

# Advanced immunophenotyping of peripheral blood leukocytes

„Rozšířená imunofenotypizace leukocytů PK“

- more than T-B-NK
- HLA-DR
- NK-cell subsets
- Monocyte subsets
- T-cell subsets (RTE, naive, memory)
- B-cell subsets (naive, mature, clonality)

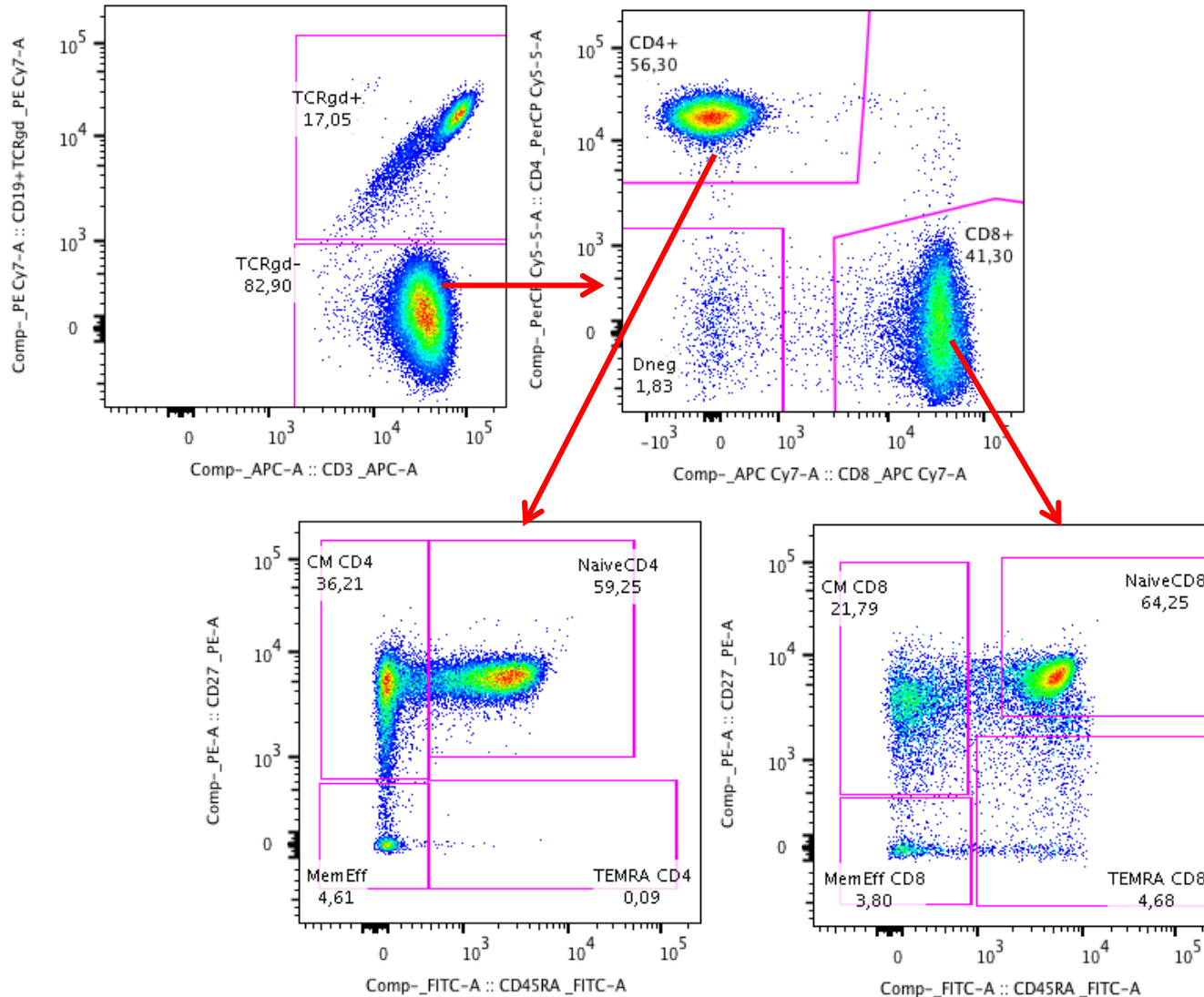
