Interference evaluation of the BD® Stem Cell Enumeration Kit on BD FACSLyric™ Flow Cytometer

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Introduction

The BD® Stem Cell Enumeration Kit is a single tube in vitro diagnostic assay intended for enumeration of viable dual-positive CD45+/CD34+ hematopoietic stem cell populations to determine viable CD34+ absolute counts (cells/µL) and the percentages of viable CD45+/CD34+ hematopoietic stem cells. The BD® Stem Cell Enumeration (SCE) Kit was evaluated using fresh cord blood, fresh bone marrow and fresh leukapheresis in the presence of endogenous and exogenous interfering substances on the BD FACSLyric™ Flow Cytometer using the BD FACSuite™ Clinical Application with the BD® Stem Cell Enumeration module for acquisition and analysis. The interferents to be evaluated included substances likely to be present in patient specimens and were evaluated according to CLSI EP07 Interference Testing in Clinical Chemistry 3rd Edition, April 2018. The concentrations tested were based on three times the maximum therapeutic dose observed in patients or the highest expected concentrations per CLSI Supplement (EP37) Supplemental Tables for Interference Testing in Clinical Chemistry 1st Edition, April 2018. The substances evaluated in this study included albumin, bilirubin cyclophosphamide, hemoglobin, doxorubicin, GCSF, intralipid and paclitaxel. For each of the specimen types, 15 replicates were stained using the BD® SCE Kit for each interferent condition. A total of nine specimens comprising of three leukapheresis, two bone marrows and four cord bloods were used to complete the evaluation. A paired difference analysis was used to evaluate the Control (no interferents) versus the Test (with interferents) conditions. There was no detectable interference from the substances tested at the highest concentrations. Precision of the measurements was also calculated with 95% CI.

Methods

Study was conducted using fresh bone marrow, fresh cord blood and fresh leukapheresis specimens anticoagulated with heparin, EDTA and ACD-A respectively. Interference performance was determined by comparing the reference sample to the same sample spiked with individual interferents or a mixture of interferents.

Reference:	Test Conditions (Interferents)		
Specimen+ DMSO	Specimen+ Interference Mix (Albumin, GCSF, Cyclophosphamide, Hemoglobin, Paclitaxel, Intralipid) at 1x, 2x and 3x concentrations	Specimen + Doxorubicin at 1x, 2x and 3x concentrations	Specimen + Bilirubin at 1x, 2x, 3x concentrations

Specimens were stained within 24 hours of collection for fresh bone marrow and fresh leukapheresis products. For fresh cord blood, samples were stained within 48 hours of collection. Stained samples were stored on wet ice and were acquired within 1 hour after lysing was complete. All samples were stained per the BD® Stem Cell Enumeration Kit Instructions for Use. The BD® SCE 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™ Flow Cytometers with 4-3-5 configurations using the BD FACSuite™ Clinical Application (v1.4.) 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 were added to the compensation matrix. A paired difference analysis was performed, i.e., mean of the test condition replicates was compared to the mean of the control sample. Precision of the measurements was also calculated with 95% CI.

Results

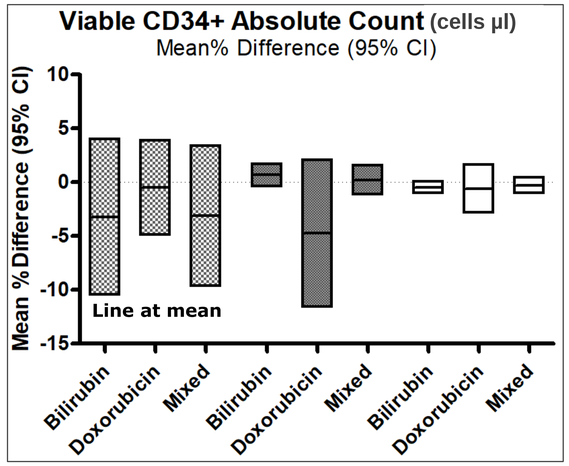
The gating strategy and analysis for the BD® Stem Cell Enumeration Assay module on BD FACSLyric™ Flow Cytometer are consistent with ISHAGE guidelines. The results for Viable CD34+ Absolute count (cells/µL) and CD34 Stem Cell Viability (%) provided by the BD FACSuite™ Clinical Application were used to assess the performance of BD® SCE Assay in the presence of endogenous and exogenous interfering substances. The SCE assay results for all specimen types with (Test) and without (Control) interfering substances were analyzed by paired difference analysis with a 95% confidence interval.

