Stability of fresh leukapheresis, fresh cord blood and fresh bone marrow specimens using BD® Stem Cell Enumeration Kit on BD FACSLyric™ Flow Cytometer

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INTRODUCTION

The BD® Stem Cell Enumeration (SCE) Kit on BD FACSLyric™ System is a single tube, in vitro diagnostic intended for enumeration of viable dual-positive CD45+/CD34+ hematopoietic stem cells to determine viable CD34+ absolute counts (cells/µL) and percentages of viable CD45+/CD34+ hematopoietic stem cells. The ability of the BD® SCE Kit to accurately measure the drift of analytes over 24 and 48 hours was evaluated on the BD FACSLyric™ System for three specimen types: Fresh leukapheresis, fresh cord blood and fresh bone marrow. Various timepoints were selected for Age of Blood (AOB: duration between specimen draw and staining with SCE kit) and Age of Stain (AOS: duration between stained sample end of lyse and acquisition). Mean difference with 95% CI was calculated between each test and reference timepoint (AOB ≤ 6h and AOS 10min) across all samples. Fresh leukapheresis and fresh bone marrow specimens exhibited a mean difference of 5.87% (1.06, 10.68) and -8.32% (-11.75, -4.89), respectively, for viable CD34+ absolute counts at 24-hour AOB and 1-hour AOS. A mean difference of -4.92% (-8.12, -1.71) at 48-hour AOB and 1-hour AOS was determined for fresh cord blood specimens. We demonstrated that stained samples from all three specimen types were stable for 1 hour when stored on wet ice. We also established that Fresh Leukapheresis and Bone Marrow specimens were stable for 24 hours and Fresh Cord Blood specimens were stable for 48 hours from collection when evaluated with the BD® Stem Cell Enumeration Kit.

MATERIALS AND METHODS

Study was conducted using de-identified purchased fresh cord blood specimens anticoagulated with EDTA, heparin, CPD, or ACD-A and de-identified remnant or procured fresh leukapheresis specimens anticoagulated with EDTA, heparin, ACD-A, or a mixture of ACD-A, heparin, and EDTA. Fresh bone marrow specimens were obtained from external vendors anticoagulated with heparin.

Specimens	Fresh Cord Blood	Fresh Leukapheresis	Fresh Bone Marrow
Number of samples at reference timepoint	65	70	33

All specimens were stored undiluted at 2 to 8 ºC and stained within 6 hours of draw for the reference timepoint (T0). Stained samples were stored on wet ice until they were acquired. All samples were stained per the BD® Stem Cell Enumeration Kit Instructions for Use. The BD® Stem Cell Enumeration Kit contains BD® Stem Cell Reagent (CD45 FITC/CD34 PE), 7-AAD reagent, 10X ammonium chloride lysing solution, and BD® Trucount Tubes. Samples were acquired on BD FACSLyric™ 10-Color or 12-Color Flow Cytometers using the BD FACSuite™ Clinical Application and BD® Stem Cell Enumeration Assay module. BD® CS&T Beads were used for daily QC and setup of the stem cell assay. BD® FC Beads 7-Color Kit was used to setup compensation and BD® Stem Cell Controls stained with 7-AAD was added to the compensation matrix.

ANALYSIS

The difference between each test timepoint and reference timepoint (T0) results were calculated for each paired specimen and pooled across all specimens to produce the mean difference for each time point with 95% confidence interval.

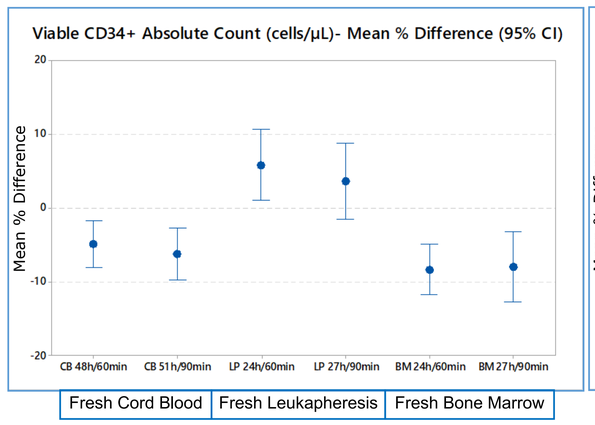
RESULTS

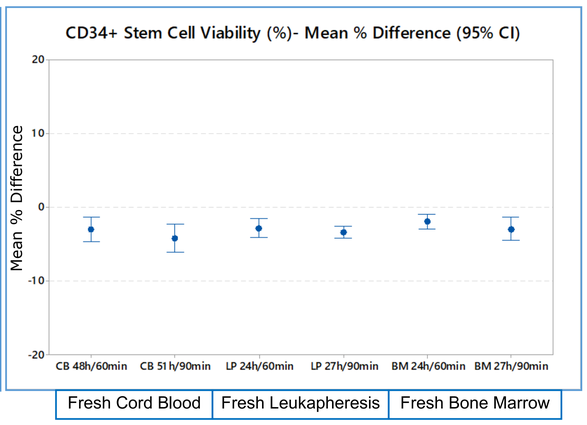
The gating strategy and analysis for the BD® Stem Cell Enumeration Assay module on BD FACSLyric™ Flow Cytometer are consistent with ISHAGE guidelines. The results for Viable CD34+ Absolute count (cells/µL) and CD34 Stem Cell Viability (%) provided by the BD FACSuite™ Clinical Application were used to assess stability of each specimen type. The reference timepoint (T0) for all specimen types was AOB ≤ 6 h and AOS 10 min.

Mean difference for each timepoint compared to T0:

Timepoint	Parameter	Number of donors	Mean %Difference (95% CI)
Fresh Cord Blood			
48 h AOB 60 min AOS	Viable CD34+ Absolute count (cells/µL)	65	-4.92 (-8.12, -1.71)
	CD34+ Stem Cell Viability (%)	65	-3.01 (-4.66, -1.36)
51 h AOB 90 min AOS	Viable CD34+ Absolute count (cells/µL)	63	-6.21 (-9.68, -2.73)
	CD34+ Stem Cell Viability (%)	63	-4.17 (-6.08, -2.25)
Fresh Leukapheresis			
24 h AOB 60 min AOS	Viable CD34+ Absolute count (cells/µL)	70	5.87 (1.06, 10.68)
	CD34+ Stem Cell Viability (%)	70	-2.80 (-4.10, -1.50)
27 h AOB 90 min AOS	Viable CD34+ Absolute count (cells/µL)	70	3.63 (-1.52, 8.79)
	CD34+ Stem Cell Viability (%)	70	-3.41 (-4.24, 2.58)
Fresh Bone Marrow			
24 h AOB 60 min AOS	Viable CD34+ Absolute count (cells/µL)	33	-8.32 (-11.75, -4.89)
	CD34+ Stem Cell Viability (%)	33	-1.92 (-2.91, -0.92)
27 h AOB 90 min AOS	Viable CD34+ Absolute count (cells/µL)	33	-8.03 (-12.76, -3.29)
	CD34+ Stem Cell Viability (%)	33	-2.93 (-4.48, -1.38)

Interval Plots for Viable CD34+ Absolute count (cells/µL) (left) and CD34+ Stem Cell Viability (%) (right):





CONCLUSION

We established that ***fresh leukapheresis and bone marrow specimens were stable for 24 hours*** and ***fresh cord blood specimens were stable for 48 hours*** from collection when stored at 2 to 8 ºC and evaluated with the BD® Stem Cell Enumeration Kit. Stained samples from all three specimen types were stable for 1 hour when stored on wet ice.

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.