## Commercial IVD antibody panels

**Custom Panels:** utilize preferentially CE-IVD certified antibodies to create a tailored flow cytometry panel



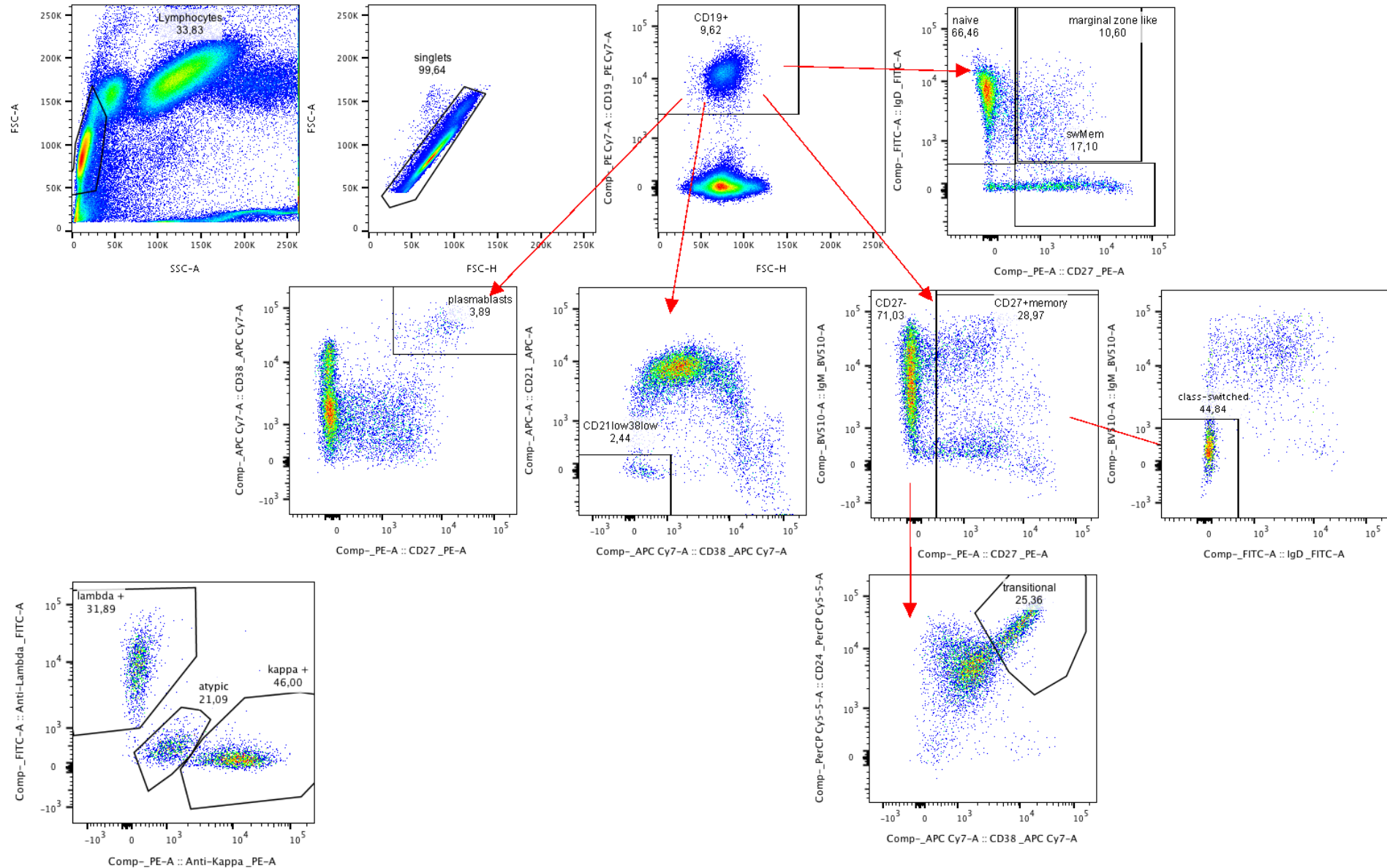
# T-cell subsets

CD3, CD4, CD8, TCRgd and/or TCRg, CD27 (or CCR7 or CD62L), CD45RA and/or CD45RO



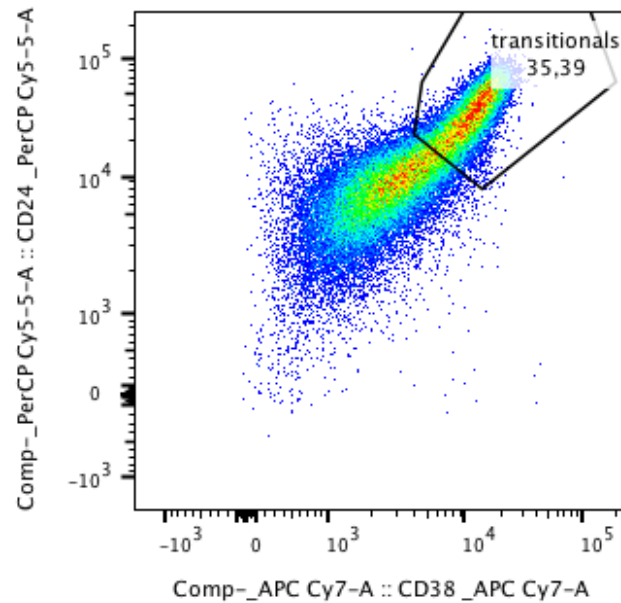
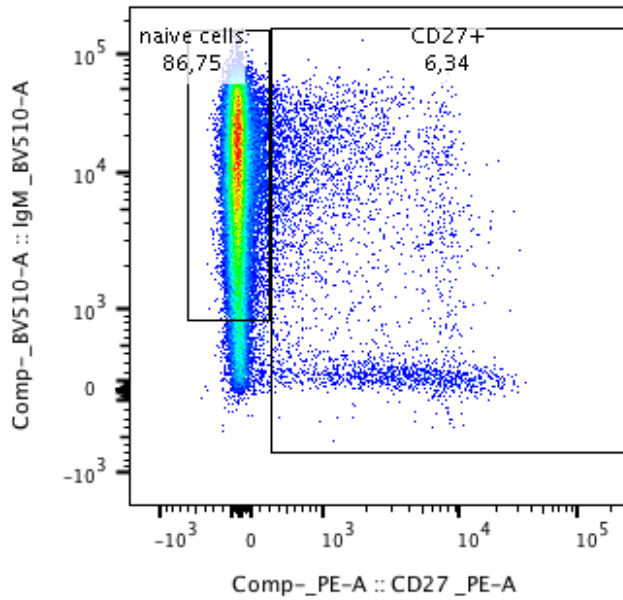
# B-cell subsets