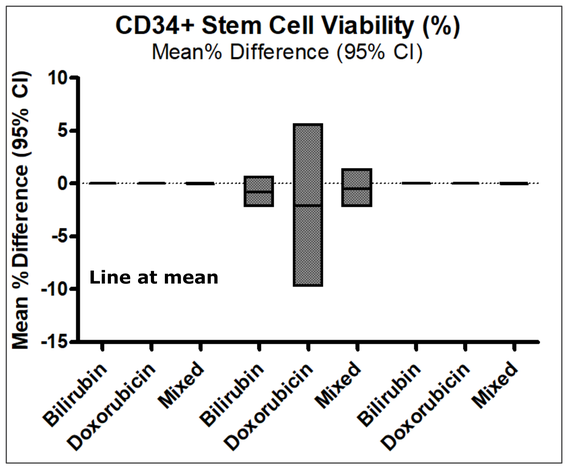
Summary of the mean % difference for each specimen with endogenous and exogenous interferents* at three times the concentration (3x) clinically observed in patients' plasma (cMax) :

Parameter	Interferent	Test Conc.	Control Mean	Mean %Difference (95% CI)
Fresh Leukapheresis Product				
Viable CD34+ Absolute count (cells/µL)	Bilirubin	3x	27.8	-3.2 (-10.5, 4.0)
	Doxorubicin	3x	29.0	-0.5 (-4.9, 3.9)
	Mixed	3x	175.4	-3.1 (-9.7, 3.4)
CD34+ Stem Cell Viability (%)	Bilirubin	3x	0.05	0.0 (-0.01, 0.0)
	Doxorubicin	3x	0.05	0.0 (0.0, 0.0)
	Mixed	3x	0.20	-0.01 (-0.02, 0.0)
Fresh Bone Marrow				
Viable CD34+ Absolute count (cells/µL)	Bilirubin	3x	29.9	0.7 (-0.4, 1.7)
	Doxorubicin	3x	84.3	-4.7 (-11.6, 2.1)
	Mixed	3x	29.9	0.2 (-1.2, 1.6)
CD34+ Stem Cell Viability (%)	Bilirubin	3x	0.42	-0.8 (-2.2, 0.6)
	Doxorubicin	3x	0.71	-2.1 (-9.7, 5.6)
	Mixed	3x	0.42	-0.5 (-2.2, 1.3)
Fresh Cord Blood				
Viable CD34+ Absolute count (cells/µL)	Bilirubin	3x	9.7	-0.5 (-1.08, 0.08)
	Doxorubicin	3x	23.2	-0.6 (-2.85, 1.65)
	Mixed	3x	9.4	-0.3 (-1.03, 0.43)
CD34+ Stem Cell Viability (%)	Bilirubin	3x	0.16	0.00 (-0.01, 0.01)
	Doxorubicin	3x	0.05	0.00 (0.00, 0.00)
	Mixed	3x	0.15	0.02 (-0.03, 0.00)

*Interference mix includes: albumin, GCSF, cyclophosphamide, hemoglobin, Paclitaxel, intralipid

Interval plots for Viable CD34+ Absolute Cap Count (cells/µL) (top) and CD34+ Stem Cell Viability (%) (bottom):





Fresh Leukapheresis
Fresh Bone Marrow
Fresh Cord Blood

Conclusion

We concluded that all exogenous and endogenous interferents tested did not interfere with the BD® Stem Cell Enumeration Assay in either leukapheresis, bone marrow or cord blood specimens at 1x, 2x or 3x (cMax) test concentrations.