

CD34+ cell analysis on the BD FACSLyric™ and BD FACSCanto™ II Flow Cytometers using the

BD® Stem Cell Enumeration (SCE) Kit

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Abstract

The guidelines of International Society of Hematology and Graft Engineering (ISHAGE) have been established to ensure a standardized flow cytometric method for quantitating CD34+ cells. The BD® SCE Kit on the BD FACSLyric™ Flow Cytometer provides automated enumeration of CD34+ cells using ISHAGE-based gating for different types of stem cell samples. To compare results of the BD® SCE Kit on the BD FACSLyric™ and BD FACSCanto™ II Systems, a total of 28 CD34+ samples provided by UK NEQAS were analyzed to demonstrate agreement between instruments and peer laboratories. The 28 CD34+ samples were stained with the BD® SCE Kit and acquired on both the BD FACSLyric™ and BD FACSCanto™ II Systems following manufacturer's instructions for use. Results from linear regression of absolute CD34+ counts and %CD34+ cells between the BD FACSLyric™ and BD FACSCanto™ II Systems were 0.9677 (slope), 0.6758 (intercept), 0.9837 (R²) for the absolute CD34+ count and 1.0692 (slope), -0.0214 (intercept), 0.9952 (R²) for %CD34+ cells. Analysis results on the BD FACSLyric™ and BD FACSCanto™ II Systems were also compared to the mean and standard deviation (SD) of greater than 110 peer laboratories that used the BD FACSCanto™ II System. For absolute CD34+ counts, the absolute Z-score of each sample ranged from 0.06 to 2.31 on the BD FACSLyric™ System and from 0 to 1.45 on the BD FACSCanto™ II System. For %CD34+ cells, the absolute Z-score of each sample ranged from 0 to 1.5 on the BD FACSLyric™ System and from 0 to 1.75 on the BD FACSCanto™ II System. The absolute Z-scores for both absolute CD34+ count and %CD34+ cells were within UK NEQAS SCE Program's acceptance criteria of ≤2.5. Our results demonstrated that the BD FACSLyric™ and FACSCanto™ II Systems generated consistent CD34+ analysis results compared to the BD FACSCanto™ II Systems used in peer laboratories of the UK NEQAS SCE Program. Linear regression results between the BD FACSLyric™ and BD FACSCanto™ II Systems were also congruent.

Methods

UK NEQAS Sample Processing and Analysis

Each sample (100 µL) was stained with 20 µL of BD® Stem Cell Reagent (CD45 FITC/CD34 PE) (Catalog No. 344563) in BD Trucount™ Tubes and incubated in the dark at room temperature for 20 min. To each sample tube, 2 mL of 1X ammonium chloride was added to lyse red blood cells for 10 min at room temperature in the dark. After lysing was completed, samples were placed on ice and acquired on the flow cytometer within one hour post-lysing. Samples were acquired side by side on the BD FACSCanto™ II and BD FACSLyric™ Flow Cytometers.

