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Flow Cytometry Solutions

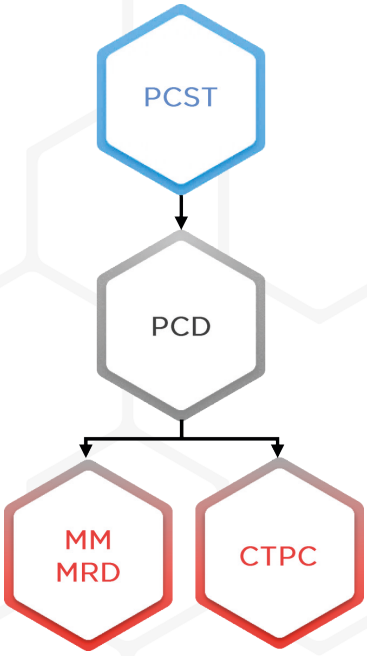
MINIMAL RESIDUAL DISEASE
MM MRD

MULTIPLE MYELOMA

Plasma cell disorders are a heterogeneous group of clinical disorders characterized by the expansion of clonal plasma cells in the bone marrow. The range of plasma cells disorders goes from Monoclonal Gammopathy of Undetermined Significance (MGUS) to symptomatic diseases such as Multiple Myeloma (MM) [1].

An important effort is being made to improve the efficacy of therapies to obtain an increased rate of remission and reduction of the number of pathological cells. Part of the success on this task depends on the sensitivity and specificity of the methodologies employed in diagnosis and follow-up of the patients [2].

IMMUNOPHENOTYPING IN MULTIPLE MYELOMA WORKFLOW



Adapted from van Dongen JJ, Leukemia. 2012 Sep; 26(9):1908-75

Flow cytometry has proved to be an important tool in the diagnosis and classification of MM and other plasma cell disorders throughout the years [1]. It has been demonstrated that abnormal plasma cells often show different phenotypes compared to their normal counterparts, namely the aberrant expression of CD56 in most patients or decreased levels of other molecules such as CD38, CD27, CD45 and CD138. These findings, together with other phenotypical changes, provide an aberrant plasma cell signature that allows for a specific and sensitive detection of the abnormal cells, crucial for an early diagnosis and a reliable follow-up [3].

In recent years, the introduction of new drugs has led to the improved survival of patients with MM [4] The treatment response of these patients has been so far evaluated by techniques that were patient specific and the Minimal Residual Disease (MRD) criteria was not well established since most patient relapsed [2, 4]. The major drawback of flow-MRD was the lack of standardization [3].

THE EUROFLOW™ MULTIPLE MYELOMA MRD PANEL

PATENTED!

BV™421	BV™510	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CD138	CD27	CD38	CD56	CD45	CD19	CD117	CD81
CD138	CD27	CD38	CD56	CD45	CD19	CyIgKappa	CyIgLambda

EuroFlow™ has designed and validated a flow cytometry based method for MRD evaluation in MM. The developed panel is composed of two separate antibody combinations orientated towards the identification of immunophenotypically aberrant clonal plasma cells in bone marrow (BM) samples. The use of two tubes is an important quality control of the process since the test is performed in two replicates of the sample and also to confirm the clonality of suspected cells. An increased sensitivity due to the high number of cells analyzed is achieved. A sensitivity of at least of 10^{-5} has shown to be clinically meaningfull [2, 5, 6].

There are treatments that specifically target surface or intracellular molecules of abnormal plasma cells. These treatments may block the binding of fluorochrome-conjugated anti-CD38 monoclonal antibodies in flow cytometry studies. Cytognos developed an anti-CD38 antibody which recognizes different epitopes of the antigen and allows for the identification of plasma cells even in the presence of therapeutic anti-CD38 antibodies [2, 6].

The combined use of CD38 and CD138 is currently recommended for the identification of plasma cells in MM workflow and for this reason both have been included in the panel [3, 6]. CD38 has shown to be valuable for the identification of both normal and abnormal plasma cells given its expression pattern significantly different from that of CD38+ precursor cells [2, 3, 6]. CD138 is very specific for plasma cells and, although it presents some downregulation on aged samples, it results useful when combined with CD38, CD45 and light scatter properties [3, 6].