CD19, CD27, CD38, IgD, IgM, CD21, CD24, CD5, opt. kappa, lambda



# B-cell subsets - quiz

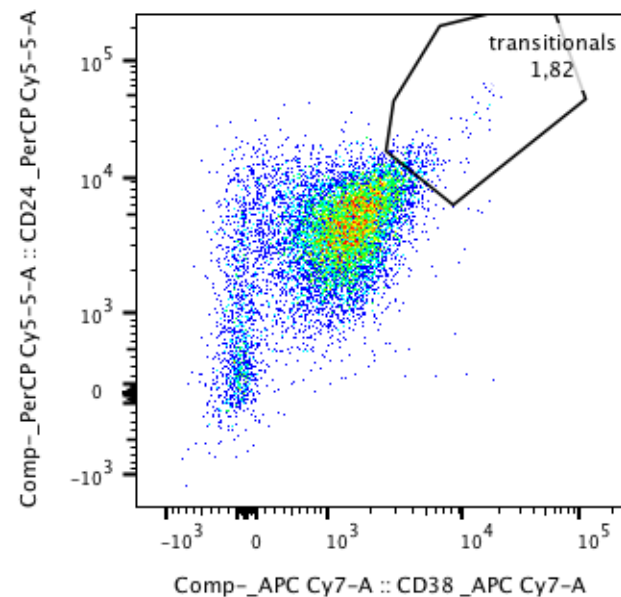
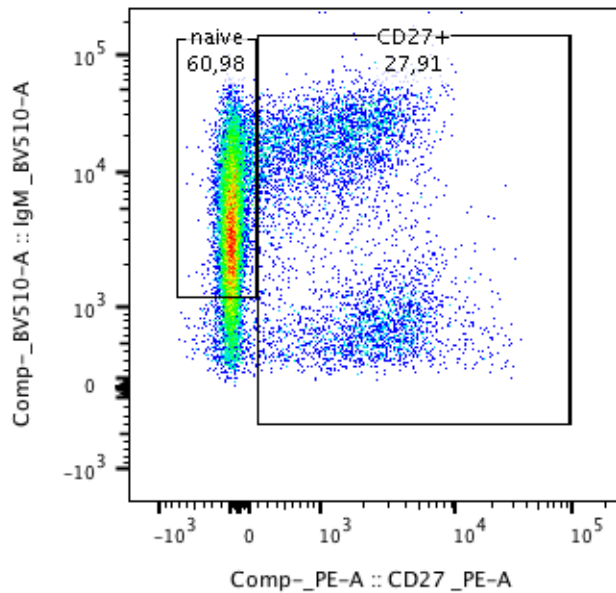
1.



Age 0.5 years

Age-matched  
reference ranges  
needed !

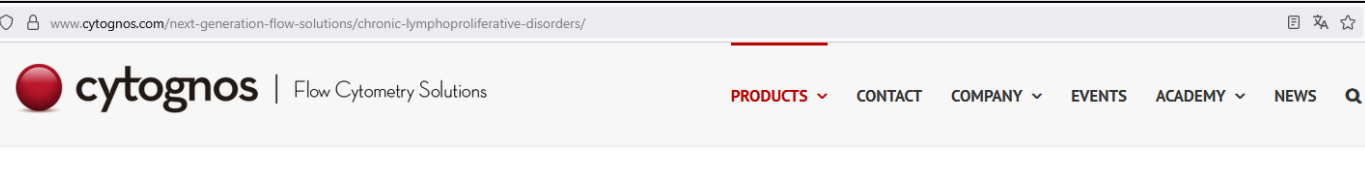
2.



Age 70 years



# LST



> Screening/Orientation: Lymphoid Screening Tube (LST) > Screening/Orientation: Small Sample Tube (SST) > Characterization/Classification

## Lymphoid Screening Tube (LST)

- Suitable for the evaluation of different type of samples (bone marrow, peripheral blood, lymph nodes, etc) with suspicion of mature lymphoid infiltration.
- An initial screening step based on a limited number of antibodies orientates efficiently towards the most appropriate EuroFlow™ characterization panel (B-CLPD, T-CLPD & NK-CLPD).
- Inclusion of two markers in the same fluorochrome allows the analysis of a larger number of antibodies in an 8-color combination.
- Good clinical sensitivity: optimized antibody combination for the dissection of lymphoid cells into their major subsets with a set of reagents that can simultaneously define aberrant and clonal phenotypes.
- High specificity: Inclusion of markers that improve the characterization of the lymphoid cell populations (CD38 for discriminating B-cell precursors or plasma cells and anti-TCRγδ for discriminating TCRγδ+ from other CD8-/low T-cells), and provide a more sensitive and robust orientation on subsequent analyses (CD5 and/or CD38 for the detection of aberrant or clonal phenotypes).



