Monitoring the immune responses to vaccination and pertussis: bordetella pertussis and beyond

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Appendices

APPENDIX 1. Overview of the EuroFlow panels and versions used in this thesis.

For transparency within this PhD thesis and convenience of the reader only!
When citing, please refer to the original publications instead of this appendix!

Table 1. EuroFlow Primary immunodeficiency orientation tube (PIDOT). Version 1
Official references for this panel: van der Burg M, Kalina T, Perez-Andres M, et al. The EuroFlow PID Orientation Tube for Flow Cytometric Diagnostic Screening of Primary Immunodeficiencies of the Lymphoid System. Frontiers in Immunology 2019;10:246.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	1	all added to membrane staining	no intracellular staining
CD45RA	BV510	HI100	BD Biosciences	563031	2.5		
CD8	FITC	SK1	BD Biosciences	345772	5		
IgD	FITC	IA6-2	BioLegend	348205	1.25		
CD16	PE	3G8	BD Biosciences	555407	5		
CD56	PE	C5.9	Cytognos	CYT-56PE	5		
CD4	PerCP Cy5.5	SK3	BD Biosciences	332772	7		
IgM	PerCP Cy5.5	MHM-88	BioLegend	314511	2		
CD19	PE-Cy7	J3-119	Beckman Coulter	IM3628	5		
TCRγδ	PE-Cy7	11F2	BD Biosciences	649806	1		
CD3	APC	SK7	BD Biosciences	345767	2.5		
CD45	APC-H7	2D1	BD Biosciences	641417	2		

Table 2. EuroFlow Primary immunodeficiency orientation tube (PIDOT). Version 2 – lyophilized version of the PIDOT with 2 drop-in antibodies (CD27 BV421 and CD45RA BV510). In the lyophilized version, different clones were used for CD8, IgD, CD4, TCRγδ, CD3, and CD45. Fluorochromes stayed the same, with exception of CD45, where APC H7 was replaced by APC C750

Official references for this panel: van der Burg M, Kalina T, Perez-Andres M, et al. The EuroFlow PID Orientation Tube for Flow Cytometric Diagnostic Screening of Primary Immunodeficiencies of the Lymphoid System. Frontiers in Immunology 2019;10;246.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Mem-brane	Intracel-lular
CD27	BV421	M-T271	Cytognos	CYT-PIDOT8-R	1	all added to mem-brane staining	no intracel-lular staining
CD45RA	BV510	HI100			2.5		
CD8	FITC	UCHT-4			50		
IgD	FITC	IADB6					
CD16	PE	3G8					
CD56	PE	C5.9					
CD4	PerCP Cy5.5	RPA-T4					
IgM	PerCP Cy5.5	MHM-88					
CD19	PE-Cy7	J3-119					
TCRγδ	PE-Cy7	TCR-1					
CD3	APC	UCHT-1					
CD45	APC-C750	HI30					

Table 3. EuroFlow PERISCOPE B-cell and plasma cell panel (BIGH) panel, version 1

Official references for this panel: Blanco E, Pérez-Andrés M, Arriba-Méndez S, et al. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. JACI 2018;141(6):2208-2219. e2216. Diks AM, Versteegen P, Teodosio C, et al. Age and Primary Vaccination Background Influence the Plasma Cell Response to Pertussis Booster Vaccination. Vaccines. 2022;10(2):136. Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	2	all added to membrane staining	
IgM	BV510	MHM-88	BioLegend	314522	2		X
CD62L	BV605	DREG-56	BioLegend	304833	5		
CD24	BV650	ML5	BD Biosciences	563720	5		
CD21	BV711	B-Ly4	BD Biosciences	563163	5		
CD19	BV786	SJ25C1	BD Biosciences	563163	4		
IgG3	FITC	SAG3	Cytognos	CYT-IGG3F	3		X
IgG2	FITC	SAG2	Cytognos	CYT-IGG2F	3		X
IgA2	PerCP Cy5.5	SAA2	Cytognos	CYT-IGA1C	3		X
IgA1	PerCP Cy5.5	SAA1	Cytognos	CYT-IGA2C	3		X
IgG1	PE	SAG1	Cytognos	CYT-IGG1PE	3		X
IgG2	PE	SAG2	Cytognos	CYT-IGG2PE	3		X
IgG4	APC	SAG4	Cytognos	CYT-IGG4AP	3		X
IgA1	APC	SAA1	Cytognos	CYT-IGA1AP	3		X
IgD	FITC	IA6-2	BioLegend	348205	1.25		X
CD20	PE CF594	2H7	BD Biosciences	562295	2.5 (1:10 dil)		
CD138	PE-Cy7	MI15	BioLegend	356513	5		
CD5	PE-Cy7	LIF7F12	BD Biosciences	348810	6		
IgD	APC	IA6-2	BD Biosciences	561303	4		X
CD38	APC H7	HB7	BD Biosciences	656646	3		