Results

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

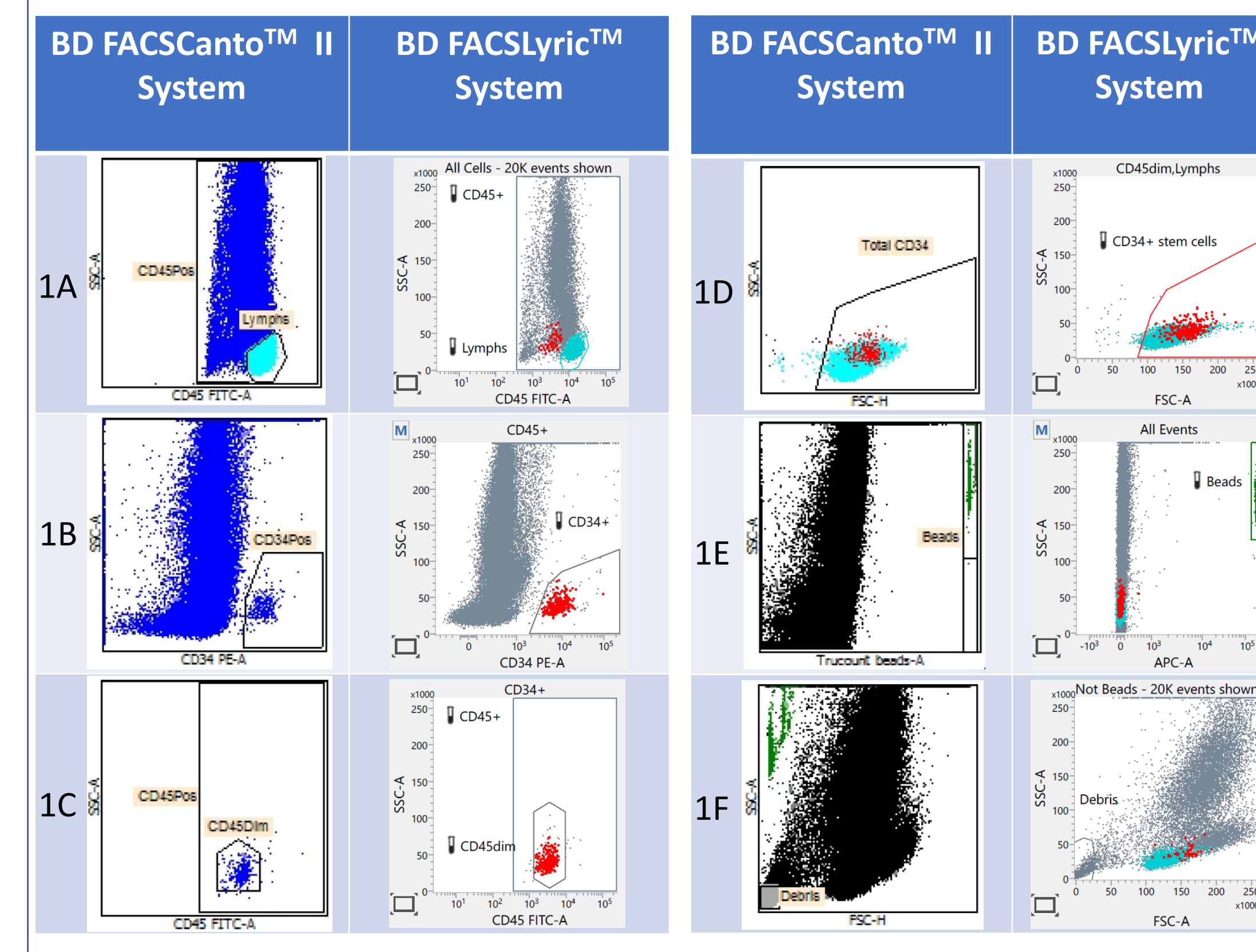
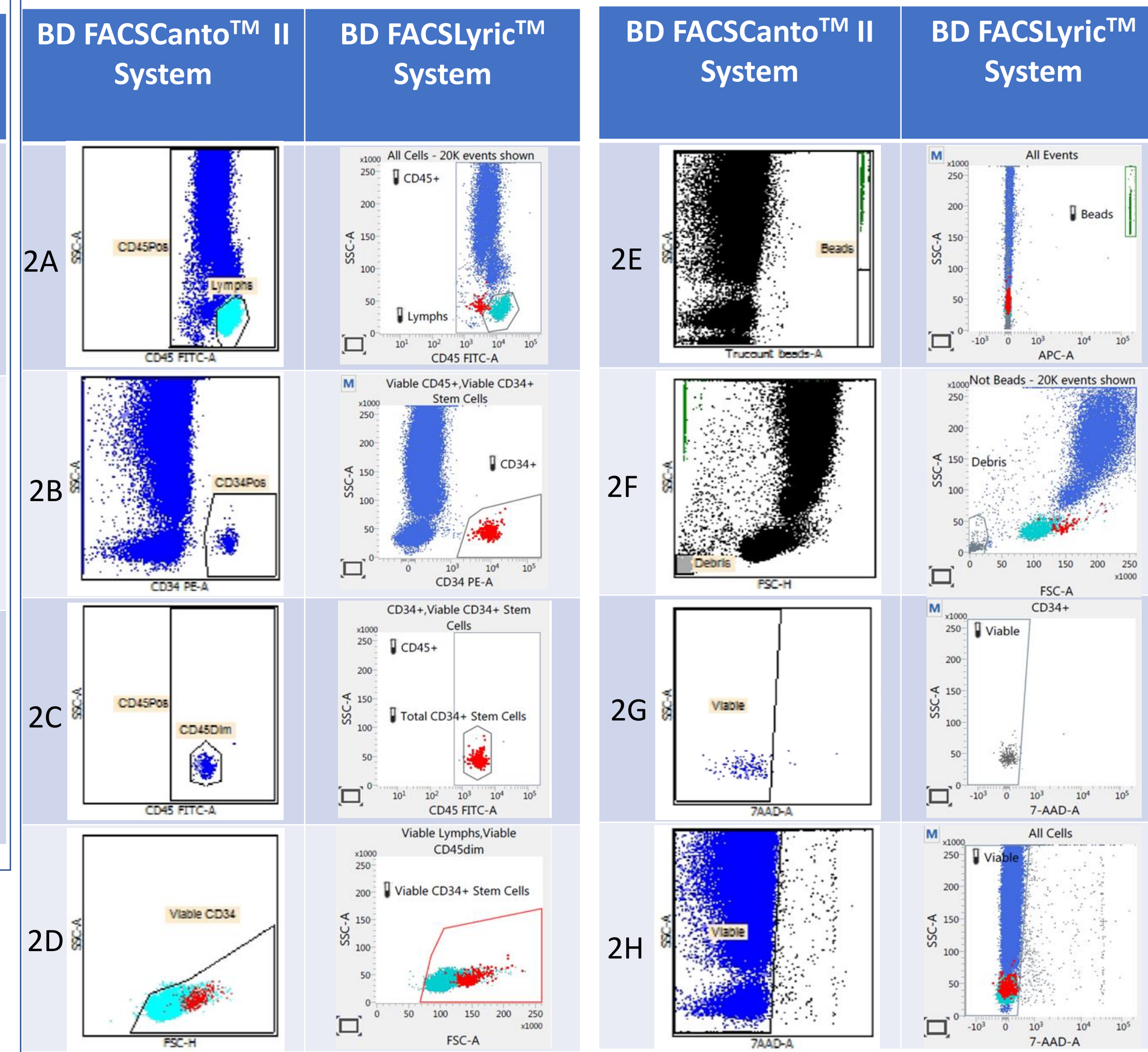


