Cross-validation of standardized EuroFlow 8-color protocols on the XF-1600 flow cytometer

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BACKGROUND

Flow cytometry (FCM) immunophenotyping has become a mainstream application in the diagnosis, classification, and follow up of hematological diseases, including leukemias, lymphomas and myelomas. In order to reduce variability and subjectivity of results, different expert groups have stablished consensus guidelines and recommendations to ensure standardization and reproducibility of FCM results between different instruments and centers. The EU-supported EuroFlow Consortium developed a project to provide optimal multicolor combinations of fluorochrome-conjugated antibodies together with adequate standard operation procedures (SOPs) that would allow reproducible and time-consistent FCM measurements by applying predefined values of light scattering and mean fluorescence intensity (MFI) using specific reference beads. The performance and reproducibility of the results were evaluated in multiple clinical diagnostic centers. Importantly, it is necessary to evaluate the performance of currently available flow cytometers, especially those made by different manufacturers, and to determine the procedures and settings that provide the most comparable results. The objective of this work is to develop a setup procedure based on EuroFlow guidelines for the XF-1600™ flow cytometer (Sysmex) in order to define reference fluorescence values comparable to those of a cross-calibration instrument (Navios™ EX flow cytometer, Beckman Coulter).

MATERIALS AND METHODS

Flow cytometers. XF-1600™ (evaluated instrument) and Navios EX (reference instrument) were the flow cytometers used in this study.

Calibration particles. Sphero™ Rainbow Calibration Particles 8-peaks (Spherotech, ref. RCP-30-50A, lot EAP01) were used to set up Navios EX settings by adjusting PMTs to reach the 7th peak reference target values of the validated lot. These calibration particles were used then to calculate XF-1600 7th peak reference target values according with stablished settings.

Spectral comparisons. CyFlow™ CompSet (Sysmex) particles were used to match spectral differences among reference and evaluated cytometers. Particles were labeled with CD8 monoclonal antibodies (Sysmex) to prepare single controls for the FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-Cy7, Pacific Blue, and Pacific Orange fluorochromes. Controls were then acquired on a reference flow cytometer. The PMTs of the evaluated flow cytometer were adjusted for each fluorochrome to achieve the same MFI.

Validation tube. Once the settings were established, the OneFlow™ Lymphoid Screening Tube (LST) (BD Biosciences, catalog number 658619) was used to prepare blood samples from healthy donors and analyze them on the evaluated flow cytometer..

Data analysis. All data were analyzed on the Infinicyt™ Software (Cytognos).

RESULTS AND DISCUSSION

Target values assessment. Both XF-1600™ (Sysmex) and Navios EX (Beckman Coulter) flow cytometers used the same excitation lines (violet, blue and red), but differed in their optical configuration as shown in **Table 1** and, consequently, the Navios™ EX reference target values could not be directly applied for the XF-1600™.

To calculate the specific MFI correction factor, unique controls for each fluorochrome prepared with compensation beads were first acquired in Navios™ EX and adjusted in XF-1600™ to fully match the reference flow cytometer (**Figure 1**). PMT values obtained on XF-1600™ were used to acquire Rainbow Calibration Particles and calculate adjusted MFI values to determine XF-1600™ target values (**Figure 2**).

Evaluation of Lymphoid Screening Tube (LST). Peripheral blood (PB) samples from healthy donors were prepared with OneFlow™ LST (BD Biosciences) and analyzed on XF-1600™, after EuroFlow setup using XF-1600™ defined target values (**Table 2**). FCS files were analyzed on Infinicyt™, demonstrating a good identification of all cell subpopulations, as shown in **Figure 3**.

Table 1. Filter configuration of the reference flow cytometer (Navios EX) and the evaluated flow cytometer (XF-1600).

Detector	Navios™ EX	XF-1600™
FL1 (FITC)	525/40	530/30
FL2 (PE)	570/30	580/30
FL4 (PerCP-Cy5.5)	695/30	700/50
FL5 (PE-Cy7)	755 LP	750 LP
FL6 (APC)	660/20	660/20
FL8 (APC-H7)	755 LP	780/50
FL9 (Pacific Blue)	450/50	450/50
FL10 (Pacific Orange)	550/40	540/80

Table 2. Reference target values (7th peak) for XF-1600.