Table 4. EuroFlow PERISCOPE B-cell and plasma cell panel (BIGH) panel, version 2 – replacement of single/individual Ig antibodies by lyophilized Ig subclass cocktail

Official references for this panel: Blanco E, Pérez-Andrés M, Arriba-Méndez S, et al. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. JACI 2018;141(6):2208-2219. e2216. Diks AM, Versteegen P, Teodosio C, et al. Age and Primary Vaccination Background Influence the Plasma Cell Response to Pertussis Booster Vaccination. Vaccines. 2022;10(2):136. Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	2	all added to membrane staining	
IgM	BV510	MHM-88	BioLegend	314522	2		X
CD62L	BV605	DREG-56	BioLegend	304833	5		
CD24	BV650	ML5	BD Biosciences	563720	5		
CD21	BV711	B-Ly4	BD Biosciences	563163	5		
CD19	BV786	SJ25C1	BD Biosciences	563163	4		
Subclasses cocktail	Fluorochromes and clones identical to version 1		Cytognos	CYT-IGS-1	25		X
IgD	FITC	IA6-2	BioLegend	348205	1.25		X
CD20	PE CF594	2H7	BD Biosciences	562295	2.5 (1:10 dil)		
CD138	PE-Cy7	MI15	BioLegend	356513	5		
CD5	PE-Cy7	LIF7F12	BD Biosciences	348810	6		
IgD	APC	IA6-2	BD Biosciences	561303	4		X
CD38	APC H7	HB7	BD Biosciences	656646	3		

Table 5. EuroFlow PERISCOPE B-cell and plasma cell panel (BIGH) panel, version 3 - addition of CD45 AF700
Official references for this panel: Blanco E, Pérez-Andrés M, Arriba-Méndez S, et al. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. JACI 2018;141(6):2208-2219. e2216. Diks AM, Versteegen P, Teodosio C, et al. Age and Primary Vaccination Background Influence the Plasma Cell Response to Pertussis Booster Vaccination. Vaccines. 2022;10(2):136. Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	2	all added to membrane staining	
IgM	BV510	MHM-88	BioLegend	314522	2		X
CD62L	BV605	DREG-56	BioLegend	304833	5		
CD24	BV650	ML5	BD Biosciences	563720	5		
CD21	BV711	B-Ly4	BD Biosciences	563163	5		
CD19	BV786	SJ25C1	BD Biosciences	563163	4		
Subclasses cocktail	Fluorochromes and clones identical to version 1		Cytognos	CYT-IGS-1	25		X
IgD	FITC	IA6-2	BioLegend	348205	1.25		X
CD20	PE CF594	2H7	BD Biosciences	562295	2.5 (1:10 dil)		
CD138	PE-Cy7	MI15	BioLegend	356513	5		
CD5	PE-Cy7	LIF7F12	BD Biosciences	348810	6		
IgD	APC	IA6-2	BD Biosciences	561303	4		X
CD45	AF700	HI30	BD Biosciences	560566	10		
CD38	APC H7	HB7	BD Biosciences	656646	3		