CE-IVD

Pacific Blue™	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC
CD20+CD4	CD45	CD8 + SmIgLambda	CD56 + SmIgKappa	CD5	CD19 + TCRγδ	SmCD3	CD38



RUO

### Reagent composition:

#### Lyophilized tubes:

- 12 antibodies against **surface antigens** (5 vials, 5 tests per vial)

#### Compensation vials (4 tubes in lyophilized format):

- PerCP-Cyanine 5.5-CD5
- PE-Cyanine7-CD19+TCRγδ
- APC-C750-CD38
- OC515-CD45

EuroFlow QA

**EuroFlow** EuroFlow QA program: Lymphoid screening tube

Certificate of participation and performance in the EuroFlow quality assesment program for

CLIP

Klinika dětské hematologie a onkologie 2. LF UK v Praze a FN Motol

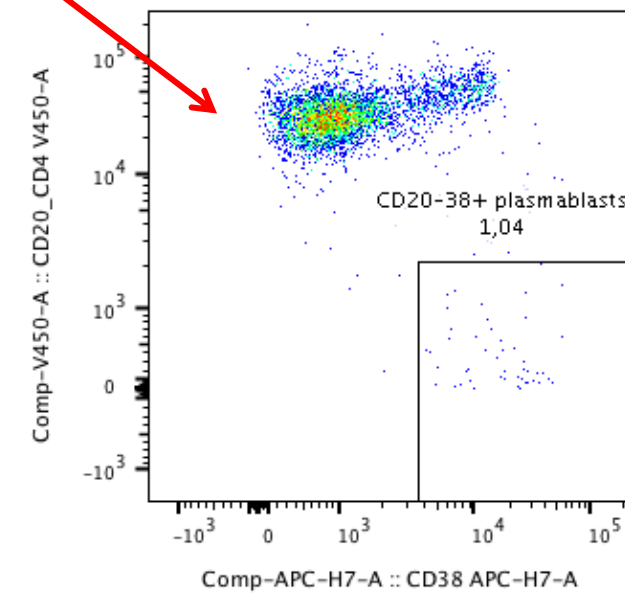
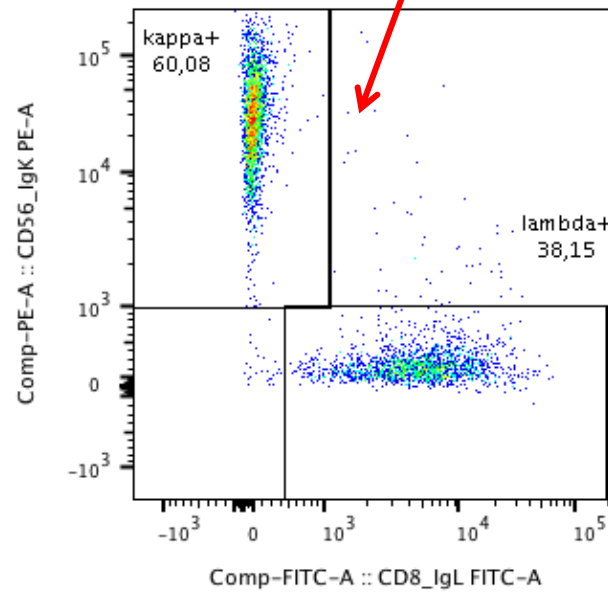
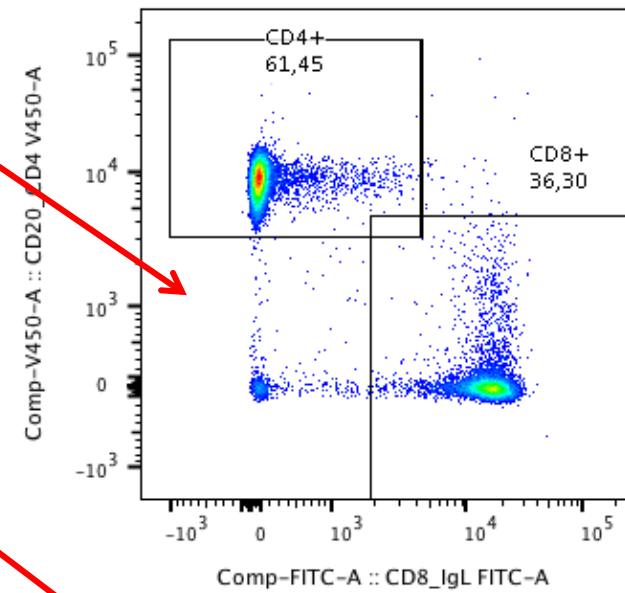
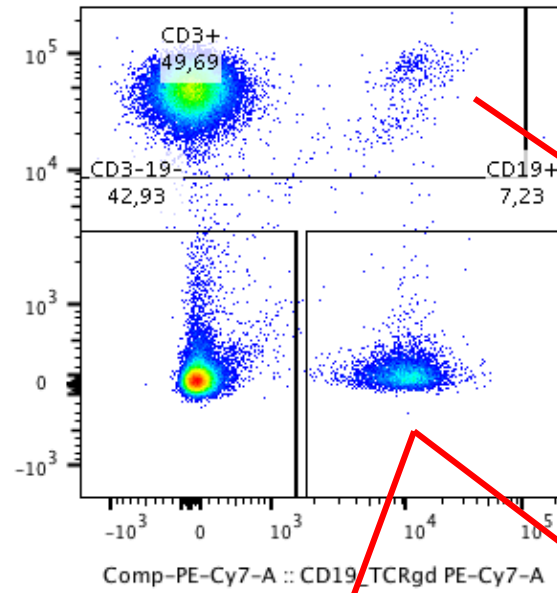
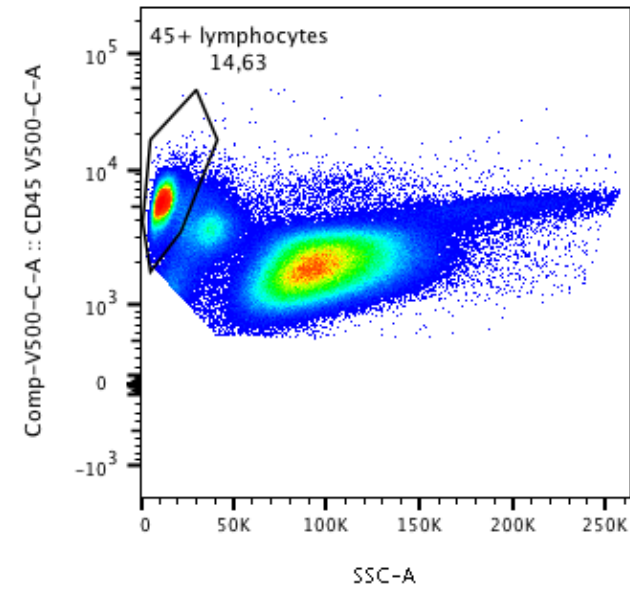
Praha 5, Czech Republic

