable CD34	Low	156	–0.2	(–0.4, –0.05)	NA	NA
	Mid	492	–0.5	(–1.3, 0.2)	–0.9	(–2.3, 0.5)
	High	257	–3.6	(–6.8, –0.3)	–1.7	(–3.1, –0.2)
%CD34 in CD45	Low	487	–0.002	(–0.01, 0.001)	–0.8	(–3.0, 1.3)
	High	418	0.2	(0.09, 0.2)	3.2	(0.9, 5.5)

Regression plots and statistics for $CD34^+$ counts and $\%CD34^+$ in $CD45^+$ on the BD FACSCalibur™ flow cytometer are shown in Figure 4 and Figure 5. The solid line in each plot is the fitted line. The dotted line in each plot is the identity line where the predicate results are equal to the BD® Stem Cell Enumeration Kit results.

Figure 4 Regression plot of all specimen types combined (BD FACSCalibur™ flow cytometer)

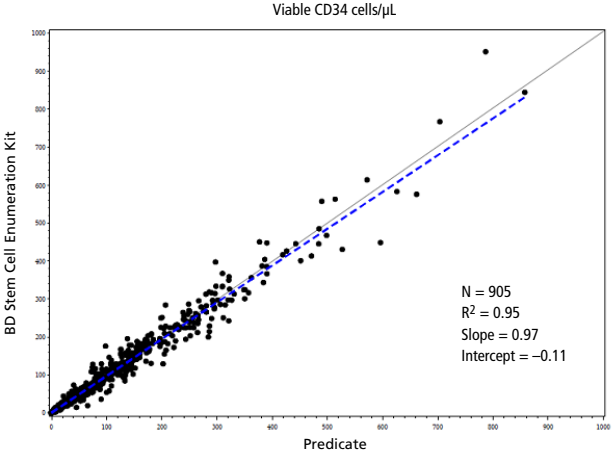


Figure 5 Regression plot of all specimen types combined (BD FACSCalibur™ flow cytometer)

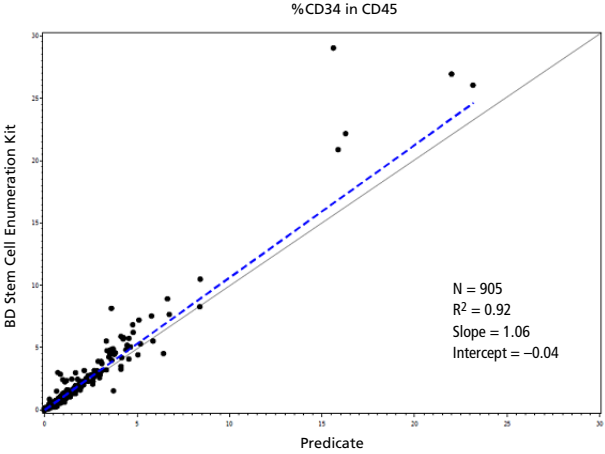


Table 26 summarizes the regression statistics of the assay for each specimen type on BD FACSCalibur™ flow cytometers.

Table 26 Regression statistics on BD FACSCalibur™ by specimen type

Specimen Type	Variable	N	R^2	Slope	Intercept
Peripheral blood	Viable CD34	167 (normal: 52, mobilized: 115)	0.94	1.00 (0.96, 1.03)	–0.13 (–0.32, 0.05)
	%CD34 in CD45	167 (normal: 52, mobilized: 115)	0.97	0.97 (0.9, 1.05)	0.00 (–0.01, 0.01)

Table 26 Regression statistics on BD FACSCalibur™ by specimen type

Specimen Type	Variable	N	R^2	Slope	Intercept
Leukapheresis products	Viable CD34	342 (fresh: 232, frozen: 110)	0.97	0.98 (0.96, 0.99)	0.04 (–0.06, 0.15)
	%CD34 in CD45	342 (fresh: 232, frozen: 110)	0.96	0.98 (0.96, 1.00)	–0.02 (–0.03, 0.00)
Cord blood	Viable CD34	245 (fresh: 122, frozen:123)	0.94	0.96 (0.93, 0.99)	–0.28 (–0.52, –0.04)
	%CD34 in CD45	245 (fresh: 122, frozen:123)	0.87	1.02 (0.92, 1.11)	–0.02 (–0.05, 0.02)
Bone marrow	Viable CD34	151 (fresh: 73, frozen: 78)	0.94	0.96 (0.92, 1.00)	–0.03 (–0.09, 0.03)
	%CD34 in CD45	151 (fresh:73, frozen: 78)	0.88	1.23 (1.15, 1.31)	–0.08 (–0.12, –0.03)