Table 6. EuroFlow PERISCOPE CD4 T-cell (TCD4) panel, version 1
Official references for this panel: Botafogo V, Pérez-Andres M, Jara-Acevedo M, et al. Age distribution of multiple functionally relevant subsets of CD4+ T cells in human blood using a standardized and validated 14-color EuroFlow immune monitoring tube. Frontiers in immunology. 2020;11:166. Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	2	All added to membrane staining	No intracellular staining
CD45RA	BV510	HI100	BD Biosciences	563031	2.5		
CD62L	BV650	DREG-56	BioLegend	304832	2.5		
CD127	BV711	HIL7RM21	BD Biosciences	563165	2.5		
CD3	BV786	SK7	BD Biosciences	563800	1		
CD25	FITC VioBright	4E3	Miltenyi	130-104-323	10		
CCR10	PerCP Cy5.5	1B5	BD Biosciences	564772	2.5		
CXCR3	PE	1C6/CXCR3	BD Biosciences	557185	10		
CCR6	PE-CF594	11A9	BD Biosciences	564816	5		
CCR4	PE-Cy7	L291H4	BioLegend	359410	1		
CXCR5*	APC	51505	R&D	FAB190A-100	10		
CD4	APC H7	SK3	BD Biosciences	641398	5		

*EuroFlow PERISCOPE CD4 T-cell (TCD4) panel, version 1a - replacement of CXCR5 from R&D by CXCR5 from Miltenyi (panel switch while studies ongoing)

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CXCR5	APC	REA103	Miltenyi	130-098-422	2.5	X	

Table 7. EuroFlow PERISCOPE CD4 T-cell (TCD4) panel, version 2 - addition of CD45 AF700 and CD154 BV605
Official references for this panel: Botafogo V, Pérez-Andres M, Jara-Acevedo M, et al. Age distribution of multiple functionally relevant subsets of CD4+ T cells in human blood using a standardized and validated 14-color EuroFlow immune monitoring tube. Frontiers in immunology. 2020;11:166. Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	2	All antibodies except for CD154 BV605	
CD45RA	BV510	HI100	BD Biosciences	563031	2.5		
CD154	BV605	24-31	BioLegend	310826	5		X
CD62L	BV650	DREG-56	BioLegend	304832	2.5		
CD127	BV711	HIL7RM21	BD Biosciences	563165	2.5		
CD3	BV786	SK7	BD Biosciences	563800	1		
CD25	FITC VioBright	4E3	Miltenyi	130-104-323	10		
CCR10	PerCP Cy5.5	1B5	BD Biosciences	564772	2.5		
CXCR3	PE	1C6/CXCR3	BD Biosciences	557185	10		
CCR6	PE-CF594	11A9	BD Biosciences	564816	5		
CCR4	PE-Cy7	L291H4	BioLegend	359410	1		
CXCR5*	APC	51505	R&D	FAB190A-100	10		
CD45	AF700	HI30	BD Biosciences	560566	2.5		
CD4	APC H7	SK3	BD Biosciences	641398	5		

*EuroFlow PERISCOPE CD4 T-cell (TCD4) panel, version 1a - replacement of CXCR5 from R&D by CXCR5 from Miltenyi (panel switch while studies ongoing)

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CXCR5	APC	REA103	Miltenyi	130-098-422	2.5	X	

Table 8. EuroFlow PERISCOPE CD8 cytotoxic T-cell (CYTOX) panel, version 1
Official reference for this panel: Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	2	X	
CD45RA	BV510	HI100	BD Biosciences	563031	2.5	X	
CD62L	BV650	DREG-56	BioLegend	304832	2.5	X	
CD16	BV711	3G8	BD Biosciences	563127	2.5	X	
CD3	BV786	SK7	BD Biosciences	563800	1	X	
CD57	FITC	HNK1	BD Biosciences	333169	10	X	
CD28	PerCP Cy5.5	CD28.2	BioLegend	302922	5	X	
Granzyme B	PE	GB11	Sanquin	M2289	15		X
CD8	PE CF594	RPAT8	BD Biosciences	562282	1	X	
TCRγδ	PE Cy7	11F2	BD Biosciences	655410	1	X	
CD56	APC H7	HCD56	BioLegend	318332	5	X	

