MACS&more

Excerpt from MACS&more Vol 14 – 1/2012

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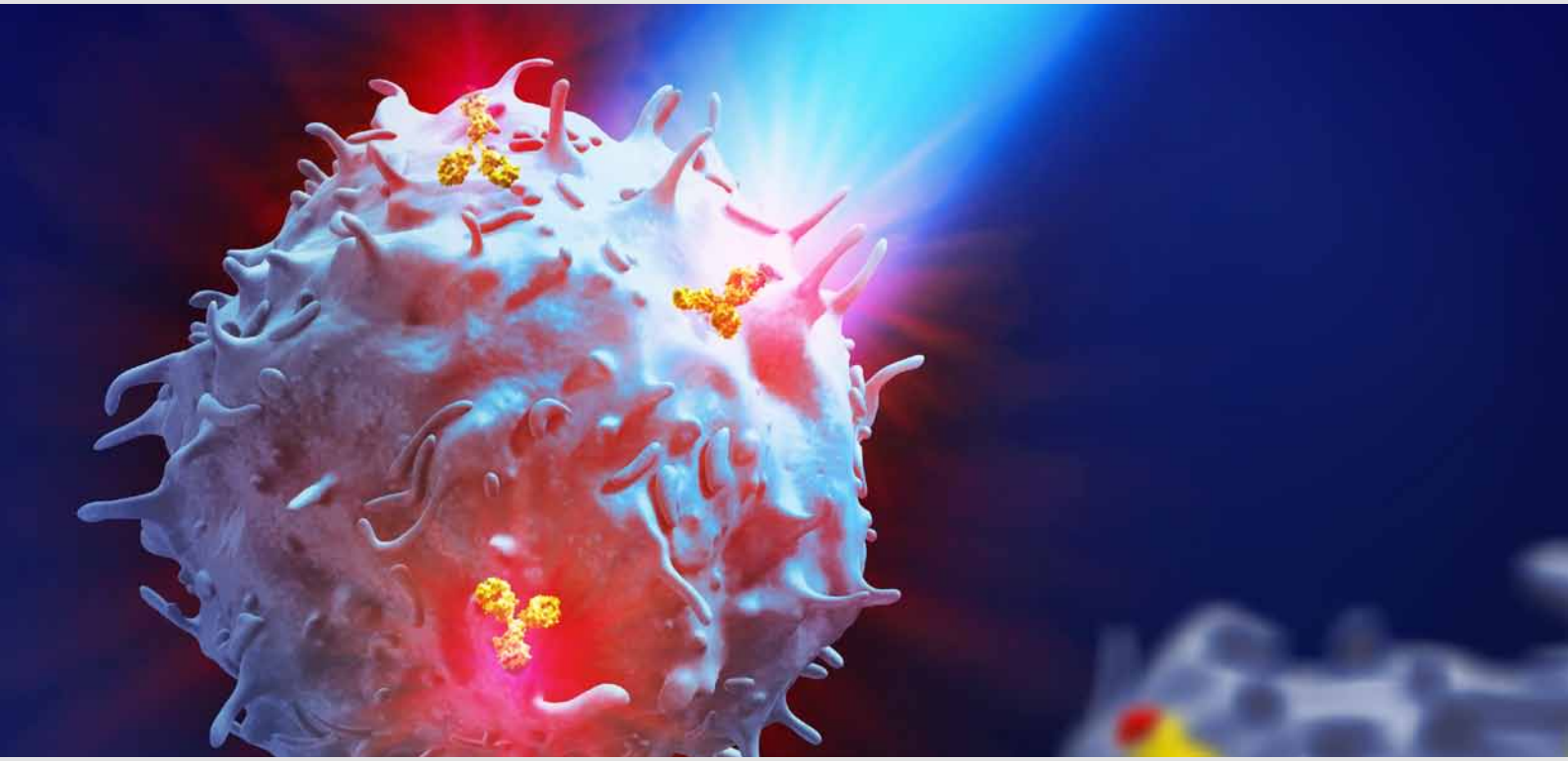

Comparison between antibodies from different providers: determination of median fluorescent intensities and stain indices

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*K.R. and S.V. performed antibody labeling and flow cytometric acquisition.