Precision (BD FACSCalibur™ flow cytometers)

Estimates of assay precision were determined at BD Biosciences using BD® SCE Control High (with a range of >10 to ≤100 cells/µL) and BD® SCE Control Custom Low (with a range of ≤10 cells/µL) process controls stained in duplicate with the BD® Stem Cell Enumeration kit and run on the BD FACSCalibur™ flow cytometer. Two separate runs were analyzed during each of the 21 days. Means, standard deviations (SDs), and coefficients of variation (CVs) are provided for within-run and total system precision in Table 27 through Table 30.

Table 27 Within-run precision of $CD34^+$ absolute counts (cells/µL)

Control	Mean (cells/µL)	SD	%CV
Control High	35.5	1.69	4.8
Control Custom Low	8.8	0.75	8.6

Table 28 Within-run precision of %CD34+

Control	Mean (%)	SD	%CV
Control High	0.58	0.03	4.7
Control Custom Low	0.14	0.01	8.2

Table 29 Total system precision of CD34+ absolute counts (cells/µL)

Control	Mean (cells/µL)	SD	%CV
Control High	35.5	2.02	5.7
Control Custom Low	8.8	0.82	9.3

Table 30 Total system precision of %CD34+

Control	Mean (%)	SD	%CV
Control High	0.58	0.03	5.1
Control Custom Low	0.14	0.01	9.4

Linearity (BD FACSCalibur™ flow cytometers)

Linearity was assessed at BD Biosciences. Triplicate measurements of multiple concentrations of frozen purified CD34+ cells spiked into normal blood were analyzed across the reportable range of the assay for CD34+ absolute counts on the BD FACSCanto™ II and BD FACSCalibur™ instruments. Low range = 0–100 cells/µL and total range = 0–1,000 cells/µL. Results were observed to be linear within the CD34+ absolute count range.

Specimen handling and collection (AOB/AOS) (BD FACSCalibur™ flow cytometers)

A study was performed to assess the Age of Blood (AOB) and Age of Stain (AOS) using the BD® Stem Cell Enumeration Kit. The stability of fresh leukapheresis products was evaluated by assessing the combined effect of:

- AOB: Time duration between specimen draw and staining
- AOS: Time duration between staining specimen (end of lysis) and acquiring the stained sample.

All specimens were maintained at 2°C–8°C before staining. Based on the results of this study using leukapheresis products, we recommend staining specimens within 24 hours of collection, keeping stained samples on wet ice, and analyzing stained samples within 1 hour of lysing.

11. TROUBLESHOOTING

Problem	Possible Cause	Solution
Resolution between debris and lymphocytes is poor.	Cells interact with other cells and platelets.	Prepare and stain another sample.
	Specimen was handled roughly during cell preparation.	Check cell viability. Centrifuge cells at lower speed.
	Inappropriate instrument settings.	Follow proper instrument setup procedures. Optimize instrument settings as required.
Staining is dim or fading.	Cell concentration was too high at staining step.	Check and adjust cell concentration or sample volume. Repeat staining.
	Insufficient reagent was used.	Repeat staining with increased amount of antibody.
	Cells were not analyzed within 1 hour of lysing.	Repeat staining with fresh specimen. Analyze promptly.

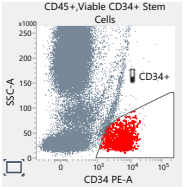
Problem	Possible Cause	Solution
Few or no cells observed.	Cell concentration is too low.	Dilute samples by a smaller dilution factor. Repeat staining and analysis.
	Cytometer is malfunctioning.	Troubleshoot instrument.

Differences in the assay between BD FACSuite™ Clinical application and BD FACSCanto™ clinical software:

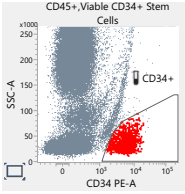
Feature	BD FACSuite™ Clinical application	BD FACSCanto™ clinical software
Acquisition stopping time	10 minutes	15 minutes
Ability to change acquisition stopping time	No	Yes
CD34+ gate (found on the CD34 PE-A vs SSC-A dot plot from the CD45+, Viable CD34+ Stem Cells population)	Yes	No

The new CD34+ gate is used to help exclude the platelet streak and other debris.

Before manual gating:



After manual re-gating to exclude platelet streak:



WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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