CD34+ cell analysis on the BD FACSLyric™ Flow Cytometer using UK NEQAS samples and the BD® Stem Cell Enumeration (SCE) Kit

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Abstract

The CD34+ analysis protocol under the guidelines of ISHAGE has been established to ensure a standardized flow cytometric method for quantitating CD34+ cells. The BD® SCE Kit was used on the BD FACSLyric™ Flow Cytometer with BD FACSuite™ Clinical Application. CD34+ cells were analyzed using consortium samples provided by UK NEQAS to demonstrate agreement results compared to peer instruments and laboratories. In six UK NEQAS trials, twelve UK NEQAS CD34+ samples were stained with the BD® SCE Kit and run on the BD FACSLyric™ System. Analysis results were compared relative to the mean and SD of more than 132 laboratories that used the BD FACSCanto™ II Flow Cytometer in the UK NEQAS SCE Program. For absolute CD34+ counts, the absolute Z-score of each sample on the BD FACSLyric™ System ranged from 0.06 to 1.99. For %CD34+, the absolute Z-score of each sample ranged from 0 to 1.5. Our results demonstrated that the BD FACSLyric™ System generated consistent CD34+ analysis results compared with peer participants.

Materials & Methods

Reagents

BD® Stem Cell Enumeration Kit (Catalog No. 344563):

- BD® Stem Cell Reagent (CD45 FITC/CD34 PE).
- 7-AAD as nucleic acid dye used to identify dead cells.
- 10X ammonium chloride diluted to 1X as lysing solution for red blood cells.
- BD Trucount™ Tubes containing fluorescent beads with known bead count.

Instrument Configurations

- BD FACSCanto™ II 8-Color 4-2H-2V Flow Cytometer.
- BD FACSLyric™ 10-Color 4-Blue 3-Red 3-Violet or 12-Color 4-Blue 3-Red 5-Violet Flow Cytometer.

UK NEQAS Sample Processing

We received UK NEQAS samples for CD34 trial, and we analyzed them on the BD FACSLyric™ Flow Cytometer as shown in Tables 3 and 4. The UK NEQAS CD34 samples are preserved and stabilized. Determination of cell viability with marker for dead cells (7-AAD dye) was not applicable.

Each sample (100 µL) was stained with 20 µL of BD® Stem Cell Reagent (CD45 FITC/CD34 PE) in BD Trucount™ Tubes and incubated in the dark at room temperature for 20 min.

To each sample tube, we added 2 mL of 1X ammonium chloride to lyse red blood cells for 10 min at room temperature in the dark. Sample was placed on ice and acquired on the flow cytometer within one hour of lysing.

Sample Acquisition and Analysis

Samples were acquired on the BD FACSCanto™ II Flow Cytometer in BD FACSCanto™ Clinical Software and on the BD FACSLyric™ Flow Cytometer in BD FACSuite™ Clinical Application at medium flow rate with threshold on CD45 FITC at 400. Acquisition targets include a minimum of 75,000 CD45+ events, 125 CD34+ events, and 1,000 bead events using BD Trucount™ Tubes. If CD45+ events, CD34+ events and bead events don't meet the target numbers, the acquisition time must be at least 600 seconds or 10 min for samples to be evaluable on the BD FACSLyric™ Flow Cytometer and 900 seconds or 15 min on the BD FACSCanto™ II Flow Cytometer.

Results (1)

Figure 1. **Example dot plots of UK NEQAS SCE sample stained with BD® SCE Kit using BD® Stem Cell Reagent and acquired on the BD FACSCanto™ II and BD FACSLyric™ Systems.**

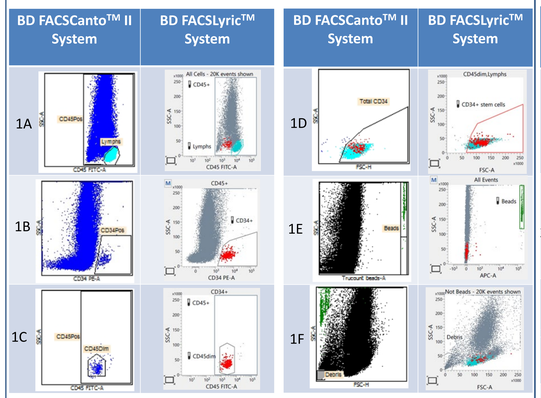
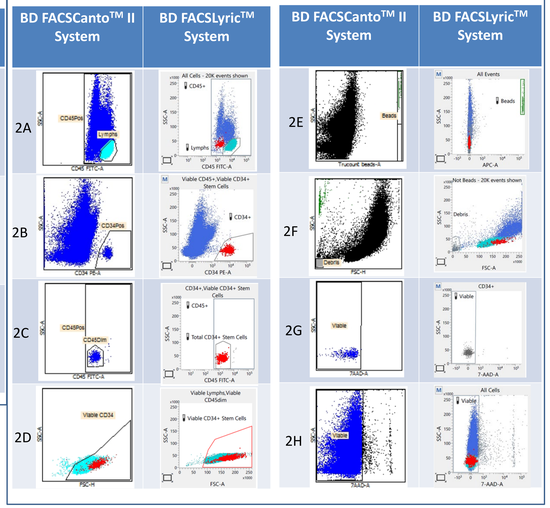


Figure 2. **Example dot plots of a fresh leukapheresis sample stained with the BD® SCE Kit using BD® Stem Cell Reagent and acquired on the BD FACSCanto™ II and FACSLyric™ Systems.**



Instrument Setup and Optimization for the BD® Stem Cell Enumeration Kit Assay

BD FACSCanto™ II System
- Setup cytometer with BD 7-Color Setup Beads (BD Cat No. 335775).
- Perform Stem Cell + 7-AAD assay optimization.
- Run BD® Stem Cell Controls (High and Low) as process controls to pass manufacturer specified ranges.

