The Importance of Being Regulated

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Introduction

Flow cytometry has become an integral part of immunological and hematological diagnostics worldwide during the last 20 years. For the purpose of standardization in diagnostics and monitoring of leukemia & lymphoma diseases, initiatives such as EuroFlow™ consortium identified optimal panels of monoclonal antibody reagents.

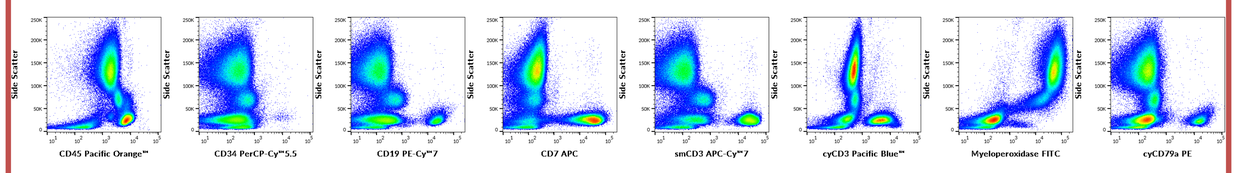
For these flow cytometry tests, single-color conjugated monoclonal antibodies are used for reagent cocktails. While many of the clinical laboratories are forced to utilize regulated in vitro diagnostic product (CE IVD) reagents, in principle, upon cocktailing to the final antibody panel they immediately breach the intended use of the CE IVD single reagent. Therefore, many clinical laboratories still use non-regulated products for research (RUO) and validate each lot before use.

Here, we introduce the new line of single-labeled monoclonal antibodies called SingleFlowEx®. SingleFlowEx® products are not registered as CE IVD reagents, however they are developed and produced in accordance to current Good Manufacture Practices. The stringent design control ensures that SingleFlowEx® reagents be suitable for cocktailing for use in leukemia & lymphoma diagnostics.

SingleFlowEx® product line suitability for cocktailing in clinical diagnostics

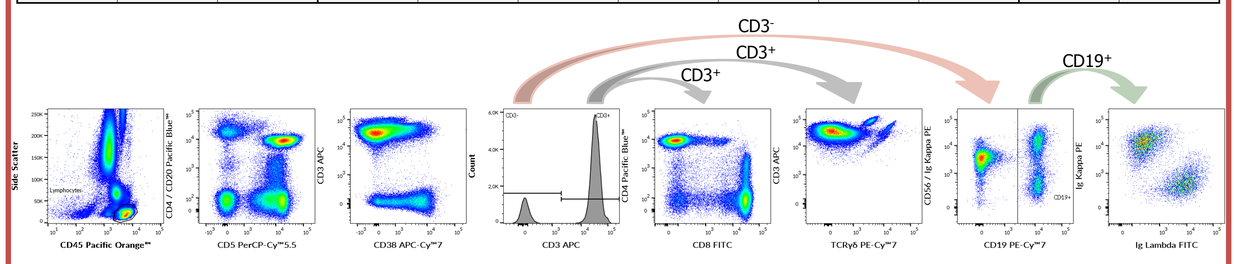
ALOT: Acute Leukemia Orientation Tube [1]

Pacific Blue™	Pacific Orange™	FITC	PE	PerCP-Cy™5.5	PE-Cy™7	APC	APC-Cy™7
cyCD3	CD45	Myeloperoxidase	CD79a	CD34	CD19	CD7	smCD3
UCHT1	2D1	MPO421-8B2	HM57	581	LT19	124-1D1	SK7
ED7158	ED7094	ED7261	ED7171	T9-664-T100*	ED7133	ED7084	ED7160
cytoplasmic	surface membrane	cytoplasmic	cytoplasmic	surface membrane	surface membrane	surface membrane	surface membrane



LST: Lymphoid Screening Tube [1]

Pacific Blue™		Pacific Orange™	FITC		PE		PerCP-Cy™5.5	PE-Cy™7		APC	APC-Cy™7
CD20	CD4	CD45	CD8	Ig Lambda	CD56	Ig Kappa	CD5	CD19	TCR γδ	CD3	CD38
2H7	MEM-241	2D1	MEM-31	1-155-2	LT56	TB28-2	L17F12	LT19	B1	UCHT1	HIT2
ED7180	ED7140	ED7094	ED7101	ED7216	ED7258	ED7222	ED7240	ED7133	ED7625	ED7162	T4-366-T100*