Represented by: Ondřej Hrušák  
 Contact person: Naděžda Brdičková  
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Certificate issued on April 20, 2024

Prof. J.J.M. van Dongen (Chairman)      Tomas Kalina (responsible for QA) euroflowqa@gmail.com

# LST



# SST

## Small Sample Tube

Categories: EuroFlow™, Multicolor cocktails

[INSTRUCTIONS FOR USE \(IFU\)](#)



[SAFETY DATA SHEET \(SDS\)](#)



The **Small Sample Tube (SST)** kit is an 8 color conjugated antibodies combination.

This kit contains a combination of 11-fluorochrome conjugated antibodies designed for detection of normal and aberrant lymphocyte populations which serves as an aid in diagnosis and follow-up of Primary Lymphoma.

It is to be used in small samples with low cell counts from patients suspected from Primary Lymphoma.

Pacific Blue™	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CD20	CD45	CD8 + SmIgLambda	CD56 + SmIgKappa	CD4	CD19	SmCD3 + CD14	CD38

### Kit composition (auxiliary reagents included)

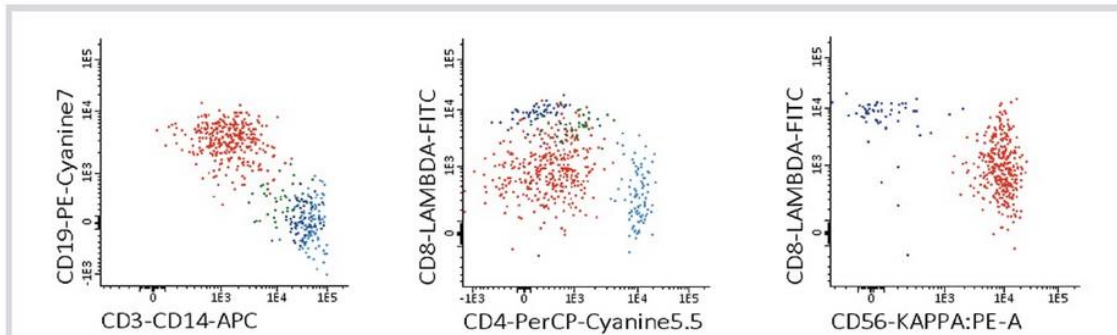
#### Lyophilized tubes

Antibodies against **surface antigens**  
(5 vials, 5 tests per vial)

#### Compensation tubes (lyophilized monostest tube)

OC515™-CD45  
PerCP-Cyanine5.5-CD4  
PE-Cyanine7-CD19  
APC-C750™-CD38

CE-IVD



## BD Cytognos™ Small Sample Tube

CE-IVD



# PIDOT



**cytognos** | Flow Cytometry Solutions

PRODUCTS ▾ CONTACT COMPANY ▾ EVENTS ACADEMY ▾

CYT-PIDOT8 Home / EuroFlow™, Multicolor cocktails

## Primary Immunodeficiency Orientation Tube

Categories: EuroFlow™, Multicolor cocktails

[INSTRUCTIONS FOR USE \(IFU\)](#)  [SAFETY DATA SHEET \(SDS\)](#) 

The Primary Immunodeficiency Orientation Tube (PIDOT) is comprised of a pre-mixed 6-color 10-antibody combination and two single antibody vials: CD27-BV421 and CD45RA-BV510. The panel is designed for the screening of suspected primary immunodeficiencies by flow cytometry using BD FACSCanto™ II and/or BD FACSLyric™. It is intended to be used for staining peripheral blood specimens. It can be used to study the changes in the relative distribution of the different leukocyte populations and sub-populations.

The Primary Immunodeficiency Orientation Tube recognizes the following antigens: CD45RA, CD27, CD8, IgD, CD16, CD56, CD4, IgM, CD19, TCR-γδ, CD3 and CD45.

drop-in BV421	drop-in BV510	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CD27	CD45RA	CD8+ IgD	CD16+ CD56	CD4+ IgM	CD19+ TCRγδ	CD3	CD45

CE-IVD

## BD Cytognos™ Primary Immunodeficiency Orientation Tube



CE-IVD




EQA certificate (before appeals)  
EuroFlow program  
ESLHO-EuroFlow PIDOT EQA scheme

PIDOT EQA certificate

Participation and performance certificate of  
the ESLHO-EuroFlow PIDOT EQA scheme

Round 2024

Childhood Leukaemia Investigation Prague

Department of Paediatric Hematology/Oncology 2nd Medical School, Charles  
University  
Prague 5, CZ

