Single Platform CD34+/CD45+ Cell Analysis on the FACSLyric™ System using UK NEQAS Samples

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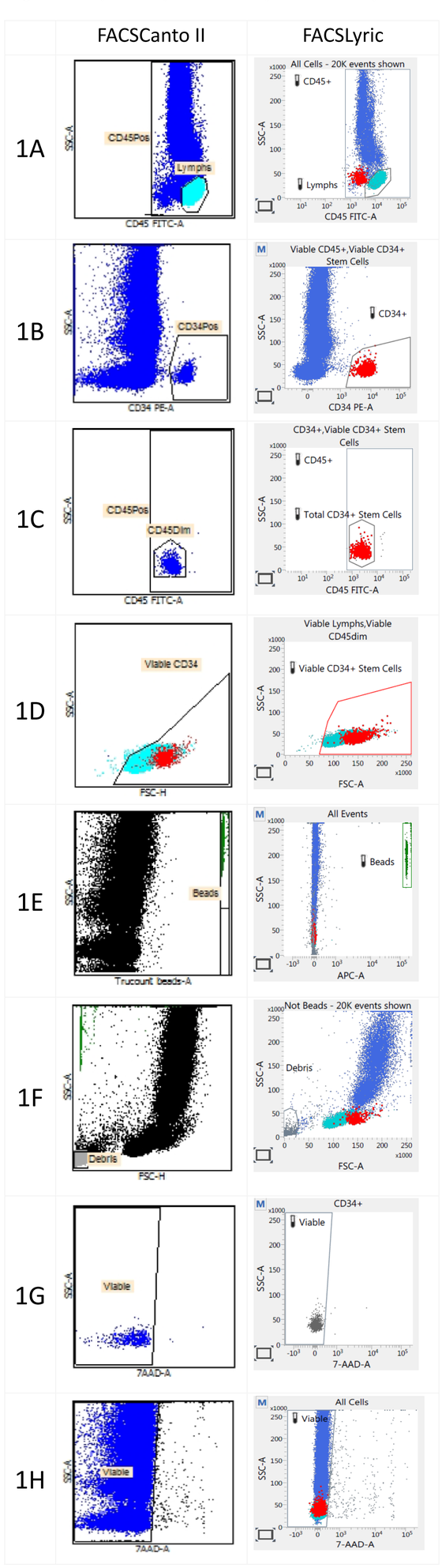
Introduction

Greater applications of flow cytometry for the immune system at the cellular level require significant efforts to standardize cell analysis methods, particularly for consistent results with minor and "dim" subsets. One example is flow-based CD34+ cell enumeration in a variety of sample types. In research settings, variations in cell counting tend to come from differences in sample handling, in instrument setup procedures, and in gating and data analysis strategies. The resulting divergent data lead to difficulties in comparing experiments across laboratories. To resolve these challenges, the CD34+ analysis protocol under the guidelines of International Society of Hematotherapy and Graft Engineering (ISHAGE) has been established to ensure a standardized flow cytometric method for quantitating CD34+ cells. The newly developed BD FACSLyric™ flow cytometer addresses the needs for instrument optimization and standardization aimed at achieving consistent results across laboratories. The BD FACSLyric system provides cytometer setup and tracking (CS&T) workflow for single-step daily instrument setup and QC, as well as automatic optimization of fluorescence compensation. Biological assays with the same tube or assay settings are transportable across different FACSLyric instruments for multisite studies. We incorporated ISHAGE-based CD34+ templates into the BD CS&T setup workflow on BD FACSLyric and assessed CD34+ analysis using consortium samples from the United Kingdom National External Quality Assessment Service (UK NEQAS) to demonstrate agreement results compared to peer instruments and laboratories.