***** Not a SingleFlowEx® line product – Soon-to-be-developed

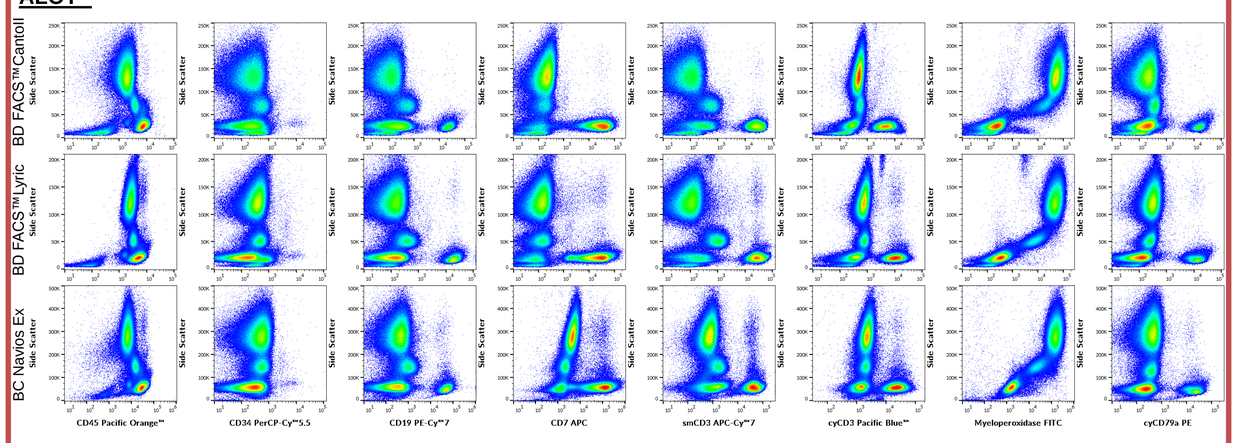
Conclusion:

We have prepared two panels ALOT and LST from SingleFlowEx single antibodies based on EuroFlow™ [1, 2] design used in common clinical practice.

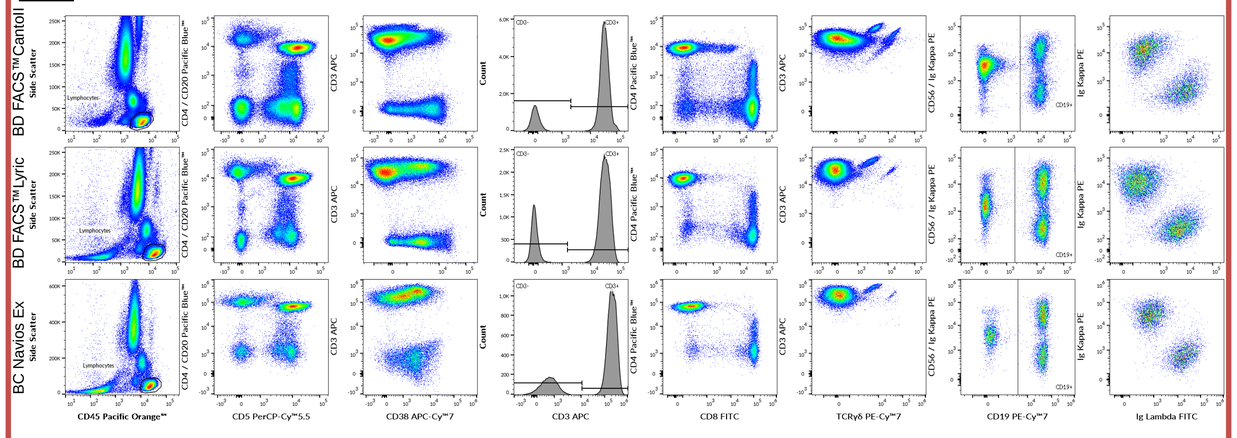
In both cases data show that both ALOT and LST tube provide staining patterns that allow for easy identification of all target cell populations when prepared using SingleFlowEx product line reagents.

