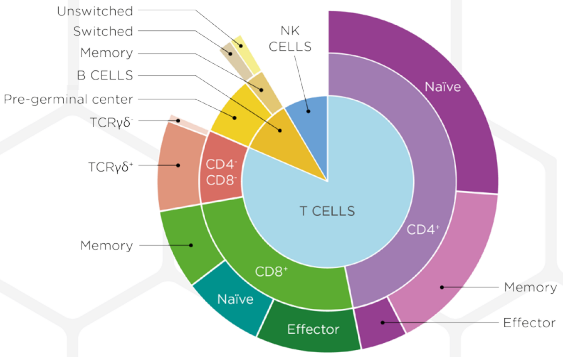
cytognos
Flow Cytometry Solutions

PRIMARY IMMUNODEFICIENCIES
PIDOT

PRIMARY IMMUNODEFICIENCIES

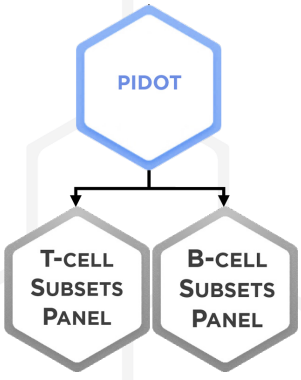
Primary Immunodeficiency (PID) disorders are a heterogeneous group of inherited conditions usually diagnosed during infancy or childhood. They are relatively rare in the general population, but extremely diverse in their underlying causes with over than 300 individual mutations described so far. In PID one or more components of either the adaptive or innate immune response is impaired and the immune system becomes unable to fight effectively infections or diseases.

The suspicion of PID usually arises from a history of recurrent or severe infections and other complications, while its confirmation demands for laboratory investigations. These may include complete blood count, immunoglobulin levels, antibody titters, assessment of cell function, among others. PID classification is based on the International Union of Immunological Societies (IUIS) criteria, which provides valuable information regarding disease-causing genotypes, immunological anomalies, and associated clinical features of PIDs. Probable diagnosis of PID can be reached by consulting the ESID (European Society for Immunodeficiencies) guidelines for diagnosis criteria.



IMMUNOPHENOTYPING IN PRIMARY IMMUNODEFICIENCIES

Flow cytometry is a highly sensitive method, playing an important role on PID diagnosis through the fast evaluation of immune system components. This includes the characterization of specific cell populations and subpopulations, specific protein expression and immune abnormalities related to cell function. Lymphoid cell-associated abnormalities might be identified among several PID cases, which makes the immunophenotypical characterization of the lymphoid compartment a mandatory test to attain an accurate diagnosis.



The EuroFlow™ group has designed a set of 8-color antibody panels for the diagnosis, classification and follow-up of PID, which can be used in combination with novel Infinicyt™ tools in order to optimize immunophenotypic evaluation of immune cells.

The use of a normal reference database helps to detect the involved celullar compartments and to orientate to further flow cytometry characterization panels or possible genetic defects.

The major advantage of the EuroFlow™ approach is that it facilitates faster, standardized immunophenotypic diagnosis of lymphoid PID and allows for a full exchange of data between different laboratories worldwide.

THE EUROFLOW™ PID ORIENTATION TUBE

PATENTED!

BV™421*	BV™510*	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
CD27	CD45RA	CD8+SmIgD	CD16+CD56	CD4+SmIgM	CD19+TCRγδ	CD3	CD45

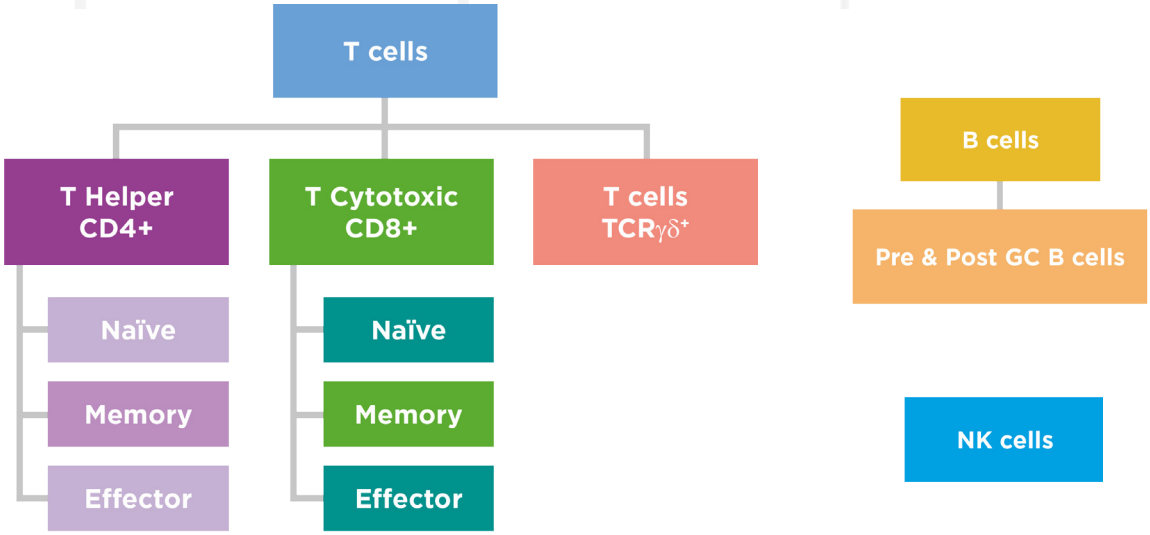
*Not included in the PIDOT kit. Should be added as drop-ins.