Every marker selected has shown to be relevant for the detection of MRD at a very high sensitivity levels contributing for the separation between normal and abnormal immunophenotypes: CD19 (97%), CD45 (89%), CD56 (86%), CD81 (86%), CyIgλ (73%), CD27 (71%), CD117 (60%) and CyIgκ (56%) [2].

STANDARDIZED OPERATING PROCEDURES FOR MRD EVALUATION

Flow cytometry immunophenotyping results are highly dependent on the sample processing protocols employed. For this reason, EuroFlow™ developed standardized protocols for each panel to assure full technical standardization in 3-laser based cytometers and in the specific case of the MM MRD to detect rare events with high sensitivity [2, 6]. The goal of EuroFlow™ was to reach a sensitivity comparable to real-time quantitative polymerase chain reaction (RQ-PCR)-based MRD analysis and, to achieve that, a minimum of 10 million total cells must be analyzed [2].

During early phases of treatment, the cellularity of BM samples are frequently low and using the standard methodology requiring the direct staining of 100 µl of whole BM will not allow the acquisition of the millions of cells needed. EuroFlow™ developed a new erythrocyte BulkLysis™ procedure to lyse sufficiently large volumes of BM, and resuspend the resulting leukocytes in a small volume of washing buffer suitable for staining. This new protocol allows for the staining of 10 million cells in 100 µL of cell suspension without compromising the data quality [6].

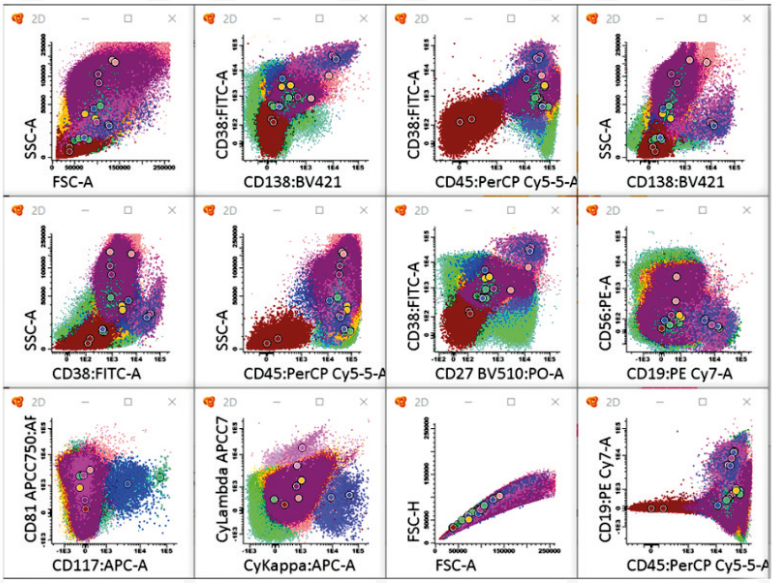
The corresponding Standard Operating Procedures (SOPs) may be found at www.euroflow.org.