#V.D.C. and P.F. performed analysis and interpretation of the data.

Introduction

Flow cytometry is a powerful technique to identify, enumerate, and characterize cells by immunophenotyping. However, data quality and reliability of the results critically depend on the quality of the antibodies used for flow cytometry. Median fluorescence intensity (MFI) and – derived from that – the stain index define the brightness, and thus quality of the fluorescence signal. We here examined 23 anti-human and 10 anti-mouse antibodies from Miltenyi Biotec or Stemgent® and compared them with the corresponding antibodies from other providers with regard to MFI and stain index.

Materials and methods

Antibodies

Antibodies used in this study are summarized in table 1. All antibodies were kept at 4 °C and protected from light. All antibodies were well within shelf life.

Cell staining

The same cell batch of either PBMCs, cell lines, thymic cells, or activated PBMCs was used for staining with either an antibody from Miltenyi Biotec/Stemgent or the corresponding antibody from another provider. Single-cell suspensions (2×10^6 cells/mL) were prepared by using wash buffer (PBS, 1% BSA, 0.1% sodium azide). Alternatively, cells were left in medium and diluted to this concentration by using wash buffer.

Human cells were then incubated with FcR Blocking Reagent, human (Miltenyi Biotec). Mouse cells were incubated with anti-CD16/CD32 (clone 2.4G2), for 15 min at 4 °C. Fifty µL of the cell suspensions (100,000 cells) were transferred to test tubes allowing for the assessment of various antibody concentrations. After the addition of 10 µL antibody solution or isotype control, cells were mixed gently and incubated for exactly 30 min at 4 °C in the dark. Subsequently, 1 mL of wash buffer was added to the cells. Cells were centrifuged for

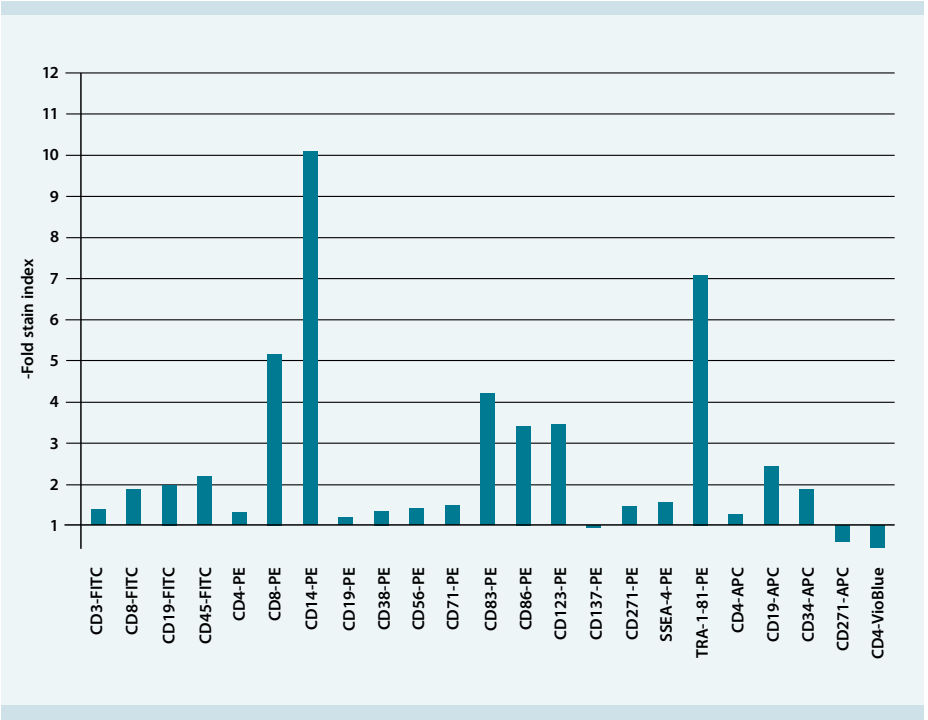

Figure 1: Stain indices of antibodies from Miltenyi Biotec/Stemgent vs. antibodies from another provider. The ratio was calculated by dividing the stain index of the Miltenyi Biotec/Stemgent antibody by the stain index of the other provider's antibody. A value of 1 thus means that both antibodies are equal. Antibodies were used at the recommended 1/1 dilutions.