Table 9. EuroFlow PERISCOPE CD8 cytotoxic T-cell (CYTOX) panel, version 2 - addition of CD45 AF700 and CD154 BV605
Official reference for this panel: Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD27	BV421	M-T271	BD Biosciences	562513	2	X	
CD45RA	BV510	HI100	BD Biosciences	563031	2.5	X	
CD154	BV605	24-31	BioLegend	310826	5		X
CD62L	BV650	DREG-56	BioLegend	304832	2.5	X	
CD16	BV711	3G8	BD Biosciences	563127	2.5	X	
CD3	BV786	SK7	BD Biosciences	563800	1	X	
CD57	FITC	HNK1	BD Biosciences	333169	10	X	
CD28	PerCP Cy5.5	CD28.2	BioLegend	302922	5	X	
Granzyme B	PE	GB11	Sanquin	M2289	15		X
CD8	PE CF594	RPAT8	BD Biosciences	562282	1	X	
TCRγδ	PE Cy7	11F2	BD Biosciences	655410	1	X	
CD45	AF700	HI30	BD Biosciences	560566	2.5	X	
CD56	APC H7	HCD56	BioLegend	318332	5	X	

Table 10. EuroFlow PERISCOPE DC-Monocyte panel, version 1
Official references for this panel: Van der Pan et al, Development of a standardized and validated flow cytometry approach for monitoring of innate myeloid immune cells in human blood, Frontiers in Immunology, 2022, 5141. Patent filed by Van Dongen et al. Means and methods for multiparameter cytometry-based leukocyte subsetting. P119646NL00 (2019), PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD141	BV421	1A4	BD Biosciences	565321	2.5	X	No intracellular stainng
CD45	OC515	GA90	Cytognos	CYT-45OC	10	X	
CD62L	BV605	DREG-56	BioLegend	304833	5	X	
HLA DR	BV711	G46-6	BD Biosciences	563696	2.5	X	
CD16	BV786	3G8	BD Biosciences	563690	5	X	
CD1c	BB515	F10/21A3	BD Biosciences	565054	5	X	
CD36	PerCP Cy5.5	CLB-IVC7	Immunostep	36PP5-100T	10	X	
SLAN	PE	DD.1	Miltenyi	130-093-029	10	X	
FcεR1	PE	EAR-37	Thermo Fisher	A18416	5	X	
CD33	PE Cy7	P67.6	BD Biosciences	333952	5	X	
CD300e (IREM2)	APC	UP-H2	Immunostep	IREM2A-100T	10	X	
CD303	APC	AC144	Miltenyi	130-090-905	10	X	
CD14	APC H7	M5E2	BD Biosciences	641349	5	X	
BV stain buffer			BD Biosciences	566349	50	N/A	

Table 11. EuroFlow PERISCOPE DC-Monocyte panel, version 2 - replacement of CD45 OC515 by CD45 AF700
Official references for this panel: Van der Pan et al, Development of a standardized and validated flow cytometry approach for monitoring of innate myeloid immune cells in human blood, Frontiers in Immunology, 2022, 5141. Patent filed by Van Dongen et al. Means and methods for multiparameter cytometry-based leukocyte subsetting. P119646NL00 (2019), PCT/NL2020/050688, priority date 5 November 2019.