INFINICYT™ DATA ANALYSIS AND REFERENCE DATABASES

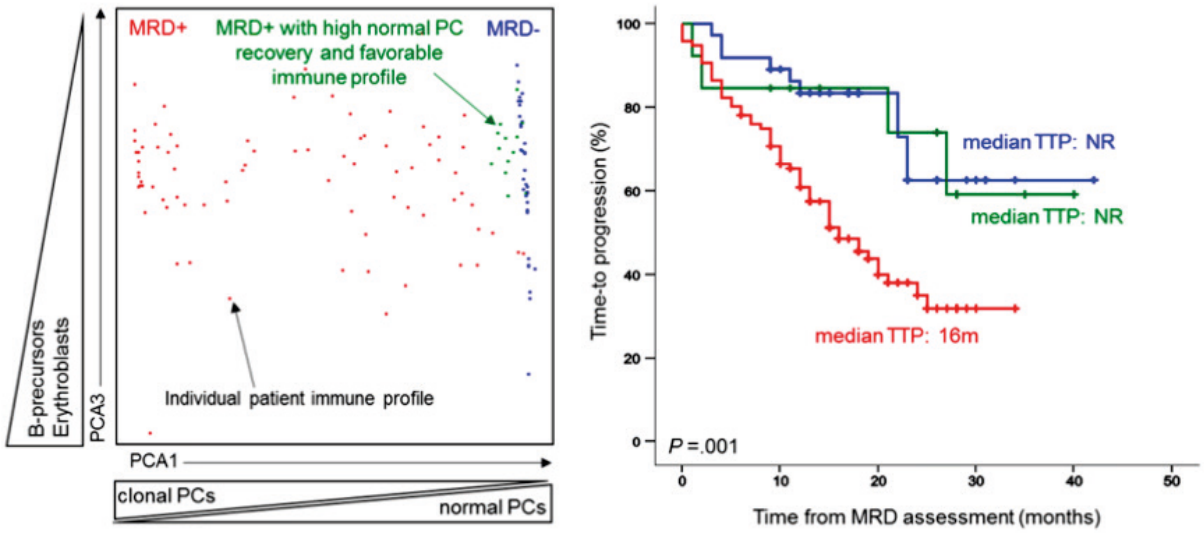
The number of immunophenotypic markers that can be evaluated in 8-color assays and the high number of cells interrogated using the MM MRD methodology increases the complexity of data analysis. EuroFlow™ developed and validated a database containing representative flow cytometry data sets from normal healthy BM samples processed in different standardized centers. The database (available through Infinicyt™) when used with files which follow SOPs allows for an automated analysis of the complete BM sample [2].

The MM MRD database is designed to be used with Infinicyt™ Automated Gating and Identification (AG&I) tool in order to provide a complete analysis of the sample. Normal populations are identified comparing them with the reference database while events which differ from normal need to be confirmed by the user.

Evaluating the complete immune profile of the sample, instead of simply looking for abnormal plasma cells, has shown to be prognostically relevant, allowing for the identification of patients with poor survival. Moreover, the flow cytometry based MRD negativity has shown to be associated with a longer time of progression independently from the risk level established by other techniques (e.g. FISH) [5].



Studies demonstrate that MRD-negative status surpasses the prognostic value of complete remission across the disease spectrum regardless of the type of treatment or patient risk group [2]. MRD negativity should be considered as one of the most relevant assessments as long as the method is performed with a high level of sensitivity and using standardized protocols as proposed by the EuroFlow™ group [5].



Images provided by Dr. Paiva (CIMA LAB diagnostics, Spain)

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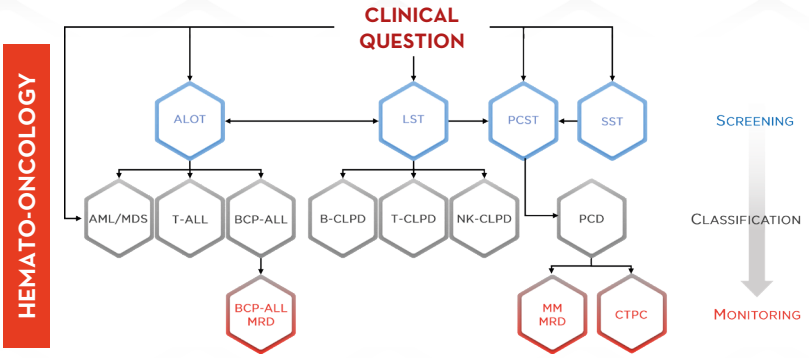
Product	Reference	Regulatory Status	Format	Size
MM MRD kit	CYT-MM-MRD8	CE-IVD	Lyophilized	20 test
CD38 multi-epitope-FITC	CYT-38F2	CE-IVD	Liquid	50 test



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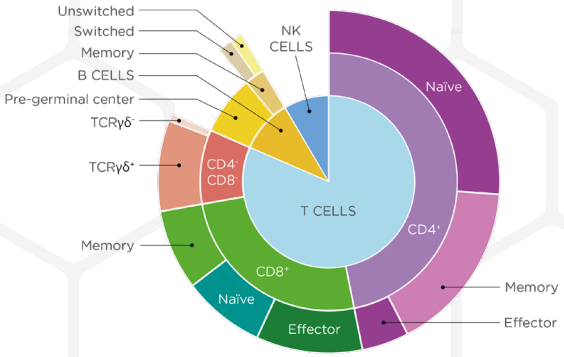
PRIMARY IMMUNODEFICIENCIES
PIDOT