Figure 2. Example dot plots of mobilized peripheral blood sample stained with the BD® SCE Kit and acquired on the BD FACSCanto™ II and FACSLyric™ Systems



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

BD FACSCanto™ II System					BD FACSLyric™ System				
• Run Cytometer Setup with BD FACSTM 7 Color Setup Beads (BD Cat. No. 335775) in BD FACSCanto™ Clinical Software					• Run performance QC with BD® CS&T Beads (BD Cat. No. 656505) in BD FACSuite™ Clinical Application				
• Optimize for BD® Stem Cell Enumeration Assay by using BD® Stem Cell Control (High) stained with BD® Stem Cell Reagent					• Run Assay/Tube Settings Setup for Stem Cell + 7-AAD and Stem Cell Controls Assays using BD® CS&T Beads				
• Run BD® Stem Cell Controls (High and Low) as process controls to pass manufacturer specified ranges					• 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
Table 2 BD FACSLyric™ System stem cell assay setup spillover values

	FITC	PE	7-AAD	APC	FITC	PE	7-AAD	APC
FITC	100.00	0.62	0.03	0.00	FITC	100.00	0.78	0.11
PE	25.63	100.00	4.00	0.01	PE	21.83	100.00	3.53
7-AAD	2.94	14.12	100.00	0.72	7-AAD	2.89	17.32	100.00
APC	0.00	0.00	8.59	100.00	APC	0.02	0.02	3.56

Absolute count and percentages of total CD34+ cells were captured using ISHAGE-based gating templates as shown in Figure 1 and Figure 2. Results are shown in Tables 3 and 4, as well as Figures 3 and 4.