Antibodies for detection of human cells

Antibody	Provider	Clone	Isotype
CD3-FITC	Miltenyi Biotec	BW264/56	Mouse IgG2a
CD3-FITC	BD Biosciences	SK7	Mouse IgG1
CD8-FITC	Miltenyi Biotec	BW135/80	Mouse IgG2a
CD8-FITC	BD Biosciences	SK1	Mouse IgG1
CD19-FITC	Miltenyi Biotec	LT19	Mouse IgG1
CD19-FITC	BD Biosciences	4G7	Mouse IgG1
CD45-FITC	Miltenyi Biotec	5B1	Mouse IgG2a
CD45-FITC	BD Biosciences	2D1	Mouse IgG1
CD4-PE	Miltenyi Biotec	M-T466	Mouse IgG1
CD4-PE	BD Biosciences	SK3	Mouse IgG1
CD8-PE	Miltenyi Biotec	BW135/80	Mouse IgG2a
CD8-PE	BD Biosciences	SK1	Mouse IgG1
CD14-PE	Miltenyi Biotec	TÜK4	Mouse IgG2a
CD14-PE	BD Biosciences	MφP9	Mouse IgG2b
CD19-PE	Miltenyi Biotec	LT19	Mouse IgG1
Anti-Human CD19 PE	eBioscience	HIB19	Mouse IgG1
CD38-PE	Miltenyi Biotec	IB6	Mouse IgG2b
CD38-PE	BD Biosciences	HB7	Mouse IgG1
CD56-PE	Miltenyi Biotec	AF12-7H3	Mouse IgG1
CD56-PE	BD Biosciences	NCAM16.2	Mouse IgG2b
CD71-PE	Miltenyi Biotec	AC102	Mouse IgG2a
CD71-PE	BD Biosciences	M-A712	Mouse IgG2a
CD83-PE	Miltenyi Biotec	HB15	Mouse IgG1
Anti-Human CD83 PE	eBioscience	HB15e	Mouse IgG1
CD86-PE	Miltenyi Biotec	FM95	Mouse IgG1
Anti-Human CD86 PE	eBioscience	IT2.2	Mouse IgG2b
CD123-PE	Miltenyi Biotec	AC145	Mouse IgG2a
CD123-PE	BD Biosciences	9F5	Mouse IgG1
CD137-PE	Miltenyi Biotec	4B4-1	Mouse IgG1
CD137-PE	BD Biosciences	4B4-1	Mouse IgG1
CD271-PE	Miltenyi Biotec	ME20.4-1.H4	Mouse IgG1
Anti-NGFR Fluorescein	Chromaprobe	ME20.4	Mouse IgG1
Stemgent Phycoerythrin (PE) anti-Human SSEA-4 Antibody	Stemgent*	MC-813-70	Mouse IgG3
Anti-Human SSEA-4 PE	eBioscience	MC-813-70	Mouse IgG3
Stemgent Phycoerythrin (PE) anti-Human TRA-1-81 Antibody	Stemgent*	TRA-1-81	Mouse IgM
Anti-Human TRA-1-81 (Podocalyxin) PE	eBioscience	TRA-1-81	Mouse IgM
CD4-APC	Miltenyi Biotec	M-T466	Mouse IgG1
CD4-Alexa Fluor	BD Biosciences	RPA-T4	Mouse IgG1
CD19-APC	Miltenyi Biotec	LT19	Mouse IgG1
CD19-APC	BD Biosciences	SJ25C1	Mouse IgG1
CD34-APC	Miltenyi Biotec	AC136	Mouse IgG2a
CD34-APC	BD Biosciences	8G12	Mouse IgG1
CD271-APC	Miltenyi Biotec	ME20.4-1.H4	Mouse IgG1
Anti-NGFR Fluorescein	Chromaprobe	ME20.4	Mouse IgG1
CD4-VioBlue	Miltenyi Biotec	VIT4	Mouse IgG2a
CD4-V450	BD Biosciences	RPA-T4	Mouse IgG1