The PID Orientation Tube (PIDOT) is a single 8-color tube developed to identify different immune cell populations helping on the selection of the most suitable characterization panel.

With this combination, lineage specific identification markers (CD3, CD19, CD16+CD56), total T, B and NK cells can be identified. Subsequently using functional (CD4, CD8, TCR, IgM, IgD) and maturation (CD27 and CD45RA) specific markers, T and B cell subsets with diagnostic value can be identified: T-helper, T-cytotoxic and their naïve, memory and effector stages, and pre-germinal center (GC), post-GC, Ig class unswitched and switched B cells.

This EuroFlow™ PIDOT combination is patent protected and has been evaluated in several multicenter rounds analysing both normal and abnormal samples. Each specific clone-fluorochrome combination was selected to provide an optimal performance using standardized EuroFlow™ protocols.

This optimized PIDOT combination is produced by Cytognos as a pre-mixed lyophilized reagent, stable and reproducible for long periods of time, and compatible with the automated gating and identification tool implemented in the Infinicyt™ analysis software.



STANDARDIZED OPERATING PROCEDURES FOR PID EVALUATION

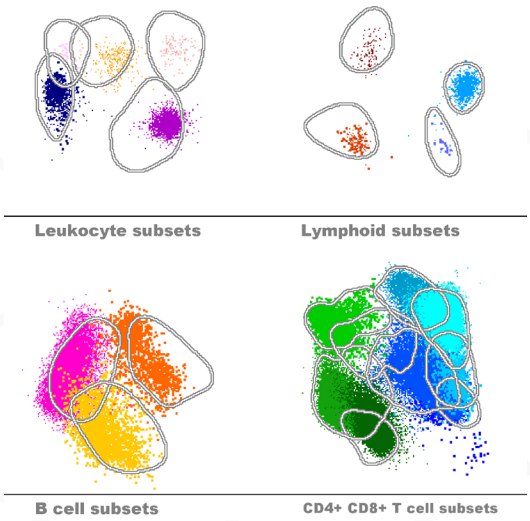
Flow cytometry immunophenotyping results are highly dependent on the sample processing protocols used. For this reason, EuroFlow™ developed standardized protocols for each panel to assure full technical standardization in 3-laser based cytometers (e.g. Omnicyt™).

The corresponding SOPs may be found at www.euroflow.org.

INFINICYT™ DATA ANALYSIS AND REFERENCE DATABASES

The manual analysis of a PIDOT file can be time consuming, experience-dependent and not easily reproducible therefore the use of more automated analysis strategies is required.

The software relies on specific algorithms and on a database of representative normal peripheral blood samples stained with the PIDOT panel and following standard operating procedures. To create this reference database, EuroFlow™ collected and merged normal samples from different age groups allowing biological and technical inter-laboratory variability including instruments and operators. First, the algorithm searches in the multidimensional space for neighbour events with similar characteristics that can be joined into the same group (clustering phase). Then, it compares each generated group with a multidimensional normal reference database and joins similar clusters under the same name (identification phase). Finally once the AG&I and review are finished, numeric alerts and the automatic report help the user to interpret the results.



Furthermore, Cytognos and EuroFlow™ developed tools for multidimensional pattern recognition of the maturation pathway of all lymphoid populations to better detect possible alterations.

REFERENCE RANGES AND REPORTING

In order to have a robust database the normal reference values have been extracted, after standard processing of samples from hundreds of normal donors belonging to different age segments. These age-related normal reference values (van der burg, M, et al. 2019) include both relative distributions and absolute counts (parameters recommended by the international consensus classification of PID).

