

WP5: Flow cytometric analysis from blood of patients with systemic autoimmune diseases



PRECISESADS

Molecular Reclassification to Find
Clinically Useful Biomarkers for
Systemic Autoimmune Diseases



Objective: elaborate protocols for multicentre flow cytometric analyses



**11 cytometry centers
6 cytometers**

Institution	City	Country	Device
FPS	Granada	Spain	FACS Verse
SAS	Córdoba	Spain	FACS Aria III
UBO	Brest	France	Navios
IDIBELL	Spain	Spain	Gallios
IRCSS	Milano	Italy	FACS Canto II
DRFZ	Berlin	Germany	FACS Canto II
UNIGE	Geneva	Switzerland	Navios
K. U. LEUVEN	Leuven	Belgium	LSR Fortessa
CH PORTO	Porto	Portugal	Navios
MHH	Hannover	Germany	FACS Canto II
UCL	Louvain	Belgium	FACS Canto II

Our challenges step by step

- 1st Challenge : Fluorescence compatibility between Beckman-Coulter and Becton-Dickinson flow cytometers**
- 2nd challenge : Panel optimization (compatibilities between dyes and markers for each panel)**
- 3rd challenge : Minimize variation among sites**
- 4th challenge : Continuous QC process**



Panel Optimization

FITC	PE	PC5.5	Pe-Cy7	APC	APC AF750	Pacific Blue	Krome Orange
525nm	575nm	700nm	770nm	660nm	780nm	450nm	550nm
Excitation 488nm				Excitation 638nm		Excitation 405nm	

Panel 1: CD16, CD15, CD56, CD14, CD19, CD3, CD4, CD8

Panel 2: CD1c, Lineage, CD141, CD11c, CD123, DRAQ7, HLA-DR

Panel 3: CD57, CD45RA, CD27, CD62L, CD38, CD3, CD4, CD8

Panel 4: TCR $\gamma\delta$, TCR $\alpha\beta$, CD25, CD127, CD69, CD3, CD4, CD8

Panel 5: IgD, TACI, CD5, CD27, CD19, CD24, CD38

Panel 6: CD43, CD69, CD5, CD27, CD11b, CD20, CD3

Panel 7: CD64, CD32, CD18, CD14, CD19, CD11b, CD16

Panel 8: CD35, CD59, CD14, CD19, CD3, CD21, CD16

Panel 9: CD46, CD55, CD14, CD19, CD3, CD16

Reference sites:
Brest and Granada

Whole blood lyse no-wash protocol

- Fluorochrome optimization
- Lysis solution selection
- Optimization of antibody cocktails

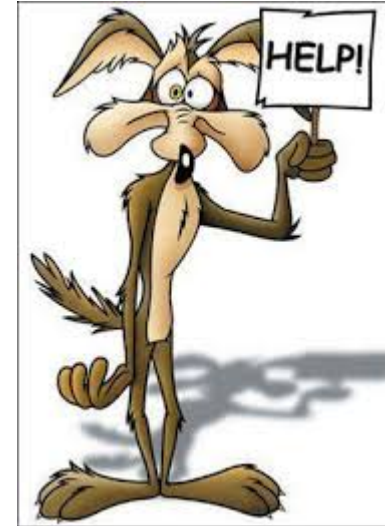
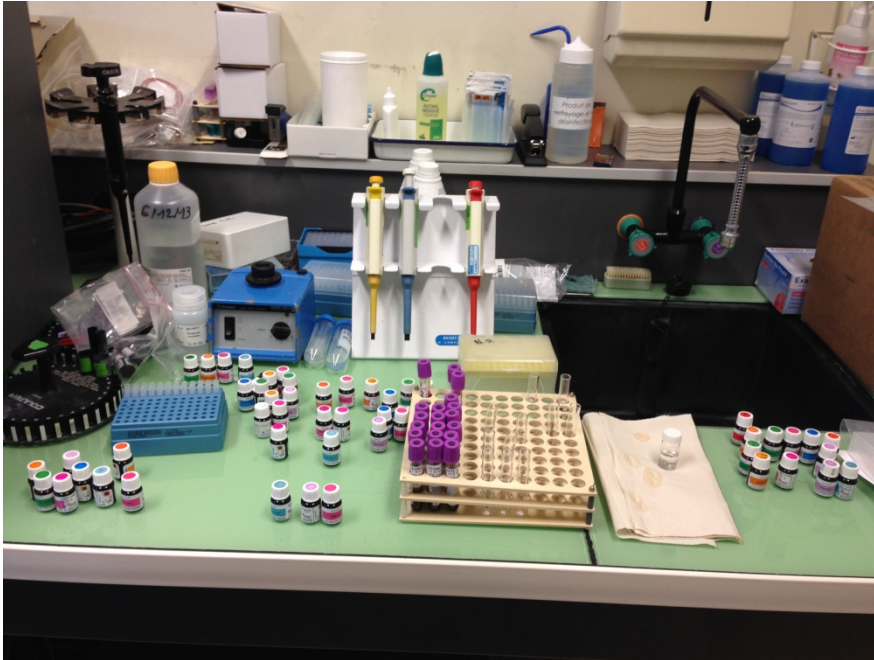
Color Doku