Antibodies for detection of mouse cells

Antibody	Provider	Clone	Isotype
CD4-PE	Miltenyi Biotec	GK1.5	Rat IgG2b
CD4-PE	BD Biosciences	RM4-5	Rat IgG2a
CD19-PE	Miltenyi Biotec	6D5	Rat IgG2a
CD19-PE	eBioscience	1D3	Rat IgG2a
CD49b-PE	Miltenyi Biotec	DX5	Rat IgM
CD49b-PE	BD Biosciences	DX5	Rat IgM
Anti-IFN-γ-PE	Miltenyi Biotec	AN18.17.24	Rat IgG
IFN-γ-PE	BD Biosciences	XMG1.2	Rat IgG1
Anti-NK1.1-PE	Miltenyi Biotec	PK136	Mouse IgG2a
NK1.1-PE	BD Biosciences	PK136	Mouse IgG2a
Anti-NKp46-PE	Miltenyi Biotec	29A1.4	Rat IgG2a
PE anti-mouse CD335 (NKp46)	BioLegend	29A1.4	Rat IgG2a
Anti-Mouse CD335 (NKp46) PE Antibody	eBioscience	29A1.4	Rat IgG2a
Anti-TNF-α-PE	Miltenyi Biotec	MP6-XT22	Rat IgG1
Anti-TNF-α-PE	BD Biosciences	MP6-XT22	Rat IgG1
CD3ε-APC	Miltenyi Biotec	145-2C11	Hamster IgG1
CD3ε-APC	BD Biosciences	145-2C11	Hamster IgG1
CD49b-APC	Miltenyi Biotec	DX5	Rat IgM
APC anti-mouse CD49b (pan-NK cells) Antibody	BioLegend	DX5	Rat IgM
Anti-NK1.1-APC	Miltenyi Biotec	PK136	Mouse IgG2a
NK1.1-APC	BD Biosciences	PK136	Mouse IgG2a

Table 1: Antibodies used in this study.
*Miltenyi Biotec is an authorized distributor of Stemgent products.

