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

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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.

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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.

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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

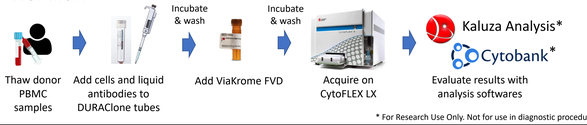
405 nm				488 nm	561 nm				638 nm			808 nm
CD57-Pacific Blue	CD45-Krome Orange	CD31-BV605	CD25-BV650	CD127-BV785	CD45RA-FITC	CD197-PE	CD28-ECD	CD279-PC5.5	CD27-PC7	CD4-APC	CD8-A700	CD3-APC-A750	ViaKrome 808 FVD

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



Thaw donor PBMC samples | Add cells and liquid antibodies to DURAClone tubes | Add ViaKrome FVD | Acquire on CytoFLEX LX | Evaluate results with analysis softwares

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RESULTS

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

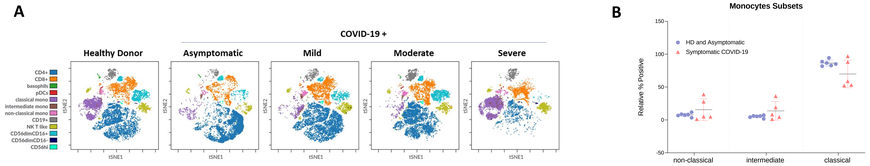

Figure 1: **A**) Overlay of manual gates on a viSNE map (Cytobank*) highlighting major cell subsets identified by staining of DURAClone tubes in PBMC from a healthy donor and four COVID-19 positive patients with different degrees of disease severity. **B**) Relative % of non-classical (CD14-CD16+), Classical (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.

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

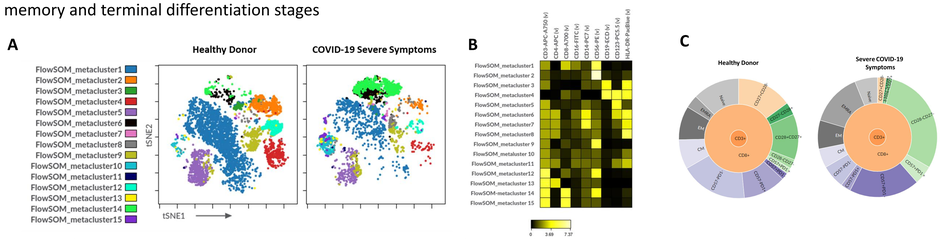

Figure 2: **A**) Overlay of FlowSOM-identified metaclusters on viSNE maps (Cytobank*) for a healthy donor (HD) and a COVID-19 positive patient with severe disease. **B**) Heatmap visualization of marker expression by FlowSOM metacluster. Data was compensated and logicle transformed using Kaluza Analysis 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.

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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.

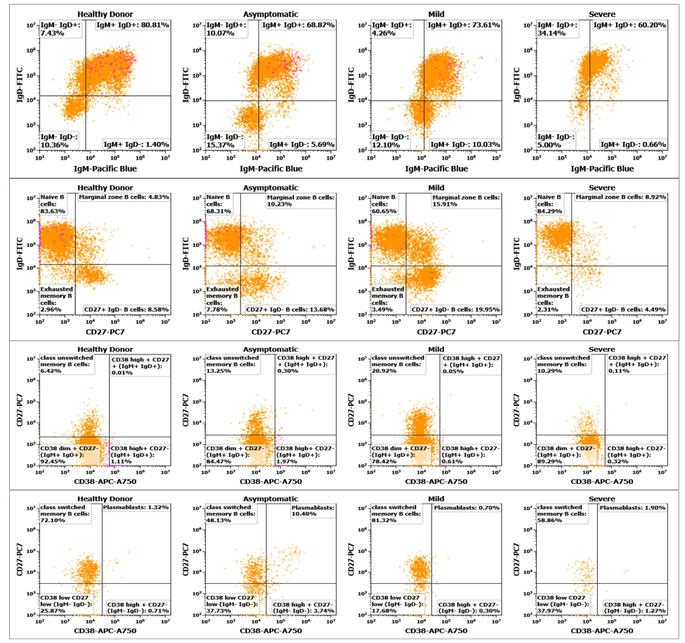

Figure 3: Representative bivariate histogram plots showing B-cell subpopulations **(A)** class-switch by IgM/IgD **(B)** Naive/memory stages by CD27/IgD **(C)** class unswitched memory B cells and **(D)** class switched memory B cells and plasmablasts in healthy and COVID19 positive donor PBMC samples.

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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.

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