PRIMARY IMMUNODEFICIENCIES

Primary Immunodeficiency (PID) disorders are a relatively rare heterogeneous group of inherited conditions usually diagnosed during infancy or childhood [1-3]. In PID, one or more components of either the adaptive or innate immune response is impaired, and the immune system becomes unable to effectively fight infections or diseases [1-5]. Therefore, PID suspicion usually arises from a history of recurrent or severe infections and other complications [1, 2]. Confirmation on the diagnosis can take months and delayed patient management leads to shortened life expectancy [6]. Novel diagnostic methodologies, which are affordable and accessible to routine laboratories, can increase the ability to diagnose PIDs earlier and thereby contribute to improve patient outcomes and survival [6].

The PID registries of different countries allow for the collaboration between centers managing PID patients, which is crucial for the study and development of improved diagnostic and treatment interventions [1, 2]. Nowadays, 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 [5]. Probable diagnosis of PID can be reached by consulting the ESID (European Society for Immunodeficiencies) guidelines for diagnosis criteria [7].



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 [3, 8]. 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 [1].

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 [1].

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 [1, 2].

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 [2].

THE EUROFLOW™ PID ORIENTATION TUBE

PATENTED!

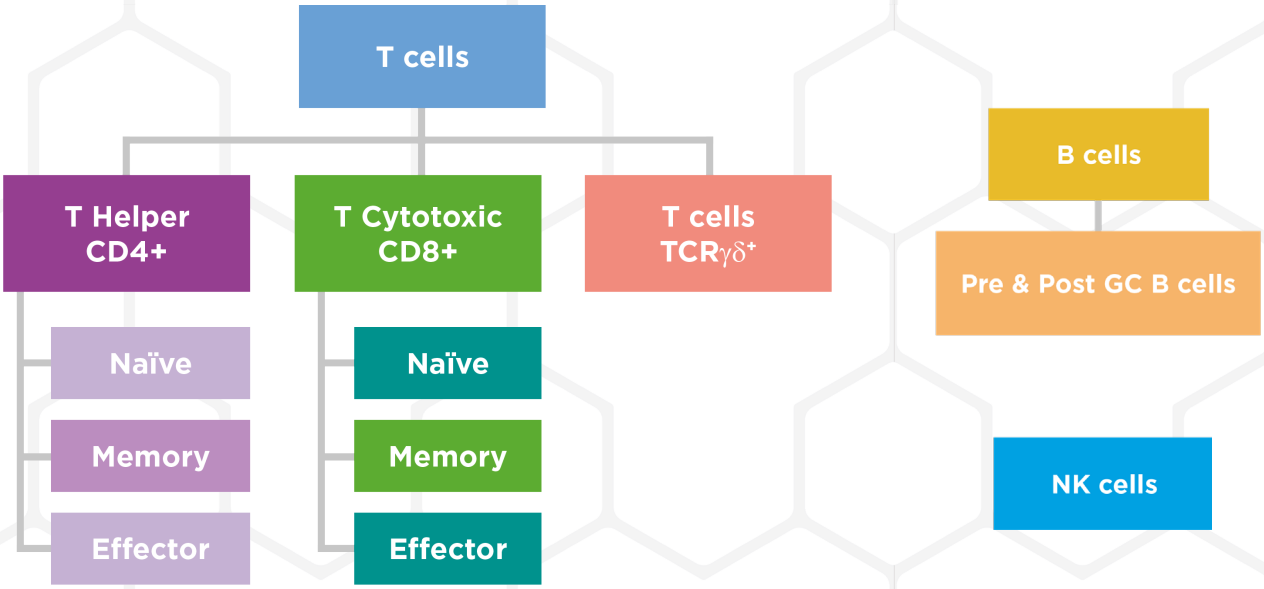
BV421	BV510	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CD27	CD45RA	CD8+SmIgD	CD16+CD56	CD4+SmIgM	CD19+TCRγδ	CD3	CD45

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 [1, 9].

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-unswitched and switched memory B-cells [1, 9].

This EuroFlow™ PIDOT combination is patent protected and has been evaluated in several multicenter rounds analysing both normal and abnormal samples [8, 9]. Each specific clone-fluorochrome combination was selected to provide an optimal performance using standardized EuroFlow™ protocols [1, 9].