The CD34+ cell count results were compared on the BD FACSLyric™ and BD FACSCanto™ II Systems relative to UK NEQAS evaluation reports using Z-score statistical analysis. The number of laboratories that used the BD FACSCanto™ II System 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 participant summary reports. Z-scores for absolute CD34 cell counts and %CD34+ cells are calculated based on the following equations:

$$\text{Z Score for absolute CD34 count:} = \frac{\text{Abs CD34 count on BD FACSLyric} - \text{mean Abs CD34 count of peers using BD FACSCanto II}}{\text{SD of Abs CD34 count from peers using BD FACSCanto II}}$$

$$\text{Z Score for \%CD34+ cells:} = \frac{\%CD34 + \text{cells on BD FACSLyric} - \text{mean \%CD34 + cells of peers using BD FACSCanto II}}{\text{SD of \%CD34 + cells from peers using BD FACSCanto II}}$$

The ISHAGE-based CD34+ template was incorporated to the BD® CS&T Beads setup workflow on the BD FACSLyric™ Flow Cytometer and CD34+ cells from UK NEQAS samples were analyzed to demonstrate agreement results compared to peer laboratories using the BD FACSCanto™ II System. In the UK NEQAS trials, results were submitted by different global laboratories. Robust mean and robust standard deviation for absolute CD34+ count and percent CD34+ cells were established by UK NEQAS based on results reported by participating laboratories. The same UK NEQAS samples were tested in BD and acquired on the BD FACSLyric™ and BD FACSCanto™ II Flow Cytometers. Results on the BD FACSLyric™ Flow Cytometer were compared to more than 110 laboratories that used the BD FACSCanto™ II System. For the absolute CD34+ cell count (Table 3), the absolute Z-score ranged from 0.06 to 2.31 on the BD FACSLyric™ System and from 0 to 1.45 on the BD FACSCanto™ II System. For percent CD34+ (Table 4), the absolute Z-score ranged from 0 to 1.5 on BD FACSLyric™ System and from 0 to 1.75 on the BD FACSCanto™ II System.

Results

Figure 3 Linear regression of absolute CD34+ cell counts: BD FACSLyric™ vs. FACSCanto™ II Systems