BD FACSLyric™ System
- Run performance QC with BD® CS&T Beads (BD Cat. No. 656505).
- Run Assay/Tube Settings Setup for Stem Cell + 7-AAD and Stem Cell Controls.
- Run BD® Stem Cell Controls (High and Low) as process controls to pass manufacturer specified ranges.

Table 1.
BD FACSCanto™ II System stem cell assay setup spillover values.

	FITC	PE	7-AAD	APC
FITC	100.00	0.62	0.03	0.00
PE	25.63	100.00	4.00	0.01
7-AAD	2.94	14.12	100.00	0.72
APC	0.04	0.07	8.59	100.00

Table 2.
BD FACSLyric™ System stem cell assay setup spillover values.

	FITC	PE	7-AAD	APC
FITC	100.00	0.78	0.11	0.00
PE	21.83	100.00	3.53	0.00
7-AAD	2.89	17.32	100.00	0.66
APC	0.01	0.02	3.56	100.00

Absolute count and percentages of total CD34+ cells were captured using ISHAGE-based gating templates as shown in Figure 1. Results are shown in Tables 3 and 4.

We compared CD34+ cell count results on the BD FACSLyric™ System relative to UK NEQAS evaluation reports using Z-score statistical analysis. The number of laboratories that used BD FACSCanto™ II Cytometers in UK NEQAS trials are shown in Tables 3 and 4. Robust mean and standard deviation of CD34+ cell absolute counts and percentages generated based on participating peer laboratories were provided by the UK NEQAS evaluation reports. Z-scores for absolute CD34 cell counts and %CD34+ cells are calculated based on the following equations, respectively:

$$\text{Z score} = \frac{Abs\ CD34\ count\ on\ FACSLyric - mean\ Abs\ CD34\ count\ of\ peers\ with\ FACSCanto\ II}{\text{SD of Abs CD34 count from peers with FACSCanto II}}$$

$$\text{Z score} = \frac{\%CD34\ on\ FACSLyric - mean\ \%CD34\ of\ peers\ with\ FACSCanto\ II}{\text{SD of \%CD34 from peers with FACSCanto II}}$$

We incorporated ISHAGE-based CD34+ template to the BD® CS&T Bead setup workflow on the BD FACSLyric™ Flow Cytometer and assessed CD34+ data from UK NEQAS samples to demonstrate agreement results compared to peer laboratories' BD FACSCanto™ II Systems. In the UK NEQAS trial, results are submitted by various laboratories. Robust mean and robust standard deviation for absolute CD34+ count and percent CD34+ are established by UK NEQAS based on results reported by participating laboratories. The same UK NEQAS samples were tested by our lab and acquired on the BD FACSLyric™ Flow Cytometer. We compared our results on the BD FACSLyric™ Flow Cytometer to more than 132 laboratories that used the BD FACSCanto™ II System. For the absolute CD34+ cell count (Table 3), the absolute z-score of the BD FACSLyric™ System ranged from 0.06 to 1.99. For percent CD34+ (Table 4), the absolute Z-score on BD FACSLyric™ System ranged from 0 to 1.5.

Results (2)

Table 3 **Comparison of CD34+ abs cell count of BD FACSLyric™ Flow Cytometer in our lab vs. BD FACSCanto™ II Flow Cytometer from UK NEQAS participating laboratories.**

	UK NEQAS Trial Statistics			BD FACSLyric™ System Results	
	CD34+ Abs Count on BD FACSCanto™ II System			CD34+ Abs Count on the BD FACSLyric™ System	
Sample	No. of Labs	Mean	SD	CD34+ Abs Count	Abs Z-Score
274	144	3.73	0.88	3	0.83
275	144	3.23	0.64	3	0.36
276	139	14.07	1.45	13	0.74
277	139	13.37	1.32	16	1.99
278	142	22.92	1.85	22	0.50
279	142	27.91	3.37	29	0.32
280	139	15.26	1.86	16	0.40
281	139	9.86	1.26	9	0.68
282	140	21.24	2.12	21	0.11
283	140	21.14	2.44	21	0.06
284	135	9.43	1.31	8	1.09
285	135	11	1.29	12	0.78

Table 4 **Comparison of percent CD34+ cells of BD FACSLyric™ Flow Cytometer in our lab vs. BD FACSCanto™ II Flow Cytometer from UK NEQAS participating laboratories.**

	UK NEQAS Trial Statistics			BD FACSLyric™ System Results	
	% CD34+ on BD FACSCanto™ II System			% CD34+ on BD FACSLyric™ System	
Sample	No. of Labs	Mean	SD	% CD34+	Abs Z-Score
274	140	0.05	0.01	0.04	1.00
275	140	0.2	0.05	0.15	1.00
276	134	0.26	0.03	0.22	1.33
277	134	0.24	0.02	0.27	1.50
278	138	0.37	0.03	0.34	1.00
279	138	0.42	0.04	0.41	0.25
280	134	0.33	0.04	0.34	0.25
281	134	0.15	0.02	0.13	1.00
282	136	0.51	0.05	0.51	0.00
283	136	0.51	0.05	0.47	0.80
284	132	0.2	0.03	0.17	1.00
285	132	0.16	0.02	0.16	0.00

Conclusions

Our results demonstrated that the BD FACSLyric™ Flow Cytometer generated consistent CD34+ analysis results when compared to peer laboratories using the BD FACSCanto™ II System in UK NEQAS trials.