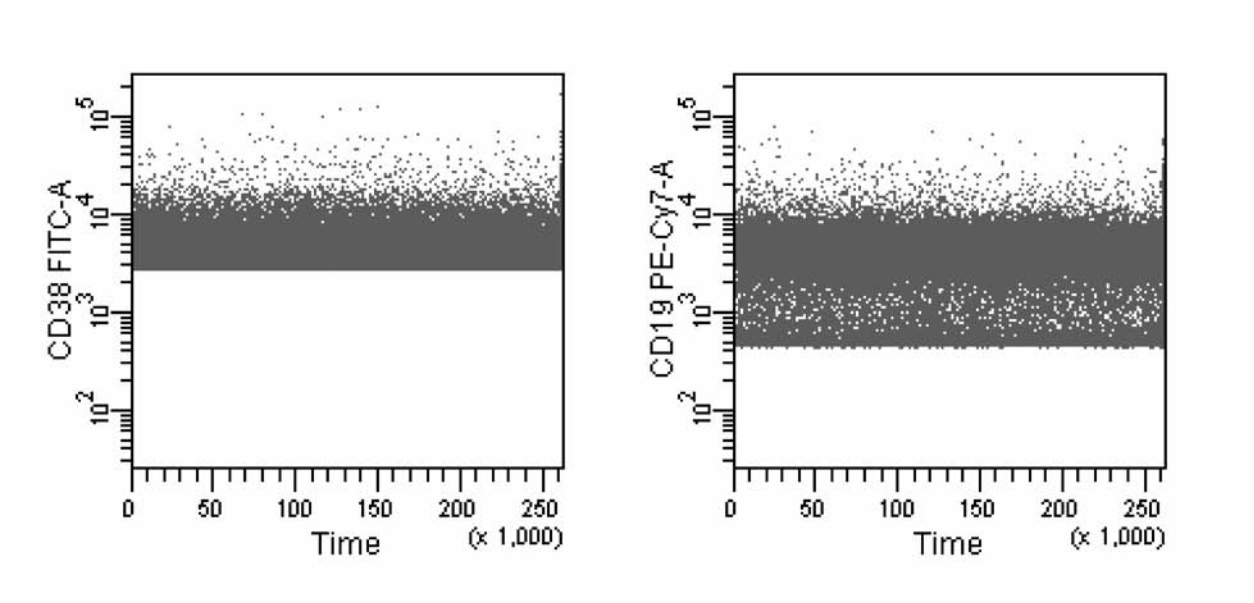
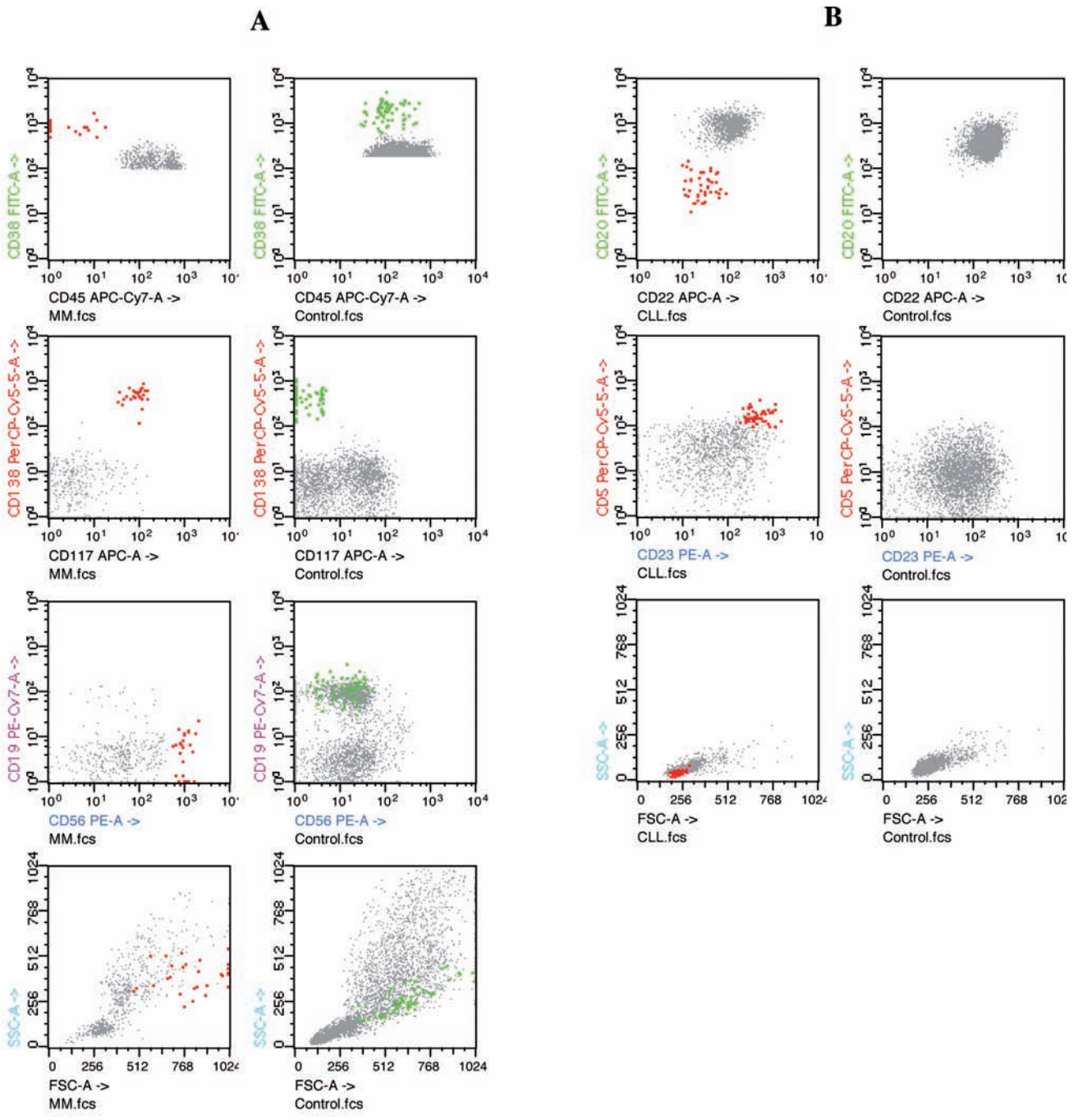
Enhanced sensitivity of flow cytometry for routine assessment of minimal residual disease