Fig. 1 Example dot plots of an apheresis sample



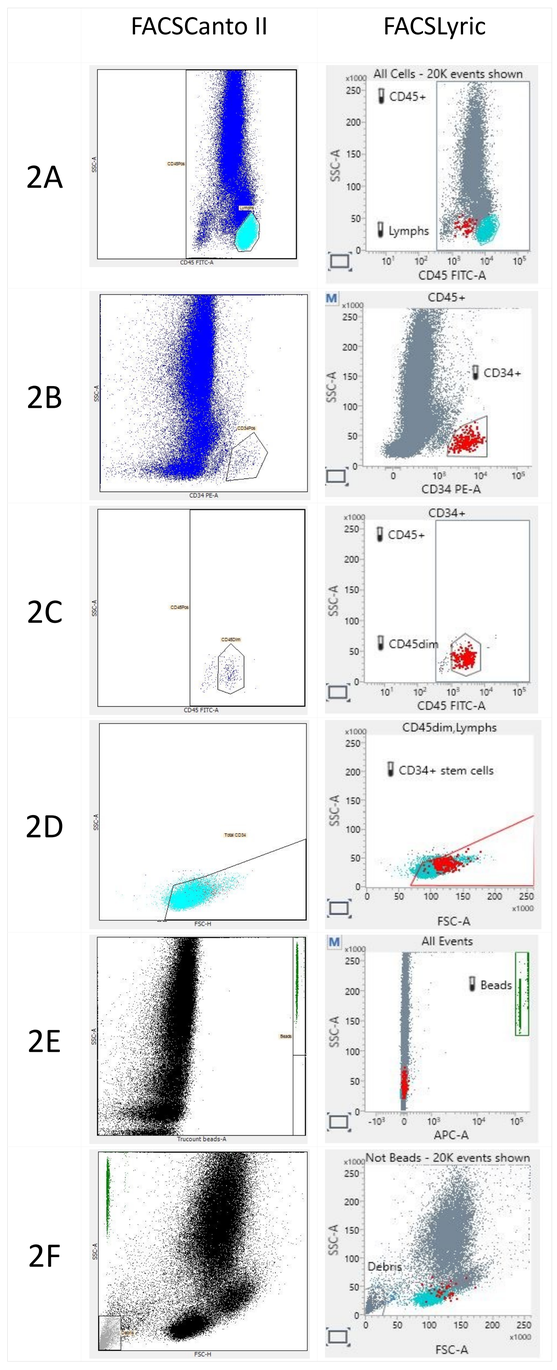
Reagents

CD45 FITC clone 2D1, CD34 PE clone 8G12, 7-AAD regent as a nucleic-acid dye used to identify dead cells, 10X ammonium chloride lysing solution diluted to 1X working solution as a fixative-free solution for red blood cell lysis, BD Trucount tubes containing a freeze-dried pellet of fluorescent beads

Instruments

BD FACSCanto II 8-color 4-2H-2V
BD FACSLyric 10-color 4-Blue 3-Red 3-Violet

Fig. 2 Example dot plots of a UK NEQAS Sample



Daily Instrument setup and optimization for the stem cell assay

BD FACSLyric

- Run Performance QC using BD CS&T beads (Cat# 656504 and 6566505)
- Run Assay/Tube Settings setup for Stem Cell +7-AAD and-Stem Cell Controls
- Run BD Stem Cell control (High and Low) as process

BD FACSCanto II

- Perform 7-color beads setup using BD 7-color Setup Beads (Cat# 335775)
- Perform Stem Cell + 7AAD assay optimization
- Run BD Stem Cell Control (High and Low) as process controls to pass manufacturer specified ranges

BD FACSLyric
Stem cell assay setup spillover values

	FITC	PE	7-AAD	APC
FITC	100.00	0.80	0.53	0.01
PE	19.49	100.00	4.88	0.01
7-AAD	2.18	15.09	100.00	0.59
APC	0.01	0.01	4.08	100.00

BD FACSCanto II
Stem cell assay setup spillover values

	FITC	PE	7-AAD	APC
FITC	100.00	0.88	0.00	0.00
PE	23.66	100.00	4.50	0.00
7-AAD	2.66	12.60	100.00	0.64
APC	0.00	0.03	8.80	100.00

UK NEQAS samples and preparation

We received UK NEQAS CD34+ samples #242—#247 from three UK NEQAS trials and analyzed these samples on two BD FACSLyric instruments in our lab, as shown in Tables 1 and 2. UK NEQAS CD34+ samples were preserved and stabilized. Determination of cell viability using 7-AAD was not applicable to these samples.

To stain a sample, add 20 uL of mixed CD45 FITC and CD34 PE reagents and 100 uL sample to a BD Trucount tube. Vortex gently and incubate for 20 minutes in the dark at room temperature (RT). Add 2 mL of 1X ammonium chloride lysing solution to each tube. Vortex gently and incubate for 10 minutes in the dark at RT. Immediately place tubes on wet ice and acquire within 1 hour of lysing.

Acquisition and analysis

Samples were acquired on BD FACSCanto and BD FACSLyric at medium flow rate with threshold on CD45 FITC 400. The default acquisition targets were set to acquired a minimum of viable CD45+ events 75,000, viable CD34+ events 125, and BD Trucount beads count 1,000. Absolute count and percentages of total CD34+ cells were analyzed using ISHAGE-based gating template, as shown in Figure 2. Results are shown in Tables 1 and 2.