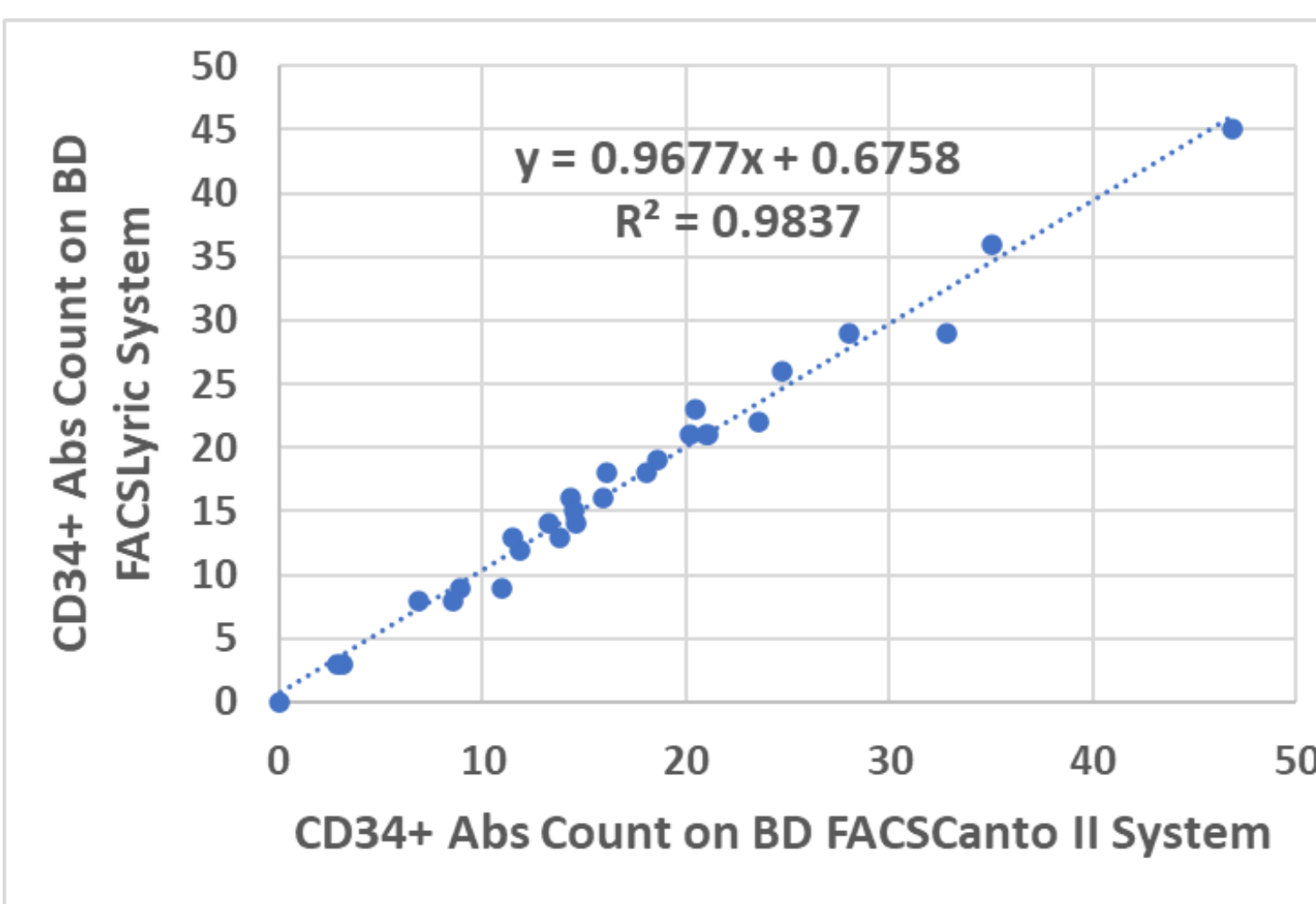


Figure 4 Linear regression of percent CD34+ cells: BD FACSLyric™ vs. FACSCanto™ II Systems

