Using Standardized Dry Antibody Panels for Flow Cytometry in the Assessment of Altered Immune Profiles in Response to SARS-CoV2 Infection

***Authors**, R Bowers[1], Giulia Grazia[2], Michael Kapinsky[3], Vashti Lacaille[4], Anis Larbi[5]*
[1]Beckman Coulter, Indianapolis, IN, [2]Beckman Coulter, Cassina de' Pecchi, Italy, [3]Beckman Coulter GmbH, Krefeld, Germany, [4]Beckman Coulter, Miami, FL, [5]Beckman Coulter, Villepinte, France

INTRODUCTION

In order to identify early indicators of disease severity in SARS-CoV2-infection, the proportions of well-established immune cell phenotypes have been subject to extensive research, utilizing flow cytometry as a core technology[1-3]. In order to ensure comparable and consistent results in the massively multi-institutional research setting of a global pandemic, the use of standardized antibody panels and procedures, as demonstrated by The ONE Study[4-6], is a promising approach that also can lower technical barriers.

AIM

As a highly standardized reagent set for comprehensive immune profiling, dry DURAClone* antibody panels (Beckman Coulter) were extended by adding antibodies in liquid format and evaluated for their utility as straightaway immune profiling research tools in normal and SARS-CoV2-positive donors.

METHOD

SAMPLES

Cryopreserved PBMCs from

- COVID 19 negative healthy donors (n=4)
- COVID 19 positive donors with different degree of symptoms: Asymptomatic (n=2), Mild (n=2), Moderate (n=1), Severe (n=2)

ANTIBODY PANELS

Panel#1: DURAClone IM Phenotyping Basic*
Drop-ins HLA-DR-PacBlue, CD123-PC5.5

405 nm		488 nm	561 nm				638 nm			808 nm
HLA-DR-Pacific Blue	CD45-Krome Orange	CD16-FITC	CD56-PE	CD19-ECD	CD123-PC5.5	CD14-PC7	CD4-APC	CD8-A700	CD3-APC-A750	ViaKrome 808 FVD

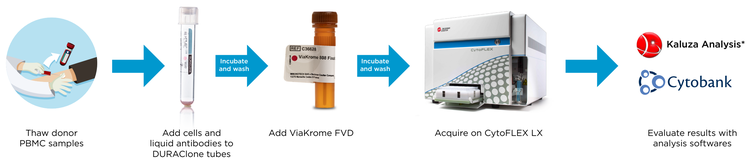
Panel#2: DURAClone IM T cell subsets*
Drop-ins CD31-BV605, CD25-BV650, CD127-BV785

405 nm					488 nm	561 nm			638 nm			808 nm
CD57-Pacific Blue	CD45-Krome Orange	CD31-BV605	CD25-BV650	CD127-BV785	CD45RA-FITC	CD197-PE	CD28-ECD	CD279-PC5.5	CD27-PC7	CD4-APC	CD8-A700	CD3-APC-A750	ViaKrome 808 FVD

Panel#3: DURAClone IM B cells*
Drop-ins CD25-PC5.5, CD71-APC-A700

405 nm		488 nm	561 nm				638 nm			808 nm
IgM-Pacific Blue	CD45-Krome Orange	IgD-FITC	CD21-PE	CD19-ECD	CD25-PC5.5	CD27-PC7	CD24-APC	CD71-APC-A700	CD38-APC-A750	ViaKrome 808 FVD

WORKFLOW



RESULTS

The **DURAClone IM Phenotyping Basic*** panel provides an overview of lymphocytes and monocytes subpopulations in healthy donors (HD) and COVID-19 positive patients.

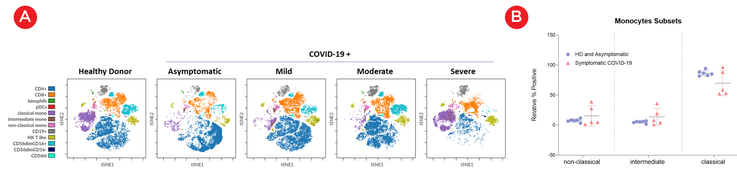


Figure 1. A) Overlay of manual gates on a viSNE map (Cytobank*) highlighting major cell subsets identified by staining of DURAClone tubes in PBMC from a healthy donor and four COVID-19 positive patients with different degrees of disease severity. **B)** Relative % of non-classical (CD14-CD16+), Classical (CD14+CD16-) and Intermediate (CD14+CD16+) monocytes in healthy donors and asymptomatic individuals compared to symptomatic COVID-19+ patients. viSNE was run on 9 population-defining markers with default settings.