Contact person(s)

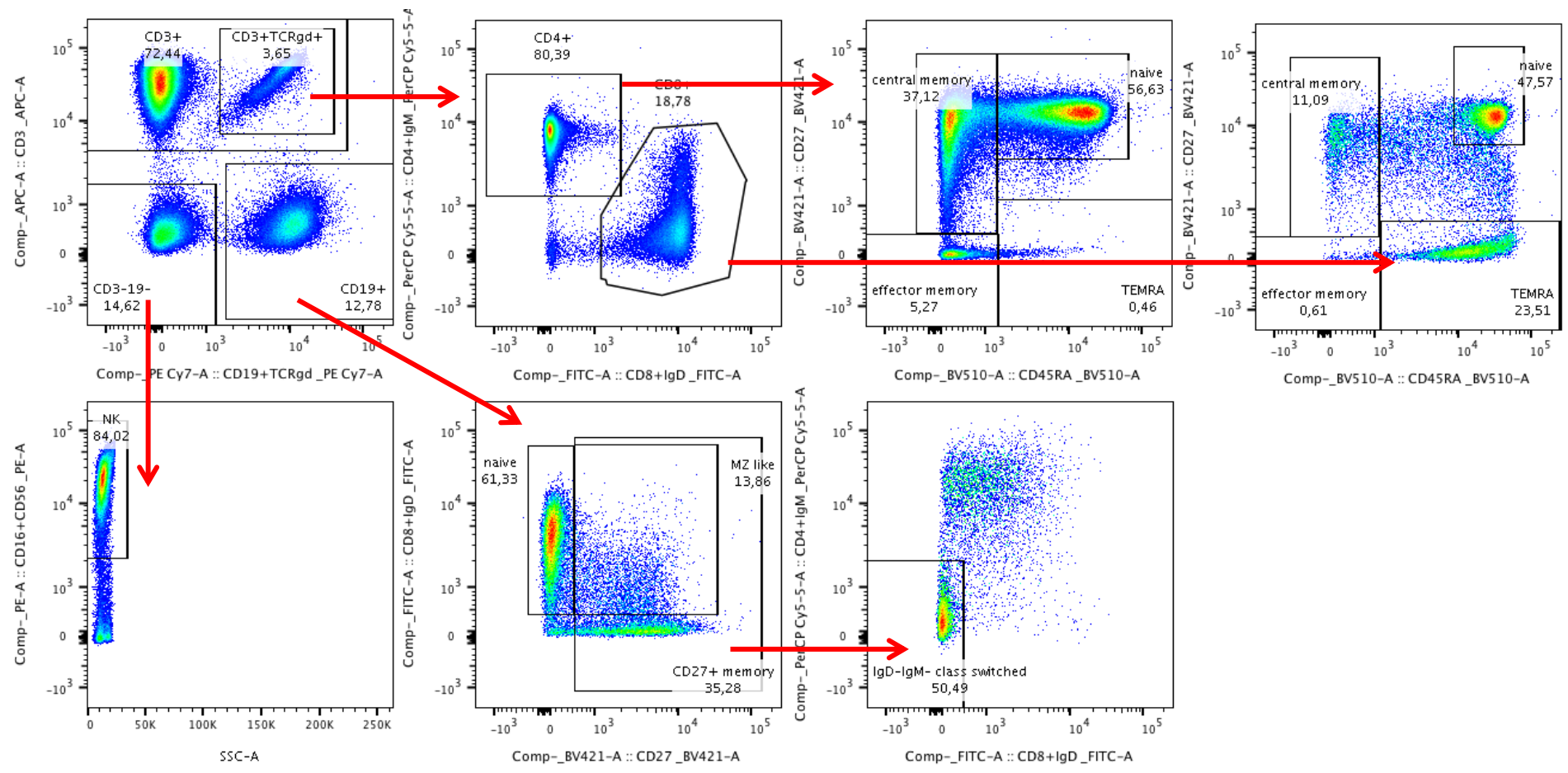
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EuroFlow QA

# PIDOT



# T-cell tubes

## T-cells tube

Category: Multicolor cocktails

INSTRUCTIONS FOR USE (IFU)



SAFETY DATA SHEET (SDS)



RUO

For Research Use Only. Not for use in Diagnostic or Therapeutic Procedures.

T-Cell Subsets Tube is a 7-antibody combination that allows the identification of different T-cell subsets for the evaluation of their relative numbers and kinetics, which can have relevance in the immune response. This tube can be complemented with different types of markers in the FITC channel.

Pacific Blue™	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
CD3	CD45	Drop-in	CD27	CD4	CD8	CD62L	CD45RO

RUO

## DURAClone IM T Cell Subsets

When studying the immune response, T cell-mediated immunity is the central component. T cells circulate in the blood and in the lymphatic system as naïve T cells expressing lymph node homing receptors. Upon exposure to antigen presenting cells in secondary lymphoid tissues, T cells become antigen-specific effector cells. The DURAClone IM T Cell panel is used to indicate maturation stages of T cells, covering naïve, effector, memory and terminal differentiation stages. It utilizes [dry reagent technology](#) that confers workflow improvements.