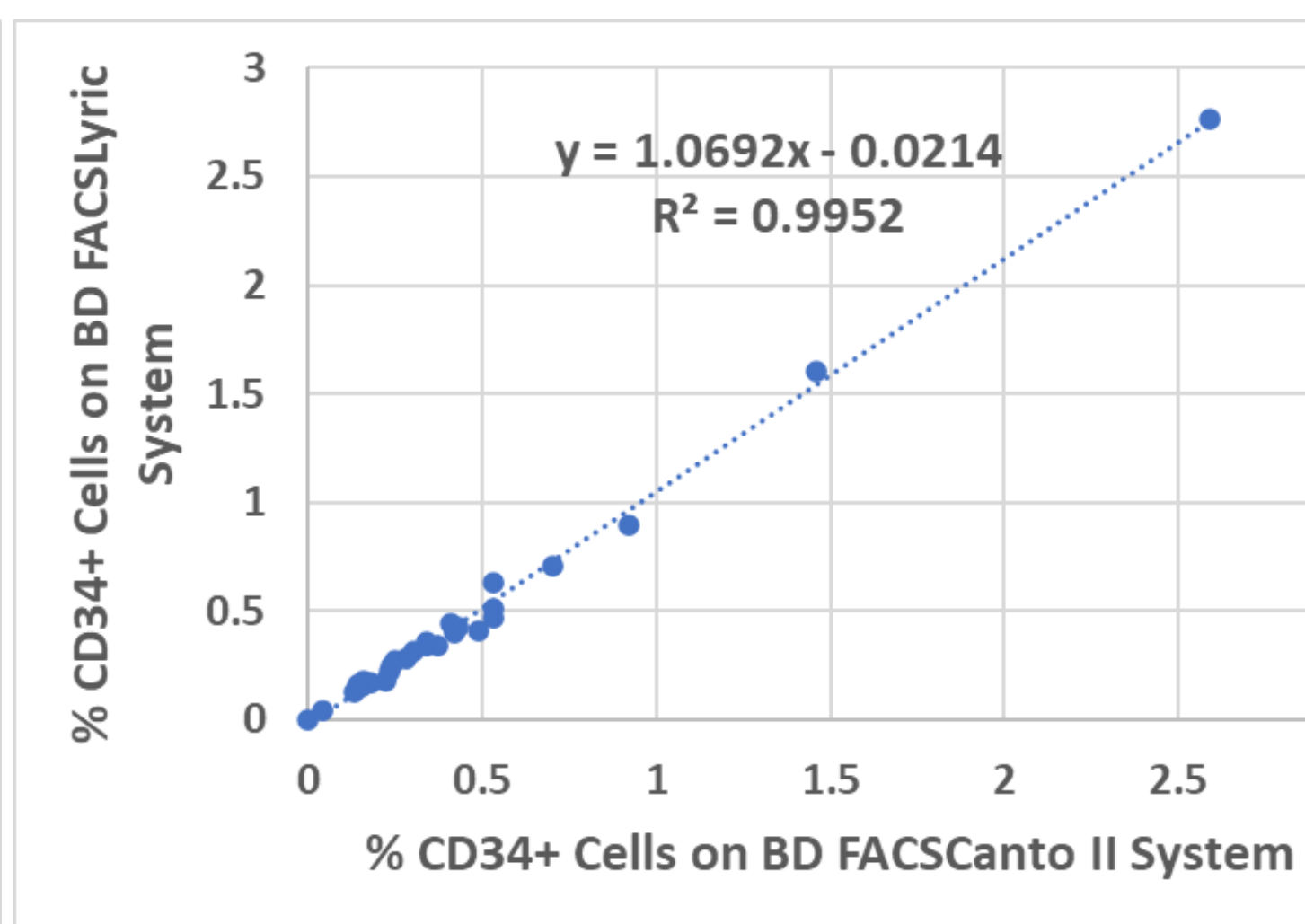


Table 3 CD34+ absolute cell counts of BD FACSLyric™ and BD FACSCanto™ II Systems compared with peer laboratories

Sample No.	UK NEQAS Trial Statistics			BD FACSLyric™ System Results		BD FACSCanto™ II System Results	
	CD34+ Abs Count on FACSCanto™ II System	Mean	SD	CD34+ Abs Count	Abs Z-Score	CD34+ Abs Count	Abs Z-Score
274	144	3.73	0.88	3	0.83	3.09	0.73
275	144	3.23	0.64	3	0.36	2.87	0.56
276	139	14.07	1.45	13	0.74	13.81	0.18
277	139	13.37	1.32	16	1.99	14.29	0.70
278	142	22.92	1.85	22	0.50	23.55	0.34
279	142	27.91	3.37	29	0.32	32.81	1.45
280	139	15.26	1.86	16	0.40	15.89	0.34
281	139	9.86	1.26	9	0.68	8.91	0.75
282	140	21.24	2.12	21	0.11	21.05	0.09
283	140	21.14	2.44	21	0.06	20.98	0.07
284	135	9.43	1.31	8	1.09	8.52	0.69
285	135	11	1.29	12	0.78	11.81	0.63
286	134	18.44	2.4	18	0.18	16.13	0.96
287	134	36.37	5.02	36	0.07	35.09	0.25
288	136	13.38	1.77	14	0.35	14.56	0.67
289	136	14.48	2.11	15	0.25	14.47	0.00
290	138	19.03	2.07	21	0.95	20.17	0.55
291	138	10.31	1.33	9	0.98	10.96	0.49
292	131	18.45	2.43	19	0.23	18.61	0.07
293	131	25.49	3.08	26	0.17	24.76	0.24
294	131	7.38	1.3	8	0.48	6.83	0.82
295	131	17.34	2.08	18	0.32	18.04	0.34
296	131	29.19	2.45	29	0.08	28.05	0.47
297	131	14.27	1.84	14	0.15	13.29	0.53
298	115	0.24	0.25	0	0.96	0.06	0.72
299	119	47.01	5.11	45	0.39	46.91	0.02
300	115	19.19	1.65	23	2.31	20.5	0.79
301	113	12.73	1.35	13	0.20	11.49	0.92