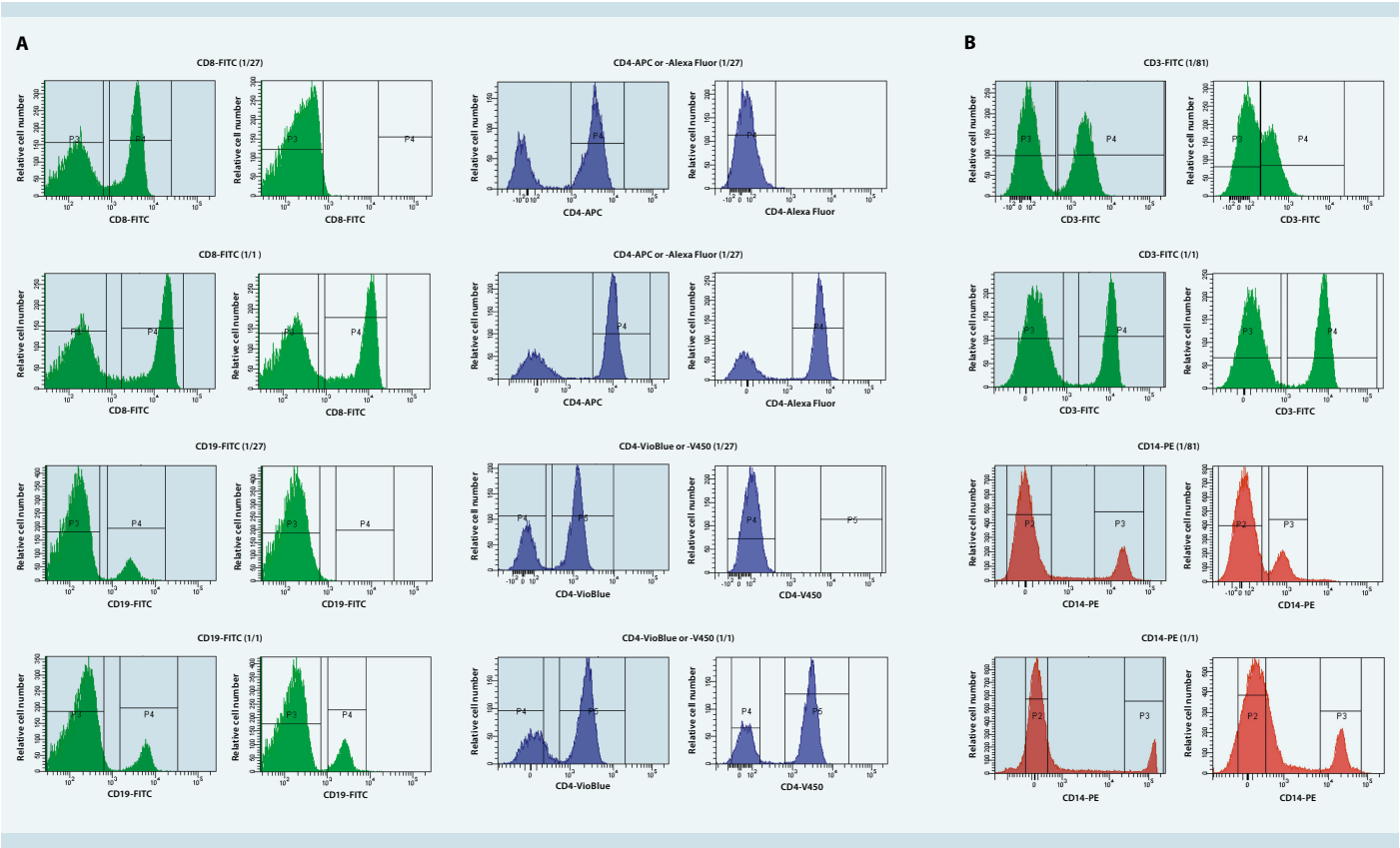

Figure 2: Flow cytometry using anti-human antibodies from different providers. Cells were stained and analyzed as indicated in materials and methods. Antibodies were used at the indicated dilutions. Left panels (dark blue background): Miltenyi Biotec antibodies; right panels: antibodies from another provider. (A) Staining at a higher dilution resulted in a loss of the positive peak with the other providers' antibodies, whereas with the Miltenyi Biotec antibodies a clear positive peak could still be detected. (B) When staining at higher dilutions, the MFIs and stain indeces of Miltenyi Biotec antibodies were higher compared to the other providers.

5 min at 360×g at 4 °C. The supernatant was poured off and the tubes were gently patted onto paper towels. Cells were resuspended in 100–200 µL of wash buffer and the tubes placed at 4 °C until analysis by flow cytometry. Immediately prior to analysis, 3 µL of 0.1 mg/mL propidium iodide (PI) solution were added to each sample. Alternatively, 20 µL of a 1/50 Live/Dead® solution (Invitrogen) were used for dead cell exclusion.

Flow cytometry

Flow cytometry was performed on BD™ LSR II or BD FACSCalibur™ flow cytometers and analyzed by using the BD FACSDiva™ software. Compensation was performed in the same way for Miltenyi Biotec/Stemgent antibodies and the corresponding antibodies from other providers. Measurements were performed using standard settings. Dead cells were excluded from the analysis based on PI fluorescence. Data were plotted as histograms.

The stain index was calculated according to the following equation:

$$SI = (MFI_{pos} - MFI_{neg})/2\times SD_{neg}$$

SI stands for the stain index and MFI_{pos} and MFI_{neg} for the median fluorescence intensities of the positive and negative peak, respectively. SD_{neg} denotes the standard deviation of the negative peak.

Results

Anti-human antibodies

We analyzed 23 antibodies directed against commonly used cell surface markers for a variety of human cell types, including T cells, B cells, plasma cells, monocytes/macrophages, NK cells, dendritic cells, mesenchymal stromal cells, and pluripotent stem cells. Antibodies from Miltenyi Biotec/Stemgent and the corresponding antibodies from other providers were tested. MFI and stain indices obtained at various antibody dilutions (1/1, 1/3, and 1/9) are summarized in table 2.