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. The software is able to distinguish between the subpopulations of B-, T- and NK-cells (see scheme below) [1]:



STANDARDIZED OPERATING PROCEDURES FOR PID EVALUATION

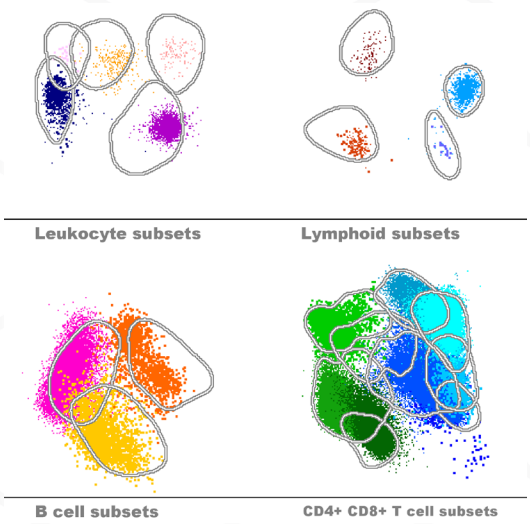
Flow cytometry immunophenotyping results are highly dependent on the sample processing protocols used. For this reason, EuroFlow™ developed standardized protocols for each panel to assure full technical standardization in 3-laser based cytometers [9].

The corresponding Standard Operating Procedures (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 [2].

The software relies on specific algorithms and on a database of representative normal peripheral blood samples stained with the PIDOT panel and following SOPs. 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 Automated Gating & Identification (AG&I) tool and review are finished, numeric alerts and the automatic report help the user to interpret the results [1, 2].



Furthermore, Cytognos and EuroFlow™ developed tools for multidimensional pattern recognition of the maturation pathway of all lymphoid populations to better detect possible alterations [1, 2].

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 include both relative distributions and absolute counts (parameters recommended by the international consensus classification of PID) [2].

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)

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Product	Reference	Regulatory Status	Format	Size
PIDOT kit	CYT-PIDOT8	CE-IVD	Lyophilized	20 test

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MINIMAL RESIDUAL DISEASE
BCP-ALL

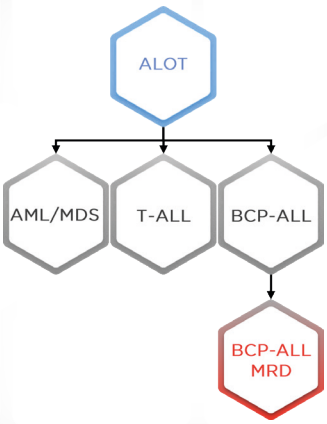
B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL)

Acute leukemias are marked by the diffuse replacement of bone marrow with abnormal immature and undifferentiated hematopoietic cells. Based on the origin of the abnormal hematopoietic cells involved, such as lymphoid, myeloid, mixed or undifferentiated, these disorders are classified accordingly.[1]

Acute Lymphoblastic Leukemia (ALL) is the most frequent type of leukemia in children but can also occur in adults.[2] In adults, 75% of cases develop from precursors of the B-cell lineage, called B-Cell Precursor (BCP) ALL, with the remainder of cases onsisting of malignant T-cell precursors.[3]

IMMUNOPHENOTYPING IN ACUTE LEUKEMIA WORKFLOW

Acute leukemia is a complex multifactorial disease and diagnosis requires an interdisciplinary approach, including review of symptoms and physical examination, blood testing, bone marrow biopsy, morphology, cerebrospinal fluid (CSF) evaluation, radiology, and genetic testing.[3, 4] Flow cytometry proves to be an important tool that is integral to leukemia diagnosis[5]: it facilitates the phenotypic characterization of leukemic cells at diagnosis, gives prognostic information relative to disease severity and allows monitoring of Minimal Residual Disease (MRD).[6, 7, 8, 9, 10, 11] Flow cytometry has been reported to be a sensitive method for the detection of MRD, in some cases providing sensitivity comparable to molecular tests.[6,11]



The EuroFlow™ Consortium has designed, tested and validated a set of 8-color antibody panels for the diagnosis, classification and MRD analysis of acute leukemia(s)[5,6]; used in combination with novel Infinicyt™ software tools, the multidimensional immunophenotypic characterisation of blast cells by flow cytometry is optimized.[12,13]