Marker	Fluorochrome	Clone	Source	Cat. number	Volume (µL)	Membrane	Intracellular
CD141	BV421	1A4	BD Biosciences	565321	2.5	X	No intracellular stainng
CD62L	BV605	DREG-56	BioLegend	304833	5	X	
HLA DR	BV711	G46-6	BD Biosciences	563696	2.5	X	
CD16	BV786	3G8	BD Biosciences	563690	5	X	
CD1c	BB515	F10/21A3	BD Biosciences	565054	5	X	
CD36	PerCP Cy5.5	CLB-IVC7	Immunostep	36PP5-100T	10	X	
SLAN	PE	DD.1	Miltenyi	130-093-029	10	X	
FcεR1	PE	EAR-37	Thermo Fisher	A18416	5	X	
CD33	PE Cy7	P67.6	BD Biosciences	333952	5	X	
CD300e (IREM2)	APC	UP-H2	Immunostep	IREM2A-100T	10	X	
CD303	APC	AC144	Miltenyi	130-090-905	10	X	
CD45	AF700	HI30	BD Biosciences	560566	10	X	
CD14	APC H7	M5E2	BD Biosciences	641349	5	X	
BV stain buffer			BD Biosciences	566349	50	N/A	

Table 12. Overview of the panels used in this thesis.

Thesis chapter	PIDOT version (V)	BIGH tube version (V)	DC-Monocyte tube version (V)	CD4 T-cell tube version (V)	CD8 cytotoxic T-cell tube version (V)
Chapter 2.1	V1, V2				
Chapter 2.2	V1				
Chapter 3.1		V1, V2	V1	V1, V1a	V1
Chapter 3.2		V3	V2	V2, V2a	V2
Chapter 3.4		V3	V2	V2, V2a	V2
Chapter 4.1		V3	V2	V2a	V2

APPENDIX 2. Phenotypic descriptions used to identify cell populations in samples stained with EuroFlow panels.

For transparency within this PhD thesis and convenience of the reader only!
When citing, please refer to the original publications instead of this appendix!

Gating strategies for the EuroFlow PIDOT panel have been published previously and can be found in the following manuscripts and in Chapter 2.2 of this PhD thesis:

- van der Burg M, Kalina T, Perez-Andres M, et al. The EuroFlow PID Orientation Tube for Flow Cytometric Diagnostic Screening of Primary Immunodeficiencies of the Lymphoid System. Frontiers in Immunology 2019;10;246.
- van der Velden VHJ, Flores-Montero J, Perez-Andres M, et al., Optimization and testing of dried antibody tube: The EuroFlow LST and PIDOT tubes as examples. Journal of Immunological Methods. 2019; 475: 112287

Official reference for the EuroFlow PERISCOPE B-cell and plasma cell panel (BIGH) panel:

- Blanco E, Pérez-Andrés M, Arriba-Méndez S, et al. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. JACI 2018;141(6):2208-2219. e2216.
- Diks AM, Versteegen P, Teodosio C, et al.
Age and Primary Vaccination Background Influence the Plasma Cell Response to Pertussis Booster Vaccination. Vaccines. 2022;10(2):136.
- Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Official reference for the EuroFlow PERISCOPE CD4 T-cell (TCD4) panel:

- Botafogo V, Pérez-Andres M, Jara-Acevedo M, et al. Age distribution of multiple functionally relevant subsets of CD4+ T cells in human blood using a standardized and validated 14-color EuroFlow immune monitoring tube. Frontiers in immunology. 2020;11:166.
- Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Official reference for the EuroFlow PERISCOPE CD8 cytotoxic T-cell (CYTOX) panel:

- Patent filed by Van Dongen et al. Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting. P119646NL00 (2019). PCT/NL2020/050688, priority date 5 November 2019.

Official references for the EuroFlow PERISCOPE DC-Monocyte panel:

- Van der Pan et al, Development of a standardized and validated flow cytometry approach for monitoring of innate myeloid immune cells in human blood, Frontiers in Immunology, 2022, 5141.
- Patent filed by Van Dongen et al. Means and methods for multiparameter cytometry-based leukocyte subsetting. P119646NL00 (2019), PCT/NL2020/050688, priority date 5 November 2019.