Cytometer platform variability-dependent performance of SingleFlowEx® product line

ALOT [1]



LST [1]



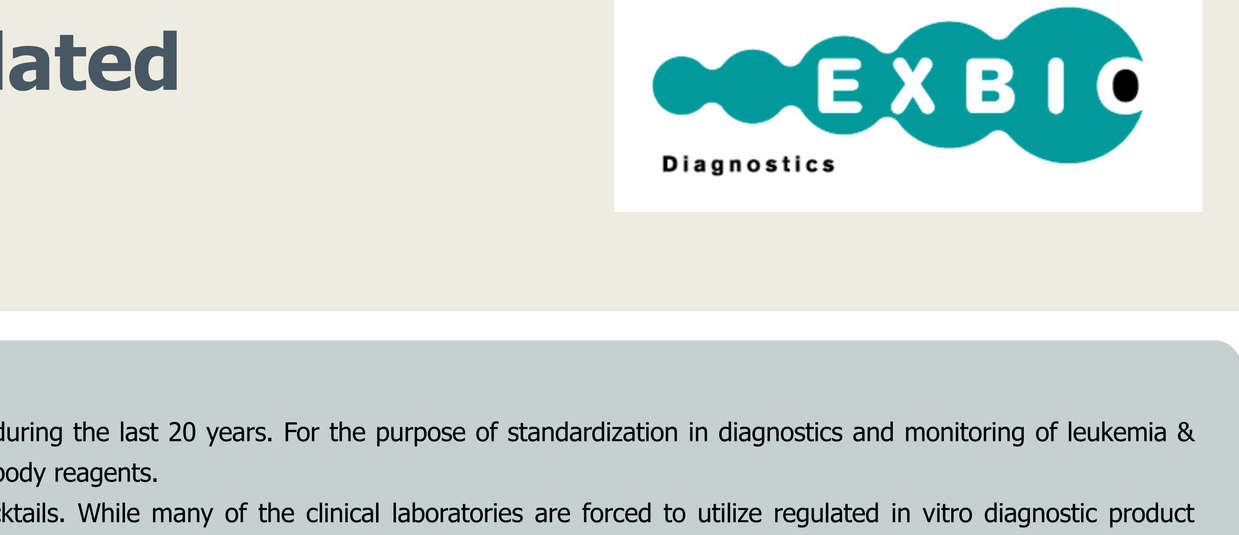
Conclusion:

In order to simulate interlaboratory variability where different cytometer platforms may be available, both ALOT and LST tubes prepared using SingleFlowEx single reagents were analysed using 3 different cytometers: Becton Dickinson (BD) FACSCanto™ II, BD FACSLyric™ and Beckman Coulter (BC) Navios Ex. All platforms are identified as clinical CE IVD and IVD 510k approved medical devices.

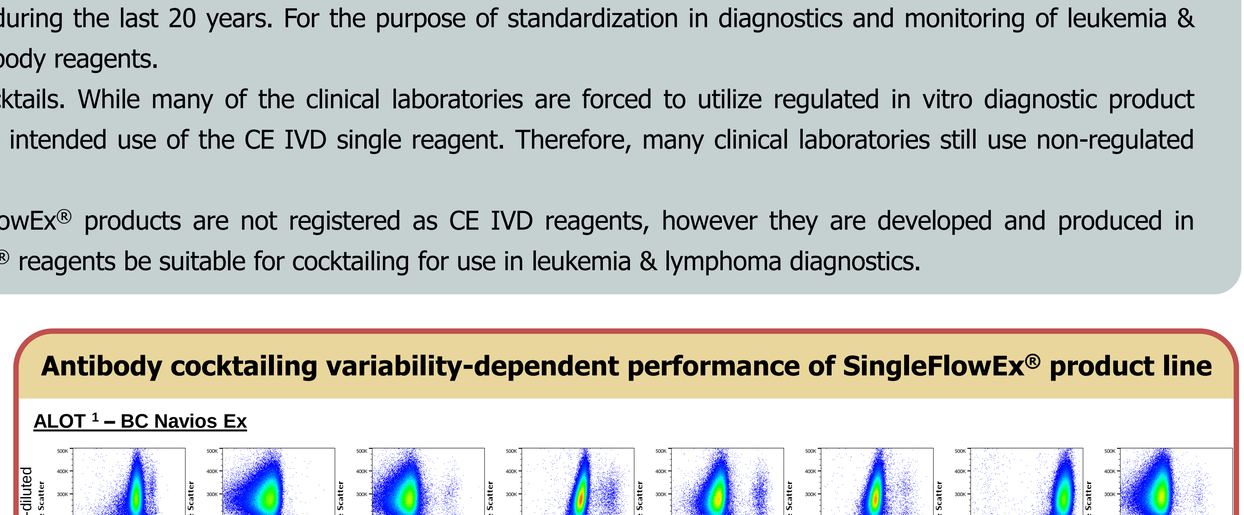
Both ALOT and LST cocktails provide comparable staining patterns and allow for easy target population identification on all 3 cytometer platforms.