The Euroflow™ BCP-ALL MRD panel

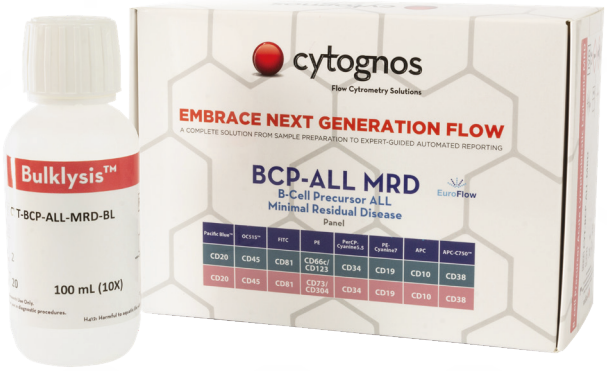
Although therapies have advanced to such an extent that survival of patients diagnosed with ALL has improved, relapses still occur in 20% of children and 40%-50% of adults.[2,14] The detection of MRD in ALL has proven to be a fundamental tool for guiding therapeutic decisions as it determines the response to initial treatment and the subsequent identification of risk groups, it allows surveillance of disease burden in relation to stem cell transplantation and early follow-up of relapse.[14]

The BCP-ALL MRD assay for bone marrow (BM) samples, as designed and validated by the EuroFlow™ Consortium, comprises two standardized antibody panels (tube 1 + tube 2) and Standard Operating Procedures (SOPs), targeting a sensitivity of at least 10^{-5} when more than 4 million BM cells are evaluated, which means comparable MRD detection as obtained with real-time quantitative polymerase chain reaction (RQ-PCR).[6]

BCP-ALL MRD	PB	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
Tube 1	CD20	CD45	CD81	CD66c+CD123	CD34	CD19	CD10	CD38
Tube 2	CD20	CD45	CD81	CD73+CD304	CD34	CD19	CD10	CD38

The BCP-ALL MRD reagent is composed of two 8-colour reagent combinations, sharing several common markers (backbone).

Among them, CD19, CD45, CD34, CD10 and CD20 are known to allow appropriate gating of BCP, characterization of several BCP subpopulations and discrimination between normal and malignant BCPALL cells.[6] Since CD66c and CD123 are both virtually negative on normal/reactive BCP cells[15,16] they are combined in the same PE channel, leaving a position available in the panel for additional markers.[6]



To optimize the composition of the BCP-ALL MRD panel with additional markers, the separation between normal and pathological populations was scored to reach the optimal combination to enhance the separation of these two entities (Figure 1).[6] Further evaluation on 78 BCPALL patients, showed that CD38 (~35% of cases), CD66c/CD123 (~30%), and CD81 (~19%) improved the separation between normal/reactive and malignant BCP cells as compared with the 5 backbone markers only. Based on the level and frequency of overexpression of CD73 and CD304 (~20% and ~40%) and their stability during follow-up, these were added to the final panel. Because of high background levels when combining these last 2 markers with CD66c and CD123 in a single fluorescence channel, a second tube was designed, identical to the first tube but with CD73/CD304 instead of CD66c/CD123 in the PE channel. Together, the two 8-color antibody tubes allowed separation between normal and malignant BCP cells in 99% of studied patients.[6]