Table 1. Phenotypic descriptions used to define B-cell subsets stained with EuroFlow PERISCOPE B-cell and plasma cell panel (BIGH) panel by manual analysis. The removal of debris and doublets is not indicated in the analysis strategy below, but should be performed to ensure high quality data. This table was previously published in: Diks et al. Age and Primary Vaccination Background Influence the Plasma Cell Response to Pertussis Booster Vaccination. Vaccines, 2022

Stepwise approach (gating in 2D plots)	Phenotypic description
#1. Identification of total plasma cells	• CD45+CD19dimCD38highCD21-CD24- • Light scatter properties are low/medium (between lymphocytes and monocytes).
#2. Definition of maturation stage	• Least mature plasma cells: CD20+CD138- • Intermediate mature plasma cells: CD20-CD138- • Most mature plasma cells: CD20-CD138+
#3. Classification of plasma cells based on isotype	• IgM+, no expression of other isotype Igs • IgG1+, no expression of other isotype Igs • IgG2+, no expression of other isotype Igs • IgG3+, no expression of other isotype Igs • IgG4+, no expression of other isotype Igs • IgA1+, no expression of other isotype Igs • IgA2+, no expression of other isotype Igs • IgD+, no expression of other isotype Igs • IgH-, no Ig expression of any isotype Igs
#4. Classification of plasma cells based on CD62L expression	• CD62L- • CD62L+
#5. Identification of total B cells	CD45+CD19+CD20+ B cells show low light scatter characteristics (lymphocyte range)
#6. Identification of switched memory B-cell (MBC) subsets based on isotype. Switched MBCs express only one isotype	• IgG1+, no expression of other isotype Igs • IgG2+, no expression of other isotype Igs • IgG3+, no expression of other isotype Igs • IgG4+, no expression of other isotype Igs • IgA1+, no expression of other isotype Igs • IgA2+, no expression of other isotype Igs
#7. Subclassification based on maturation/functional CD markers	• CD20+CD21+ Homogenous CD24 staining • CD20++CD21-/dim CD24+ CD24-
#8. Subclassification based on CD62L/CD27 positivity	• CD27+CD62L+ • CD27+CD62L- • CD27-CD62L- • CD27-CD62L+
#9. Identification of non-switched MBCs	CD27+IgM++IgD+ Of note, a minor subset of IgD+IgM- MBCs may be found as well. These can be classified separately.

#10. Subclassification based on maturation/functional CD markers	• CD20+CD21+ Homogenous CD24 staining • CD20++CD21-/dim CD24+ CD24- No further subclassification in these populations.
#11. Classification of pre-germinal center (preGC) B cells	CD27-IgM+IgD+
#12. Subclassification based on maturation/functional CD markers	• Immature preGC B cells: CD38+CD24+CD5+CD21-/+ • Naive CD5+ B cells: CD38-/dim CD24-/dimCD5+ • Naive CD5- B cells:CD38-/CD24-/dimCD5-
#13. Subclassification of naive B cells based on maturation/functional CD markers	• CD20+CD21+ Homogenous CD24 staining • CD20++CD21-/dim CD24+ CD24-

Table 2. Phenotypic descriptions used to define T-cell subsets stained with EuroFlow PERISCOPE CD4 T-cell (TCD4) panel by manual analysis. The removal of debris and doublets is not indicated in the analysis strategy below, but should be performed to ensure high quality data.

Stepwise approach (gating in 2D plots)	Phenotypic description
#1 Identification of total CD4 T cells	CD3+CD4+CD45+ Light scatter properties are low ('lymphocyte gate').
#2 Identification of regulatory T cells (Tregs) within CD4 T cells	CD25+CD127dim
#3 Identification of follicular helper T cells (TFHs) within CD4 T cells	CD25-/dim CD185+ CCR10-