For a more direct comparison of antibody properties, we divided the stain index of the Miltenyi Biotec/Stemgent antibody by the stain index of the corresponding antibody from another provider (fig. 1). The results show that 20 out of 23 tested Miltenyi Biotec/Stemgent antibodies displayed an equal or higher stain index (up to 10.1-fold). To examine the performance of the antibodies in more detail, we also tested them at a higher dilution (1/27 or 1/81). Examples of histograms are shown in figure 2. At the recommended 1/1 dilution, the CD8-FITC, CD19-FITC, CD4-APC, and CD4-VioBlue antibodies from both Miltenyi Biotec and the other providers showed a clear positive peak. For these antibodies, staining at higher dilutions was better with the Miltenyi Biotec antibodies (fig. 2A). For nine other antibodies the MFI of the

Antigen, fluorochrome	Antibody provider	MFI	Stain index	MFI	Stain index	MFI	Stain index
		1/1 dilution (recommended)		1/3 dilution		1/9 dilution	
CD3, FITC	Miltenyi Biotec	11,805	27.72	10,833	40.03	8,963	40.19
	BD Biosciences	7,543	20.02	5,584	25.09	2,793	14.23
CD8, FITC	Miltenyi Biotec	17,820	54.17	17,349	58.98	10,493	34.65
	BD Biosciences	10,288	28.99	3,115	9.27	1,158	4.18
CD19, FITC	Miltenyi Biotec	5,812	17.54	4,174	13.47	3,564	13.61
	BD Biosciences	2,455	8.86	1,221	4.72	866	3.27
CD45, FITC	Miltenyi Biotec	25,608	149.38	20,865	121.30	11,308	65.54
	BD Biosciences	12,151	68.94	9,910	56.15	3,770	21.13
CD4, PE	Miltenyi Biotec	39,585	113.33	35,418	120.25	22,947	90.81
	BD Biosciences	20,184	86.55	7,395	34.41	2,446	11.10
CD8, PE	Miltenyi Biotec	61,925	296.78	31,811	239.04	19,932	85.81
	BD Biosciences	18,529	57.44	7,463	32.27	2,346	10.86
CD14, PE	Miltenyi Biotec	141,329	382.22	99,691	364.64	60,463	235.28
	BD Biosciences	19,774	37.97	17,312	36.62	4,930	19.35
CD19, PE	Miltenyi Biotec	18,647	31.07	12,945	34.14	7,577	25.81
	eBioscience	10,301	25.83	4,653	15.98	1,921	6.48
CD38, PE	Miltenyi Biotec	4,891	47.28	2,568	25.72	1,403	13.81
	BD Biosciences	4,118	34.93	3,104	27.70	2,147	18.75
CD56, PE	Miltenyi Biotec	7,421	16.96	6,307	24.12	3,797	17.01
	BD Biosciences	4,064	11.97	3,972	13.83	3,681	15.13
CD71, PE	Miltenyi Biotec	158,218	887.58	96,728	549.02	35,902	203.34
	BD Biosciences	110,939	605.54	44,790	245.65	13,664	74.87
CD123, PE	Miltenyi Biotec	13,127	94.72	6,831	21.21	3,382	14.85
	BD Biosciences	3,743	27.57	1,923	14.80	1,431	11.29
CD271, PE	Miltenyi Biotec	4,860	38.96	2,399	18.73	652	4.70
	Chromaprobe	4,623	26.73	3,700	20.58	1,177	8.89
SSEA-4, PE	Stemgent*	24,864	46.37	10,669	19.66	3,785	5.99
	eBioscience	26,452	29.68	7,974	8.45	3,229	2.95
TRA-1-81, PE	Stemgent*	105,368	129.36	38,846	48.60	16,442	21.11
	eBioscience	21,785	18.26	9,165	7.69	3,867	2.08
CD4, APC	Miltenyi Biotec	9,613	27.97	8,899	47.77	6,880	42.41
CD4, Alexa Fluor	BD Biosciences	5,292	22.21	660	4.27	-	-
CD19, APC	Miltenyi Biotec	3,027	15.73	1,036	5.36	636	3.44
	BD Biosciences	2,321	6.50	1,615	5.66	961	5.81
CD34, APC	Miltenyi Biotec	8,557	39.31	3,663	20.59	959	10.80
	BD Biosciences	3,798	21.07	1,224	7.39	484	3.72
CD271, APC	Miltenyi Biotec	9,904	54.84	6,396	35.32	1,649	8.74
	Chromaprobe	15,503	90.14	13,884	80.85	11,250	65.75
CD4, VioBlue	Miltenyi Biotec	2,273	10.26	2,220	12.90	1,936	13.78
CD4, V450	BD Biosciences	2,762	22.94	882	9.50	300	2.77

Table 2: Median fluorescence intensities (MFI) of the positive peaks and stain indices of anti-human antibodies at various dilutions. The stain index was calculated as indicated in materials and methods. *Miltenyi Biotec is an authorized distributor of Stemgent products.