We compared CD34+ cell count results on FACSLyric relative to UK NEQAS reports using z score statistical analysis. The number of laboratories that used BD FACSCanto II cytometers in the UK NEQAS trials are shown in Tables 1 and 2. Robust mean and standard deviation of CD34+ cell absolute count and percentages generated based on participating peer laboratories were provided by the UK NEQAS trial reports .

z score of CD34+ Abs Cnt for the BD FACSLyric (or FACSCanto) is defined as
z = (CD34+ Abs Cnt on FACSLyric — Robust mean of CD34+ Abs Cnt of peer laboratories)/Standard deviation of CD34+ Abs Cnt of peer laboratories

Table 1 Comparison of CD34+ absolute count between FACSLyric and UK NEQAS results

	UK NEQAS trial statistics			BD FACSLyric results			
	CD34+ Abs Cnt on FACSCanto			FACSLyric A		FACSLyric B	
Sample	No. Labs	Mean	SD	CD34+ Abs Cnt	z-score	CD34+ Abs Cnt	z-score
#242	140	23.02	2.64	23	-0.0076	25	0.75
#243	140	9.03	0.98	9	-0.031	8	-1.05
#244	143	56.85	3.94	55	-0.47	56	-0.22
#245	143	26.33	2.51	26	-0.13	26	-0.13
#246	138	36.76	4.04	36	-0.19		
#247	138	65.28	4.89	65	-0.057		

Table 2 Comparison of CD34+% between FACSLyric and UK NEQAS results

	UK NEQAS trial statistics			BD FACSLyric results			
	%CD34+ on FACSCanto			FACSLyric A		FACSLyric B	
Sample	No. Labs	Mean	SD	%CD34+	z-score	%CD34+	z-score
#242	136	0.33	0.03	0.34	0.33	0.37	1.33
#243	136	0.24	0.02	0.25	0.50	0.21	-1.50
#244	138	0.88	0.06	0.84	-0.67	0.88	0.00
#245	138	2.16	0.22	2.21	0.23	2.09	-0.32
#246	134	0.33	0.03	0.31	-0.67		
#247	134	1.97	0.21	1.83	-0.67		

Discussion

We incorporated ISHAGE-based CD34+ templates into the BD CS&T setup workflow on BD FACSLyric and assessed CD34+ analysis using the UK NEQAS consortium samples to demonstrate agreement results compared to peer instruments and laboratories. During UK NEQAS trials, stabilized CD34+ samples were tested in different laboratories worldwide. Robust mean and robust standard deviation (rSD) for the absolute CD34+ counts and percent CD34+ were established by UK NEQAS based on results reported by participating laboratories. The same UK NEQAS samples were delivered to our laboratory, where they were stained with CD34/CD45 reagents and run on the BD FACSLyric system. We compared analysis results relative to the robust mean value of more than 134 BD FACSCanto™ II instruments. For the absolute CD34+ count of six trial samples, the z score of the BD FACSLyric system ranged from l–0.0076l to l–1.05l. For percent CD34+, the z score ranged from 0.00 to l–1.50l.

Conclusion

Our results demonstrated that BD FACSLyric generated consistent CD34+ analysis results compared with peer laboratories and FACSCanto instruments in UK NEQAS trials.

Figure 1 ISHAGE-based gating scheme as demonstrated by a fresh apheresis sample stained with CD45 FITC/CD34 PE with 7-AAD viability dye

1A: Rectangle gate for the viable CD45+ events and Lymphocyte gate to identify lymphocytes in the CD45 FITC vs SSC plot
1B: CD34+ gate to identify CD34+ events in the CD34 PE vs SSC plot
1C: CD45dim gate to further define CD34+ cells as CD45dim events in the CD45 FITC vs SSC plot. Same rectangle gate as in 1A is displayed.
1D: Polygon gate to further identify SSC^{low} CD34+ events in the FSC vs SSC plot. Lymphocytes are also displayed in the plot.
1E: Bead gate to show Trucount beads in green
1F: Debris gate to identify and exclude debris in the FCS vs SSC plot
1G: Viable cell gate in the 7-AAD vs SSC plot to show CD34+ cells. This gate is the same as viable cell gate in 1H.
1H: Viable cell gate in the 7-AAD vs SSC plot to determine CD45+ events

Figure 2 ISHAGE-based gating scheme for the UK NEQAS consortium CD34+ sample stained with CD45 FITC/CD34 PE without 7-AAD

2A: Rectangle gate for the viable CD45+ events and Lymphocyte gate to identify lymphocytes in the CD45 FITC vs SSC plot
2B: CD34+ gate to identify CD34+ events in the CD34 PE vs SSC plot
2C: CD45dim gate to further define CD34+ cells as CD45dim events in the CD45 FITC vs SSC plot. Same rectangle gate as in 1A is displayed.
2D: Polygon gate to further identify SSC^{low} CD34+ events in the FSC vs SSC plot. Lymphocytes are also displayed in the plot.
2E: Bead gate to show Trucount beads in green
2F: Debris gate to identify and exclude debris in the FCS vs SSC plot

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