Difficulty 1 2 3 4 5



Minimize variation among sites

More than 50 different tubes/sample



Take dry unitized
dried-down
reagent tube

Add blood
sample

Vortex &
incubate

Add lysis
solution &
incubate

Acquire &
analyze by flow
cytometry

Change batch and
validate every 2 years

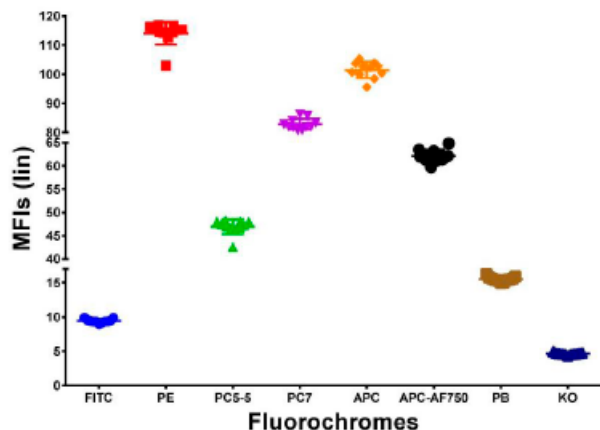


PRECISESADS

Molecular Reclassification to Find
Clinically Useful Biomarkers for
Systemic Autoimmune Diseases

Coordinated flow cytometry acquisition

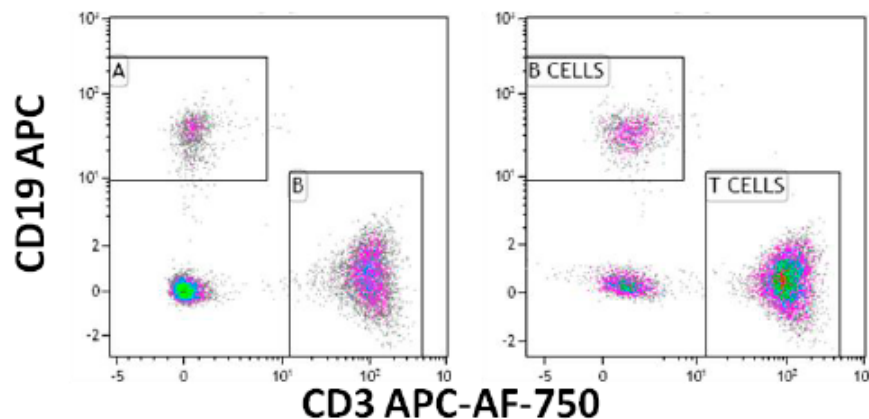
Capture beads



Personalized report to every site to fix deviations

VERSE

NAVIOS



Same donor, same day

[Data Set 2] [B CELLS] CD19 APC-A Median	33,39
[Data Set 1] [B CELLS] CD19-APC-A Median	32,90
[Data Set 2] [T CELLS] CD3 APC-AF750-A Median	98,25
[Data Set 1] [T CELLS] CD3-APC-AF750-A Median	98,40



Continuous quality control process



- PMT set-up using 8 peaks beads **every day** before sample acquisition



- Intercalibration verification **every 3 months** using capture beads



- Intercalibration verification **every 6 months** using a blood sample



- Coordination teleconference **once a month**