Antibody cocktailing variability-dependent performance of SingleFlowEx® product line

ALOT [1] – BC Navios Ex



LST [1] – BC Navios Ex



Conclusion:

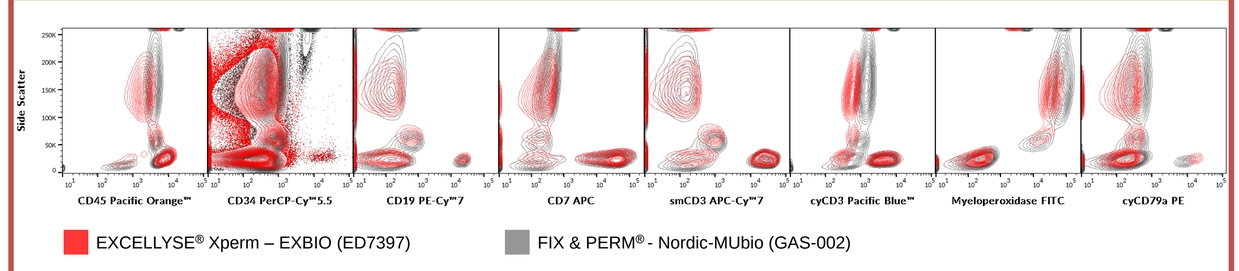
To further simulate inter-laboratory variability where operator-generated pipetting errors and different antibody concentrations have to be taken into consideration, both ALOT and LST tubes were 2x, 5x and 10x diluted from the antibody stock solution.

In the case of ALOT tube separation of positive population from negative events decreased with dilution. However, even at 10x dilution one may successfully identify positive population from negative events.

Similar titration profile was observed with 10 antibodies (out of 12) of LST tube except for CD38 APC-Cy™7 and TCR γδ PE-Cy™7, with the former being prone to signal loss due to possibly low concentration in the cocktail and hence considerable signal loss after cocktail dilution and the latter being a conjugate of a rather weaker antibody clone. The anti-Hu TCR γδ clone B1 identifies two discrete populations on the CD3 APC vs. TRC γδ PE-Cy™7 dot-plot with different degree of positivity. After ten-fold dilution only the more positive TCR γδ population was clearly distinguishable.

Presented results show great robustness of SingleFlowEx product line possibly allowing decrease in inter-laboratory variability in leukemia & lymphoma antibody panels.

Fixation and permeabilization staining protocol variability-dependent performance of SingleFlowEx® product line



Conclusion:

Furthemore, two different fixation and permeabilization reagents have been used for ALOT tube preparation and compared to simulate reagent variability impact on target cell identification.

Dot-plot overlays show minimum signal and background variability when different fixation and permeabilization reagent is used. Myoeloperoxidase (MPO) positive neutrophils and monocytes, CD79a positive B-lymphocytes and cytoplasmic CD3 positive T-lymphocytes are well separated from negative events allowing for easy identification using fixation and permeabilization reagents from both vendors.

References

1) Dongen, J J M Van, et al. "EuroFlow Antibody Panels for Standardized n-Dimensional Flow Cytometric Immunophenotyping of Normal, Reactive and Malignant Leukocytes." *Leukemia*, vol. 26, no. 9, Mar. 2012, pp. 1908–1975., doi:10.1038/leu.2012.120.

2) Kalina, T, et al. "EuroFlow Standardization of Flow Cytometer Instrument Settings and Immunophenotyping Protocols." *Leukemia*, vol. 26, no. 9, 2012, pp. 1986–2010., doi:10.1038/leu.2012.122.

Summary

The data presented confirms the importance of regulated environment for development and manufacture of products to be used in clinical applications, hence allowing the health institutions to have the possibility of preparing and using products in-house as addressed in the new EU Directive 2017/746 on In Vitro Diagnostic Medical Devices.