Infinicyt™ includes an automatic report of PIDOT findings with the following information:

- alerts set-up based on normal ranges **(Reference age-related values)**.
- warnings when cell populations are missing from the sample **(Absent populations)**.
- a description of the main findings related to the studied populations **(Comments)**.
- warnings of sample and sample processing quality **(Alerts for Debris percentage)**.

CELLULARITY
(Data referred to 100% of nucleated cells)

Population	Frequency (%) Reference	Events / µl Reference
Lymphocytes	35,3 (13,1 - 68,8)	2.190 (877 - 4.792)
B cells	3,9 (0,69 - 13,8)	240 (41 - 1.194)
preGerminal	2,7 (0,23 - 12,1)	168 (13,5 - 1.040)
postGerminal	1,2 (0,4 - 3,1)	72,2 (23 - 232)
Unswitched	0,48 (0,13 - 1,5)	30,1 (8 - 124)
Switched	0,68 (0,17 - 1,8)	42,1 (12 - 125)
T cells	28,5 (9,1 - 46,2)	1.764 (611 - 3.477)
CD4+CD8-	15,9 (5,7 - 31)	983 (361 - 1.900)
Naïve	8,4 (1,3 - 21)	524 (89 - 1.484)

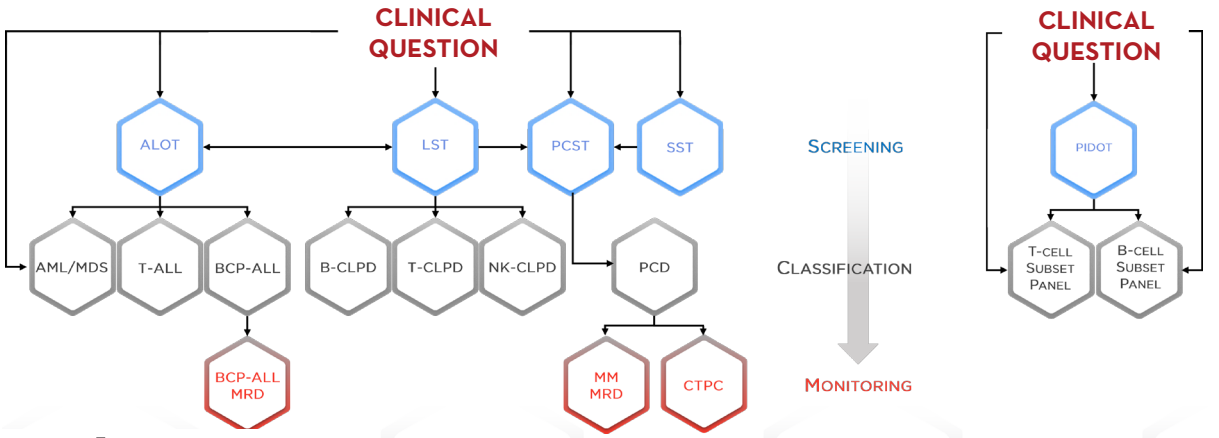
REFERENCES

1. van Dongen JJM, et al. EuroFlow-Based Flowcytometric Diagnostic Screening and Classification of Primary Immunodeficiencies of the Lymphoid System. Front. Immunol. 2019 Jun;10:1271.
2. van der Burg M, et al. on behalf of the EuroFlow PID consortium. The EuroFlow PID Orientation Tube for Flow Cytometric Diagnostic Screening of Primary Immunodeficiencies of the Lymphoid System. Frontieres in Immunology, 2019 Mar; 10 (436); 1-11.
3. Kanegane H, et al. Flow cytometry-based diagnosis of primary immunodeficiency diseases. Allergology International. 2018 Jan; 67 (1); 43-54.
4. Oliveira JB, et al. Laboratory evaluation of primary immunodeficiencies. Journal of Allergy and Clinical Immunology. 2010 Feb; 125 (2); S297-S305.
5. Erasmus University Medical Center Rotterdam. Reagents, methods and kits for diagnosing primary immunodeficiencies. Patent WO 2016068714 A3. Filled October 30, 2015, and issued July 14, 2016.
6. Mukherjee S and Thrasher AJ. Gene therapy for PIDs: progress, pitfalls and prospects. Gene. 2013 Aug 525 (2); 174–181.
7. EuroFlow™ Consortium website: www.euroflow.org.
8. ESID website: www.esid.org.
9. IUIS website: www.iuisonline.org.

Product	Reference	Format	Size
PIDOT kit	CYT-PIDOT	Lyophilized	20 test
Infinicyt™ Advanced License	CYT-INFINICYT-ADVANCED		
EuroFlow™ Database Accesses	CYT-INFINICYT-EFDB		

HEMATO-ONCOLOGY

CLINICAL IMMUNOLOGY



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