Citation: Domingo E, Moreno C, Sánchez-Ibarrola A, Panizo C, Páramo JA, and Merino J. Enhanced sensitivity of flow cytometry for routine assessment of minimal residual disease. Haematologica. 2010; 95:691-692. doi:10.3324/haematol.2009.018911



Online Supplementary Figure S1. Time-resolved dot plots of CD38 intensity in 6 million bone marrow leukocytes from a representative minimal residual disease-positive (MRD^+) multiple myeloma sample acquired through a live gate drawn on $CD38^{++}/^{+++}$ cells (left), and of CD19 intensity in 6 million peripheral blood leukocytes from a representative MRD+ B-cell chronic lymphocytic leukemia sample acquired through a live gate drawn on CD19+ cells (right). In both cases, data derived from several acquisition tubes were appended to the same file using FACSDiva software. The absence of fluctuations of fluorescence intensity between acquisition tubes is illustrated.



Online Supplementary Figure S2. The specificity of detection of minimal residual disease of multiple myeloma and B-cell chronic lymphocytic leukemia when a total of 10 million leukocytes are acquired. Panel A: Representative dot plots of 2 million bone marrow leukocytes from a MRD$^+$ multiple myeloma (MM) sample (left column) and 10 million normal bone marrow leukocytes (right column) stained with CD38/CD56/CD138/CD19/CD117/CD45. Recorded events correspond to gated CD38^{+++} cells. The red dots are myelomatous plasma cells (CD38^{++} CD138^{++} CD56^{++} CD19- CD117$^+$ CD45-). The green dots are normal plasma cells (CD38^{+++} CD138^{++} CD56- CD19$^+$ CD117$^-$ CD45$^+$). Panel B: Representative dot plots of 2 million peripheral blood leukocytes from a MRD+ B-cell chronic lymphocytic leukemia (B-CLL) sample (left column) and 10 million normal peripheral blood leukocytes (right column) stained with CD20/CD23/CD5/CD19/CD22/CD3. The recorded events correspond to gated CD19$^+$ cells. The red dots are B-CLL cells (CD20d$^+$ CD22d$^+$ CD5$^+$ CD23$^+$). The gray dots are normal B-lymphocytes. No clusters of events mimicking malignant cells were detected in the normal samples.

Online Supplementary Table S1A. Assessment of the percentage of electronic aborts when normal leukocytes were acquired at increasing event rates.

Cell Concentration (million cells/mL)	Event Rate (events/sec)	Electronic Aborts (%)
0.25	600	0.4
0.5	1200	0.8
1	2250	1.5
2	3800	2.6
4	6300	4.3
5	7500	5.3

Increasing event rates were achieved by increasing the concentration of leukocytes in the acquisition tubes. Results are the mean of three independent trials. Based on these results, we considered that 4000 events/sec could be a reasonable flow rate for routine analysis.

Online Supplementary Table S1B. Influence of acquisition event rate on minimal residual disease measurements.

		Event Rate	
		2000 (events/sec)	4000 (events/sec)
MRD (%)	MM	0.001	0.0098
		0.01	0.01
		0.04	0.04
	B-CLL	0.01	0.0097
		0.01	0.0087
		0.006	0.006

Minimal residual disease was measured in samples from 3 patients with multiple myeloma (MM) and 3 patients with B-cell chronic lymphocytic leukaemia (B-CLL), acquired at 1 or 2 million leucocytes/ml and therefore with an event rate of 2000 or 4000 events/sec, respectively. Similar results were obtained with both event rates.