Table 4 Percent CD34+ cells of BD FACSLyric™ and BD FACSCanto™ II Systems compared with peer laboratories

Sample No.	UK NEQAS Trial Statistics			BD FACSLyric™ System Results		BD FACSCanto™ II System Results	
	% CD34+ on FACSCanto™ II System	Mean	SD	% CD34+ on FACSLyric™ System	Abs Z-Score	% CD34+ on FACSCanto™ II System	Abs Z-Score
274	140	0.05	0.01	0.04	1.00	0.04	1.00
275	140	0.2	0.05	0.15	1.00	0.15	1.00
276	134	0.26	0.03	0.22	1.33	0.23	1.00
277	134	0.24	0.02	0.27	1.50	0.25	0.50
278	138	0.37	0.03	0.34	1.00	0.37	0.00
279	138	0.42	0.04	0.41	1.25	0.49	1.75
280	134	0.33	0.04	0.34	0.25	0.34	0.25
281	134	0.15	0.02	0.13	1.00	0.13	1.00
282	136	0.51	0.05	0.51	0.00	0.53	0.40
283	136	0.51	0.05	0.47	0.80	0.53	0.40
284	132	0.2	0.03	0.17	1.00	0.18	0.67
285	132	0.16	0.02	0.16	0.00	0.16	0.00
286	129	0.62	0.09	0.63	0.11	0.53	1.00
287	129	3.57	0.71	2.76	1.14	2.59	1.38
288	133	0.23	0.03	0.25	0.67	0.24	0.33
289	133	0.34	0.04	0.36	0.50	0.34	0.00
290	136	0.66	0.06	0.71	0.83	0.7	0.67
291	136	0.21	0.03	0.18	1.00	0.22	0.33
292	128	0.44	0.05	0.43	0.20	0.43	0.20
293	128	0.33	0.03	0.32	0.33	0.3	1.00
294	129	0.15	0.02	0.16	0.50	0.14	0.50
295	129	0.93	0.13	0.9	0.23	0.92	0.08
296	128	1.61	0.11	1.6	0.09	1.46	1.36
297	128	0.43	0.05	0.44	0.20	0.41	0.40
299	118	0.44	0.04	0.4	1.00	0.42	0.50
300	110	0.27	0.03	0.28	0.33	0.28	0.33
301	110	0.18	0.02	0.18	0.00	0.16	1.00

Conclusions

- Analysis of CD34+ absolute count and percentages on the BD FACSLyric™ and FACSCanto™ II Flow Cytometers showed consistent results compared to peer laboratories using the BD FACSCanto™ II Flow Cytometer in the UK NEQAS CD34 trials (Tables 3 and 4).
- The linear regression analysis between the BD FACSLyric™ and FACSCanto™ II Flow Cytometers demonstrated agreement results on the two systems.

BD FACSLyric™ Flow Cytometers and BD FACSCanto™ II Flow Cytometers are Class 1 Laser Products. 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 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® Stem Cell Enumeration Kit is for In Vitro Diagnostic Use with the BD FACSLyric™ Flow Cytometer, BD FACSCanto™ II Flow Cytometer and BD FACSCalibur™ Flow Cytometer.

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