#4 Division of total CD4 T cells into T-helper (TH) subsets	• Divide based on chemokine receptor expression (CD183, CD194, CD196, and CCR10) Naive: CD27+CD45RA+CD62L+CD127+CD183-CD194-CD196-CCR10- TH1: CD183+ CD194-CD196-CCR10- TH2: CD183- CD194+CD196-CCR10- TH17: CD183- CD194+CD196+CCR10- TH1/17: CD183+CD194-CD196+CCR10- TH22: CD183- CD194+CD196+CCR10+ CD183+CD194+CD196+CCR10+ CD183+CD194+CD196+CCR10- CD183+CD194+CD196-CCR10+ CD183+CD194+CD196-CCR10- CD183+CD194-CD196+CCR10+ CD183+CD194-CD196-CCR10+ CD183-CD194-CD196+CCR10- CD183-CD194+CD196-CCR10+ Non-naive CD4+CD183-CD194-CD196-CCR10-CD27-/+CD45RA-CD62L-/+
#5 Division of total Tregs in TH-like subsets	• Divide primarily based on chemokine receptor expression (CD183, CD194, CD196, and CCR10) Naive Treg: CD27+CD45RA+CD62L+ CD183-CD194-CD196-CCR10- TH1-like: CD183+CD194-CD196-CCR10- TH2-like: CD183-CD194+CD196-CCR10- TH17-like: CD183-CD194+CD196+CCR10- TH22-like: CD183-CD194+CD196+CCR10+ CD183+CD194+CD196-CCR10+ Treg CD183+CD194+CD196-CCR10- Treg CD183+CD194+CD196+CCR10- Treg CD183+CD194+CD196+CCR10+ Treg CD183-CD194+CD196-CCR10+ Treg
#6 Division of total TFHs in Treg-/TH-like subsets	• Divide primarily based on chemokine receptor expression (CD183, CD194, CD196, and CCR10) CD185+CD27+CD45RA+CD62L+ T cells (CD183-CD194-CD196-CCR10-) Treg TFH: CD127+/dimCD183-/+CD194-/+CD196-/+CCR10- TH1-like: CD183+CD194-CD196-CCR10- TH2-like: CD183-CD194+CD196-CCR10- TH17-like: CD183-CD194+CD196+CCR10- TH1/17-like: CD183+CD194-CD196+CCR10- CD183+CD194+CD196-CCR10- TFH CD183+CD194+CD196+CCR10- TFH CD183-CD194-CD196+CCR10- TFH CD183+CD194+CD196-CCR10- TFH
#7 division of each subset into different maturation stage	• Divide based on CD27, CD45RA, and CD62L Central memory (CD27+CD45RA-CD62L+) Transitional memory (CD27+CD45RA-CD62L-) Effector memory (CD27-CD45RA-CD62L-/+) Terminal effector (CD27-CD45RA+CD62L-/+)

Table 3. Phenotypic descriptions used to define T-cell and NK-cell subsets stained with the EuroFlow PERISCOPE CD8 cytotoxic T-cell (CYTOX) panel by manual analysis. The removal of debris and doublets is not indicated in the analysis strategy below but should be performed to ensure high quality data.

Stepwise approach (gating in 2D plots)	Phenotypic description
#1 Identification of total T cells	• CD3+CD45+ • Light scatter properties are low ('lymphocyte gate').
#2 Identification of total TCRγδ+ T cells within total T cells	CD3+ TCRγδ+
#3 Identification of total CD8+ and CD8- T cells within total T cells	• CD8+ T cells: CD8+ TCRγδ- • CD8- T cells: CD8- TCRγδ-
#4 Identification of total NK cells	CD3- TCRγδ- CD45+ Light scatter properties are low ('lymphocyte gate'). CD56-/+ CD16-/+ (lower than neutrophils) CD45RA-/+ (most NK cells are CD45RA positive) CD62L-/+
# 5 Identification of additional lymphocytes	Light scatter properties are low ('lymphocyte gate'). CD45+CD3-CD4-
#6 Identification of myeloid cells	Neutrophils: high SSC, CD16+CD45+ Eosinophils: high SSC, CD45+, Autofluorescence results in double positive population in CD57 vs cy Granzyme B plot Monocytes: intermediate SSC, CD45+CD16+/- CD45RA-/+ and mostly CD62L+
#7 Subsetting of TCRγδ+ T cells	• Naive: CD27+CD28+CD45RA+CD62L+GranzB-CD57- • Central memory: CD27+CD28+CD45RA-CD62L+ CD57-cyGranzB-/+ CD57+CyGranzB+ • Transitional memory: CD27+CD28-/+CD45RA-CD62L-/dim CD57-cyGranzB- CD57-CyGranzB+ CD57+CyGranzB+ • Peripheral memory: CD27-CD28+CD45RA-CD62L-/+ CD57-cyGranzB- CD57-CyGranzB+ CD57+CyGranzB+ • Early effector: CD27+CD28-CD45RA+CD62L-/+ CD57-CyGranzB+ CD57+CyGranzB+ • Terminal effector: CD27-CD28-CD45RA+CD62L-/+ CD57-CyGranzB+ CD57+CyGranzB+