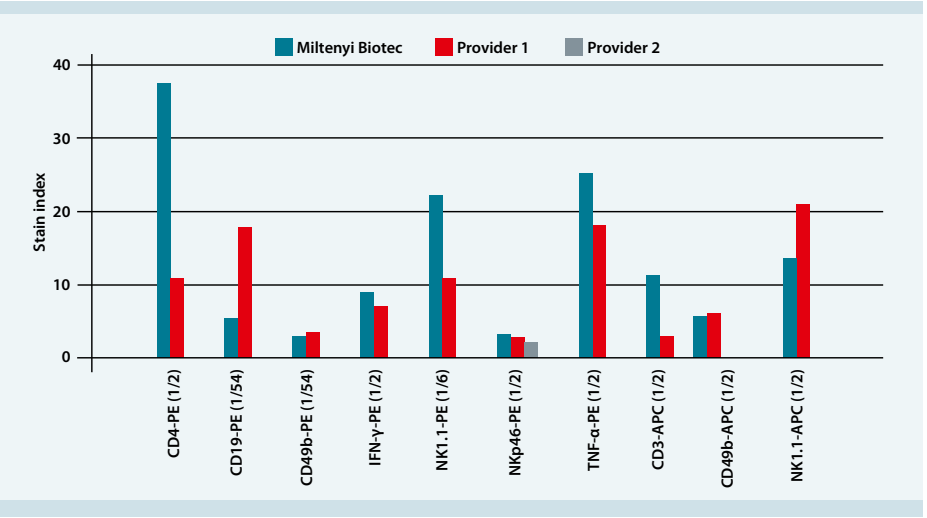

Figure 3: Stain indices of anti-mouse antibodies. Anti-mouse antibodies were tested at the optimal dilutions, as indicated. The stain index was calculated as described in materials and methods.

Miltenyi Biotec/Stemgent antibody was higher, leading to a greater resolution of positive and negative peaks (examples are shown in fig. 2B).

Anti-mouse antibodies

We also tested ten anti-mouse antibodies (fig. 3), each from at least two different providers. The antibodies allow the identification of T cells, B cells, NK cells, or the detection of cytokine secretion. We found that four of the Miltenyi Biotec antibodies showed higher stain indices than the corresponding antibodies from another provider at the tested dilutions. Four of the Miltenyi Biotec antibodies yielded similar results as the ones from other providers. Only two Miltenyi Biotec antibodies had a lower stain index than the corresponding antibody from another provider.

Conclusion

In this study we tested 33 antibodies detecting markers for the identification of particular human or mouse immune or stem cell types. We compared antibodies from at least two different providers. Twenty-nine out of the 33 tested antibodies from Miltenyi Biotec/Stemgent showed equal or higher stain indices compared to the corresponding antibodies from other providers. The brighter fluorescence signals allowed for a more sensitive cell detection and reliable analysis.

Perspectives from a frequent user of MACS® Antibodies

"As manager of several flow cytometry facilities over the years, projects have included cell characterization of stem cells and additionally neonatal tissues. Through the process of testing multiple antibodies from several vendors at serial dilutions, I have found that on all occasions, if the antibody and fluorochrome conjugate that was needed was available through Miltenyi Biotec, this was the superior or one of the superior antibodies to choose. Because of their high-quality products I continue to be a very satisfied customer."

Maria A. Giovino-Doherty
Quality Control Manager
Massachusetts Human Stem Cell Bank & Registry
University of Massachusetts Medical School

For further information on the Massachusetts Human Stem Cell Bank & Registry see: www.umassmed.edu/MHSCB