Detector	Lower MFI (-15%)	MFI	Upper MFI (+15%)
FL1 (FITC)	339.35	1658.20	459.11
FL2 (PE)	461.21	1796.77	623.99
FL4 (PerCP-Cy5.5)	927.78	3494.22	1255.24
FL5 (PE-Cy7)	96.65	400.62	130.76
FL6 (APC)	1847.48	5570.75	2499.54
FL8 (APC-H7)	431.79	1536.30	584.19
FL9 (Pacific Blue)	1758.92	5282.16	2379.72
FL10 (Pacific Orange)	1062.14	3616.15	1437.02

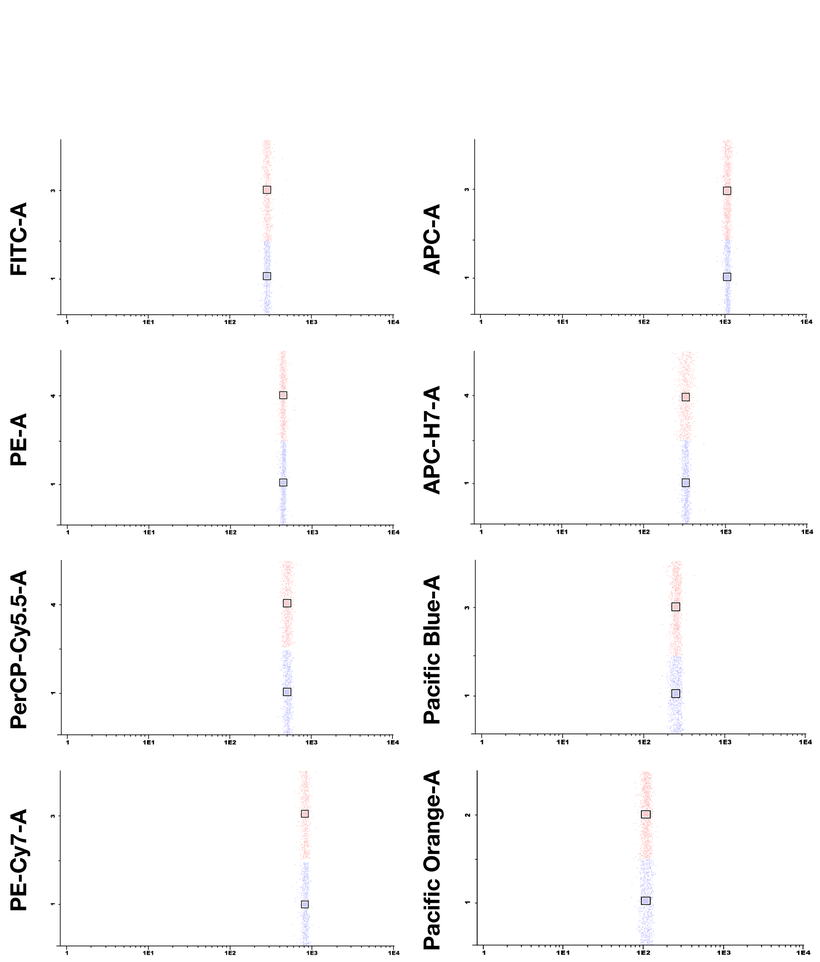

Figure 1. Single fluorescence controls measured on the reference flow cytometer and the evaluated flow cytometer. CyFlow™ Compset beads were stained with monoclonal antibodies to prepare single controls and acquired on Navios™ EX to obtain MFI patterns (purple dots). Then, single controls were acquired on XF-1600™ (pink dots) and PMT values were adjusted to match MFI from reference flow cytometer.