#8 Subsetting of CD8+ T cells	• Naive: CD27+CD28+CD45RA+CD62L+ (NB: in some donors the naive population can be divided into CD62Lhigh and CD62Llow) • Central memory: CD27+CD28+CD45RA-CD62L+ CD57-CyGranzB-/+ CD57+ CyGranzB-/+ • Transitional memory:CD27+CD28+C-D45RA-CD62L-/dim CD57-cyGranzB- CD57+CyGranzB- CD57-CyGranzB+ CD57+CyGranzB+ • Peripheral memory: CD27-CD28-/+CD45RA-CD62L-/+ CD57-cyGranzB- CD57-CyGranzB+ CD57+CyGranzB+ • Early effector: CD27+CD28-CD45RA+CD62L-/+ CD57-cyGranzB- CD57-CyGranzB+ CD57+CyGranzB+ • Terminal effector: CD27-CD28-CD45RA+CD62L-/+ CD57-cyGranzB- CD57-CyGranzB+ CD57+CyGranzB+
#9 Subsetting of NK cells	• CD56+ bright NK cells: CD56brightCD16lo/dim CD57-cyGranzB- CD57-CyGranzB+ • CD56+dim NK cells: CD56dimCD16+ CD57-cyGranzB- CD57-CyGranzB+ CD57+CyGranzB+

Table 4. Phenotypic descriptions used to define innate immune cell (sub)sets stained with the EuroFlow PERISCOPE DC-Monocyte panel by manual analysis. The removal of debris and doublets is not indicated in the analysis strategy below, but should be performed to ensure high quality data.

Stepwise approach (gating in 2D plots)	Phenotypic description
#1 Identify eosinophils	SSC high, CD45+ neg. for all other markers in the panel
#2 Identify mature neutrophils	SSC high CD45+CD16+
#3 Identify immature neutrophils	• CD45+CD33+CD16-/+HLA DR-CD14-SLAN&FcER1- CD62L- CD62L+
#4 Identify monocytes	• SSC intermediate • CD45+CD33+CD16-/+HLA DR+ CD14-/+SLAN&FcER1-/+
# 5 Divide the monocytes based on CD14/ CD16 expression	• ncMo: CD14-/dimCD16+CD62L- SLAN &FcER1-/+ SLAN+CD36+ SLAN-CD36+ SLAN-CD36- SLAN+CD36- • iMo:CD16+CD14+HLA DR+Slan&F-cER1-CD300e&CD303+CD36+ • cMo:CD16-CD14+62L-/+ CD62L+FcER1+ CD62L-FcER1+ CD62L+FcER1- CD62L-FcER1-
#6 Identify the CD1c+ myeloid DCs	• SSC intermediate • CD45+CD33+CD141-/dimFcER1+HLA DR+CD16-CD14-/dim CD14dim CD14-
#7 Identify the plasmacytoid DCs	SSC intermediate CD45+CD303+CD14-HLA DR+CD16-CD36+
#8 Identify the CD141+ myeloid DCs	CD141+CD33+CD300e-CD303-CD14-HLA DR+CD16-
#9 Identify the Axl+ DCs within the plasmacytoid DCs	CD33hiCD141hiCD36dim
#10 Identify the basophils	SSC intermediate CD45dimCD33+CD303-CD300e-CD14-HLA DR-
#11 Identify 'unspecified nucleated cells'	Left-over CD45+ events that fit the singlet gate