- ✓ Panel of 10 markers in fluorochrome combinations that provide robust population identification, including CD3, CD4, CD8, CD27, CD28, CD45, CD45RA, CD57, CD197 (CCR7), CD279 (PD-1)
- ✓ Pre-formulated antibody panel in a unitized assay eliminating antibody cocktailing
- ✓ 25 tests per kit including 3 compensation kits with lot matched tandem dyes

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



# T regs

## DURAClone IM Treg

Regulatory T cells (Tregs) play a crucial role in the induction and maintenance of immunological tolerance. The DURAClone IM Treg panel enables identification and characterization of FoxP3+ regulatory T cells using a fast permeabilization protocol. It utilizes **dry reagent technology** that confers workflow improvements.

- ✓ Combination of 8 markers in fluorochrome combinations that provide robust population identification, including CD3, CD4, CD25, CD39, CD45, CD45RA, FoxP3 and Helios
- ✓ Pre-formulated antibody panel in a unitized assay eliminating antibody cocktailing
- ✓ 25 tests per kit including 3 compensation kits with lot matched tandem dyes

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



**exbio** CLINICAL PRODUCTS RESEARCH PRODUCTS DRY REAGENTS ABOUT EXBIO

EXBIO - CLINICAL PRODUCTS - HEMATOLOGY - KITS - TREGFLOWEX KIT

## TregFlowEx Kit

Regulatory status RUO  
Reactivity Human  
Application FC (QC tested)



Product details Images References IFU download SDS download

Application FC (QC tested)  
Reactivity Human  
Description The TregFlowEx Kit is designed for the detection of regulatory T cells (CD4+CD25+FOXP3+ cells) in human peripheral blood or human umbilical cord blood using flow cytometry.  
Contents ED7417-1 Fix and Lysing Solution - 1 vial containing 10 ml of 10x concentrated reagent.  
ED7417-2 Permeabilizing Solution - 1 vial containing 25 ml of reagent, ready-to-use.  
ED7417-3 Blocking Buffer - 1 vial containing 2.5 ml of reagent, ready-to-use.  
ED7417-4 CD4 FITC/CD25 PE - 1 vial containing 0.50 ml of cell surface staining reagent, a mixture of mouse monoclonal antibody against CD4, FITC labelled (clone MEM-241, isotype IgG1) and antibody against CD25, RPE labelled (clone MEM-181, isotype IgG1).



## BD Human Th17/Treg Phenotyping Kit

Simultaneously view TH17 and regulatory T cells in human model systems

Supplier: BD 560762

# B-cell tubes

## DURAClone IM B Cell

The major function of B cells is to recognize and remember immunogenic structures and to terminally differentiate into plasma cells that produce antibodies against these structures.

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. It utilizes **dry reagent technology** that confers workflow improvements.

- ✓ Panel of 8 markers in fluorochrome combinations that provide robust population identification, including CD19, CD21, CD24, CD27, CD38, CD45, IgM and IgD
- ✓ Pre-formulated antibody panel in a unitized assay eliminating antibody cocktailing
- ✓ 25 tests per kit including 3 compensation kits with lot matched tandem dyes

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



## Primary Immunodeficiency Orientation Tube

Categories: EuroFlow™, Multicolor cocktails

[INSTRUCTIONS FOR USE \(IFU\)](#)

[SAFETY DATA SHEET \(SDS\)](#)

The Primary Immunodeficiency Orientation Tube (PIDOT) is comprised of a pre-mixed 6-color 10-antibody combination and two single antibody vials: CD27-BV421 and CD45RA-BV510. The panel is designed for the screening of suspected primary immunodeficiencies by flow cytometry using BD FACSCanto™ II and/or BD FACSLyric™. It is intended to be used for staining of peripheral blood specimens. It can be used to study the changes in the relative distribution of the different leukocyte populations and sub-populations.

The Primary Immunodeficiency Orientation Tube recognizes the following antigens: CD45RA, CD27, CD8, IgD, CD16, CD56, CD4, IgM, CD19, TCR-γδ, CD3 and CD45.

drop-in BV421	drop-in BV510	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CD27	CD45RA	CD8+ IgD	CD16+ CD56	CD4+ IgM	CD19+ TCRγδ	CD3	CD45

**CE-IVD**

# Tubes for hematopoietic neoplasms

www.mybeckman.uk/reagents/coulter-flow-cytometry/antibodies-and-kits/clearlab-antibody-solutions/clearlab-10c-system/panel-design

Cytometry Reagents > Antibodies and Kits > ClearLLab Panels > ClearLLab 10C System > **Panel Design**

CE-IVD

## ClearLLab 10C Panel Design

The integrated L&L\* immunophenotyping solution for both lymphoid and myeloid lineages

ClearLLab 10C panels can aid in the differential diagnosis of hematologically abnormal patients having or suspected of having hematopoietic neoplasms:

- Chronic leukemia
- Acute leukemia
- Non-Hodgkin lymphoma
- Myeloma
- Myelodysplastic syndrome (MDS)
- Myeloproliferative Neoplasms (MPN)



These specimen types can be used:

- Peripheral whole blood (collected in K2EDTA, ACD or heparin)
- Bone marrow (collected in K2EDTA, ACD or heparin)
- Lymph node specimens



Part#	Blue Laser					Red Laser			Violet Laser	
	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700	APC-A750	PB	KrO
B Cell Tube <a href="#">B96805</a>	Kappa	Lambda	CD10	CD5	CD200	CD34	CD38	CD20	CD19	CD45
T Cell Tube <a href="#">B96806</a>	TCRγδ	CD4	CD2	CD56	CD5	CD34	CD7	CD8	CD3	CD45
M1 Cell Tube <a href="#">B96807</a>	CD16	CD7	CD10	CD13	CD64	CD34	CD14	HLA-DR	CD11b	CD45
M2 Cell Tube <a href="#">B96808</a>	CD15	CD123	CD117	CD13	CD33	CD34	CD38	HLA-DR	CD19	CD45