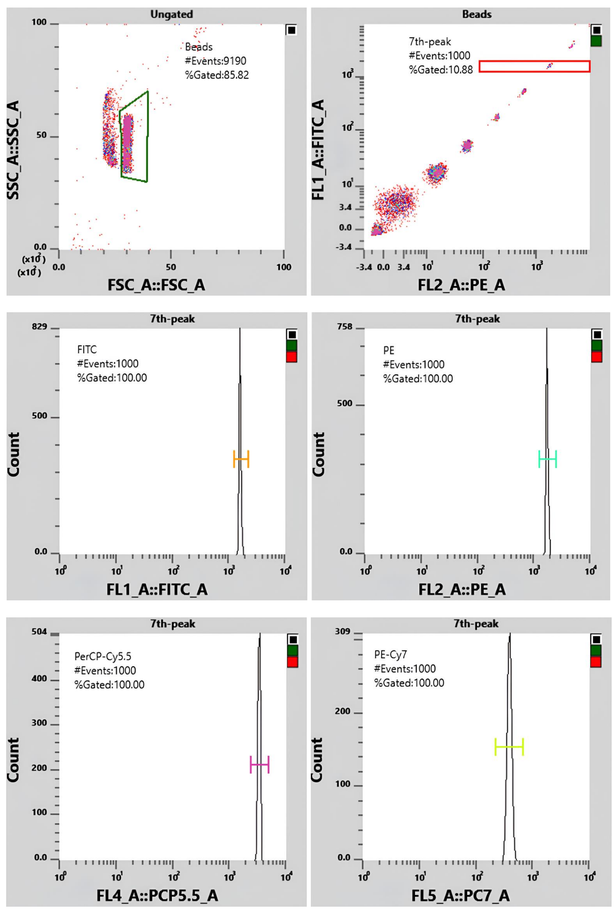

Figure 2. Rainbow Calibration Particles (RCP) analysis. RCP (lot. EAP01) were analyzed on XF-1600™ using obtained PMT values to calculate XF-1600™ 7th peak reference target values for each fluorochrome (FITC, PE, PerCP-Cy5.5, PE-Cy7 – data shown; and APC, APC-Cy7, Pacific Blue and Pacific Orange – data not shown).

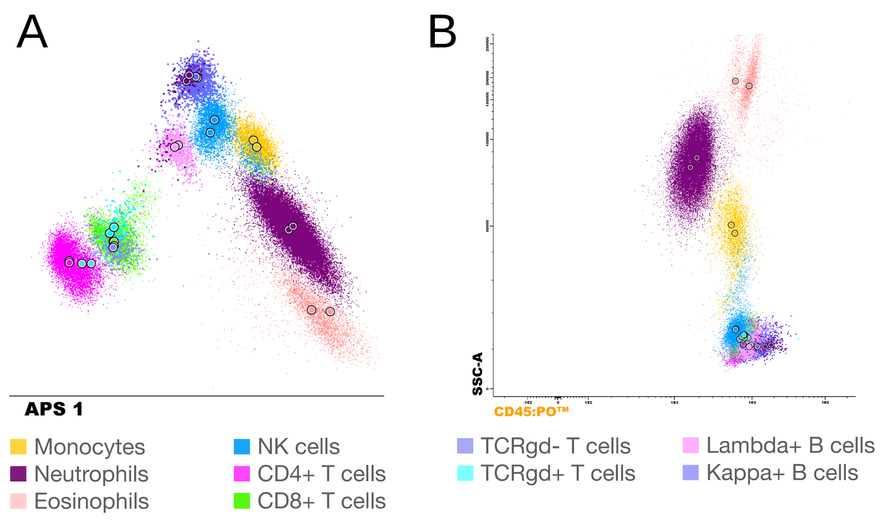

Figure 3. Evaluation of the Lymphoid Screening Tube (LST). Peripheral blood samples were prepared with OneFlow™ LST (BD Biosciences), acquired on XF-1600™, and analyzed on Infinicyt™ Software to detect leukocyte subpopulations. Dual dotplots showing the Automatic Population Separator 1 **(A)** and a CD45-PO vs SSC-A **(B)**. Classified cell subpopulations are identified by different colors (legend).

CONCLUSIONS

In this study we have demonstrated that the standardized EuroFlow approach based on specific target MFIs can be used on other flow cytometers equipped with >8 colors, such as the XF-1600™. Further experiments will be necessary to evaluate the performance of this setup providing superimposable data files when analyzing samples labeled with specific monoclonal antibodies.

ACKNOWLEDGEMENTS

AGAUR, Generalitat de Catalunya, Catalan Research Group (SGR) 2021 SGR 00002