The **DURAClone IM T Cell Subsets*** panel allows the delineation of maturation stages of T cells, covering naïve, effector, memory and terminal differentiation stages

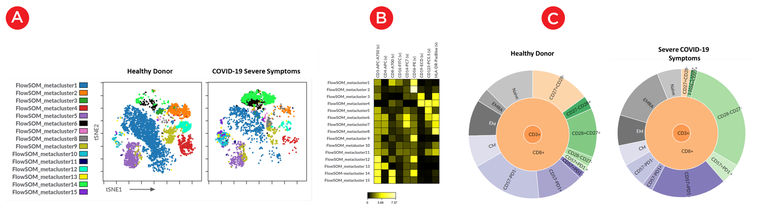
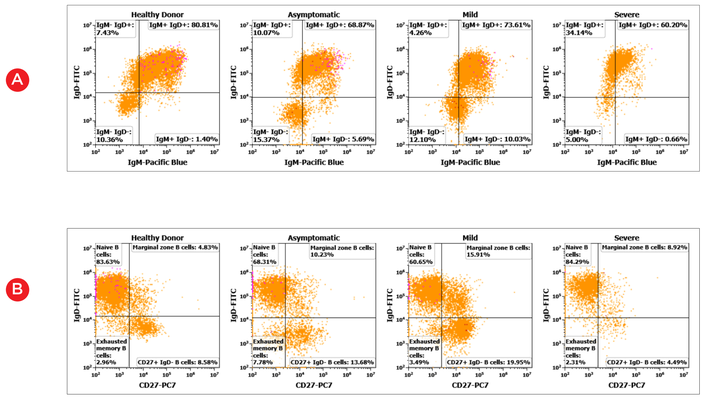


Figure 2. A) Overlay of FlowSOM-identified metaclusters on viSNE maps (Cytobank*) for a healthy donor (HD) and a COVID-19 positive patient with severe disease. **B)** Heatmap visualization of marker expression by FlowSOM metacluster. Data was compensated and logicle transformed using Kaluza Analysis Software* and uploaded to the Cytobank platform through the Kaluza* Cytobank Plugin. viSNE was run on 12 population-defining markers with default settings. FlowSOM was used with hierarchical consensus clustering. **C)** Sunburst plots (Cytobank) are used to display hierarchical relationships of manual gates in two representative samples.

The **DURAClone IM B Cell*** panel allows for identification of late maturation stages of B cells, such as transitional stage, isotype class-switch, naïve and memory stages.



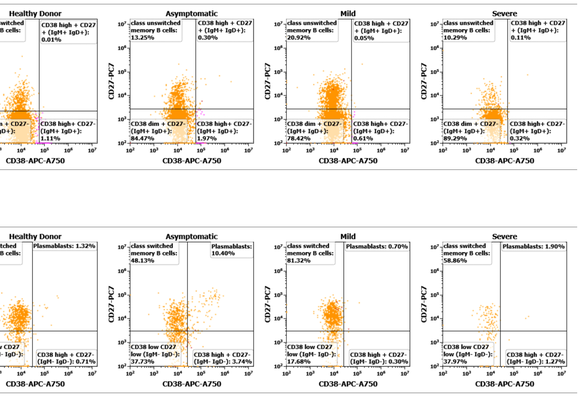


Figure 3. Representative bivariate histogram plots showing B-cell subpopulations **(A)** class-switch by IgM/IgD **(B)** Naive/memory stages by CD27/IgD **(C)** class unswitched memory B cells and **(D)** class switched memory B cells and plasmablasts in healthy and COVID19 positive donor PBMC samples.

CONCLUSIONS

- The DURAClone IM antibody panels Phenotyping Basic, T Cell Subsets and B Cell (all RUO*) allow for straightaway standardized immune profiling for research purposes, including flexible antibody additions.
- In this research context, cryopreserved healthy and SARS-CoV-2-positive samples revealed marked differences by manual population gating as well as by unsupervised analysis (non-significant, small n).
- The dry DURAClone* reagent format reduces sources of human error, thus ensuring observed differences are due to biological variation as opposed to inconsistent staining protocol execution.

REFERENCES

1. Cossarizza A et al. SARS-CoV-2, the Virus that Causes COVID-19: Cytometry and the New Challenge for Global Health. Cytometry A. 2020;97(4): 340-343.
2. Schulte-Schrepping J et al. Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. Cell. 2020;182(6):1419-1440
3. Mathew D et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science. 2020;369(6508)
4. Streitz M et al. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. Transplant Res. 2013;2(1):17.
5. Kverneland AH et al. Age and gender leucocytes variances and references values generated using the standardized ONE-Study protocol. Cytometry A. 2016;89(6):543-564.
6. Sawitzki B et al. Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. Lancet. 2020; 395(10237):1627-1639.

CONTACT INFORMATION

For questions on:

- Data acquisition and manual population analysis, please contact Rita Bowers at rbowers@beckman.com
- Unsupervised data analysis & Cytobank, please contact Giulia Grazia at GGRAZIA@beckman.com
- DURAClone* antibody panels, please contact Michael Kapinsky at mkapinsky@beckman.com

* For Research Use Only. Not for use in diagnostic procedures.

12.04.1511.FLOW

BECKMAN COULTER Life Sciences