# Tubes for hematopoietic neoplasms


CYT-ALOT-R

Home / EuroFlow™ /

## Acute Leukemia Orientation Tube **CE-IVD**

Category: EuroFlow™

[INSTRUCTIONS FOR USE \(IFU\)](#) 

[SAFETY DATA SHEET \(SDS\)](#) 

*For Research Use Only. Not for use in Diagnostic or Therapeutic Procedures.*

The ALOT kit designed by the EuroFlow™ consortium contains a combination of 8 antibodies for initial assessment of the nature of immature populations of hematopoietic cells in acute leukemia research purpose.

Pacific Blue™	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
CyCD3	CD45	CyMPO	CyCD79a	CD34	CD19	CD7	SmCD3

### BD OneFlow™ ALOT



RESET FILTERS

Product category

Product Region

Target/CD marker

EuroFlow™ Panel

- ALOT (1)
- AML/MDS (15)
- B-CLPD (23)
- BCP-ALL (11)
- BCP-ALL MRD (3)
- IGS (19)
- LST (5)
- MM MRD (4)
- NK-CLPD (0)
- PCD (0)
- PCST (12)
- PIDOT (1)
- SST (2)
- T-ALL (7)
- T-CLPD (0)

Show less

# Tubes for hematopoietic neoplasms

## A 19-Color Multiparameter White Blood Cell Panel Designed for the Immunophenotyping of Normal and Malignant Leukocytes by Flow Cytometry

Michelle Scott, Chris Brampton PhD, Kelly Kroeger PhD  
Bio-Rad Laboratories, Life Science Group, 2000 Alfred Nobel Dr., Hercules, CA 94547



### Cell Analysis

Bulletin 6993

#### Abstract

Flow cytometry is an important tool for the diagnosis and classification of leukemia. While many leukocyte markers have been identified and are used to determine the exact subtypes of leukemia a patient may have, high-dimensional multicolor analysis has been limited by instrument capabilities. This has often resulted in splitting a valuable limited sample into multiple independent tests to fully define the patient's phenotype.

A 19-antibody white blood cell panel was developed using the ZE5™ Cell Analyzer from Bio-Rad Laboratories. With up to five lasers and 30 analysis parameters (of which 27 channels are for fluorescence detection), all the markers necessary to classify chronic/acute lymphoproliferative leukemia disorders can be combined into a single tube. Using this panel, a patient with chronic B-cell lymphocytic leukemia was successfully identified from a normal PBMC sample.

#### Introduction

With the advent of flow cytometry into the clinical arena as a diagnostic tool, high-dimensional multicolor analysis has become increasingly recognized as invaluable for the diagnosis and classification of leukemia. In the past, clinicians were restricted by the number of channels available to construct multicolor panels of leukocyte markers. As a result, defining populations of normal and aberrant blood cells often required splitting valuable samples into multiple tubes to achieve the needed resolution.

The ZE5™ Cell Analyzer, when configured with five lasers and 27 channels of fluorescence detection, allows all the markers necessary to classify chronic/acute lymphoproliferative leukemia disorders (CLL/ALL) to be mixed into a single tube. This panel combines 19 surface membrane markers of both cluster of differentiation and Ig species with TCR expression to successfully dissect aberrant leukocyte populations present in a patient with chronic B-cell lymphocytic leukemia (B-CLL).

#### Materials and Methods

##### Antibodies

CD3 BUV496, CD4 BUV661, CD19 BUV737, TCRgd BUV395, CD8 BV711, CD45 VioGreen, CD56 BV785, Kappa Light Chain BV421, CD7-BV650, HLADR VioBlue, CD117 BV605, CD33 FITC, CD34 PE-Vio770, CD64 PE-CF594, CD11b PE, CD14 PE-Cy5, CD20 APC-Vio770, CD38 APC-R700, Lambda Light Chain APC

##### Blocking Buffer

PBS + 50 µg/ml mouse, rat, rabbit IgG + 1% BSA + 1% glucose + 20 mM EDTA + 0.1% azide

##### Washing Buffer

PBS + 1% BSA + 1% glucose + 20 mM EDTA + 0.1% azide

##### Beads

AbC Total Antibody Compensation Bead Kit  
(Thermo Fisher Scientific)

##### Media

RPMI 1640 or DMEM + 10% FBS

##### Cell preparation

Normal PBMCs and B-CLL patient PBMCs were thawed and stained using standard procedures. Thawed samples were placed into 5 ml of prewarmed cell media in 50 ml polypropylene centrifuge tubes. Cells were counted and assessed for viability using the TC20™ Automated Cell Counter

# Conclusion

Advanced immunophenotyping of leukocyte subpopulations in PB is

- an important tool in modern diagnostics and monitoring of various diseases, especially in the fields of hematology, immunology and infectious medicine
- enabling a more accurate diagnosis and better patient management compared to classic TBNK

CE-IVD commercial antibody panels are more or less unavailable.

In-house combinations of fluorescently-labeled antibodies are essential.

Their use in clinical diagnostics strictly depends on

- a review of the latest scientific publications including reference ranges
- a good education of laboratory personnel
- clinical needs of the institutions