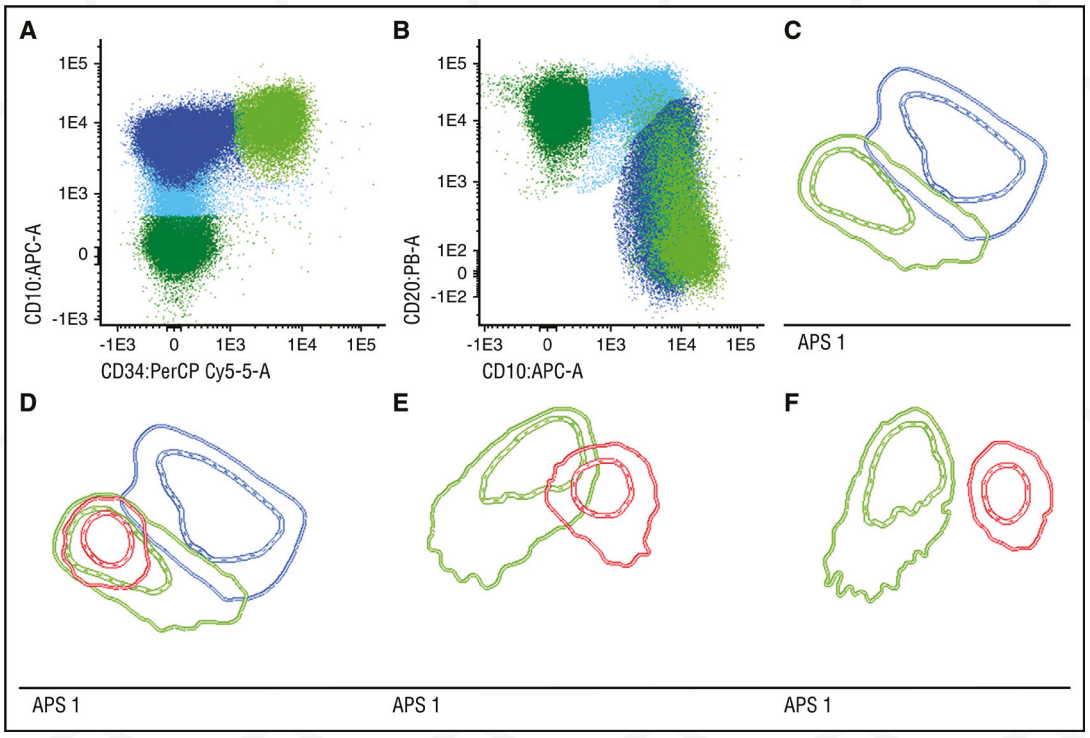


***Figure 1**[6] shows the strategy followed by the EuroFlow™ Consortium to optimize the composition of the BCP-ALL MRD panel. **Figures A and B** show the different subpopulations of B cells (CD19+) from various normal/reactive or regenerating bone marrow samples. **Figure C** represents a view of the Automatic Population Separator (APS) plot for the most immature BCP populations (pre-B-I in light green and pre-B-II in blue). The dotted line represents 1 standard deviation of the cases being plotted and the solid line 2 standard deviations. In **Figure D**, each individual BCP-ALL case was plotted in a fixed APS together with the normal populations. Then, each BCP-ALL case was visualized in separate unfixed APS with the nearest normal population, using the backbone markers (**Figure E**) and the 8 markers (**Figure F**). The separation between normal and pathological populations was scored to reach the optimal combination to enhance the separation of these two entities.*

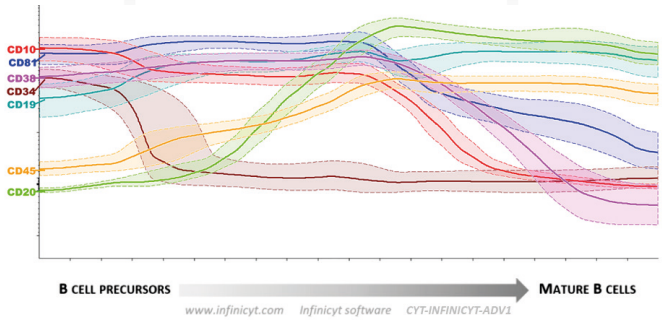


Figure 2 *shows the maturation pattern of normal BCP cells in BM when using tools available in the Infinciyt™ software.*

For flow cytometric MRD data analysis using the Euroflow™ BCP-ALL MRD panel, the presence of abnormal leukemic BCP in a given BM sample can be determined by looking at the changes in the pattern expression of the markers. The marker expression on normal BCP in BM as well as their maturation pattern can be found in the Infinciyt™ software (Figure 2) (for more information refer to the Infinicyt™ software Instruction for Use (IFU)).

STANDARDISED OPERATING PROCEDURES FOR MRD evaluation

Flow cytometry immunophenotyping results are highly dependent on the protocols used for sample processing. For this reason, the BCP-ALL MRD assay was designed in collaboration with the EuroFlow™ Consortium and the protocol was developed following general EuroFlow™ standardized recommendations and Standard Operating Procedures* (SOPs) for flow cytometers equipped with three-laser (blue, red, violet). Using these fully standardized SOPs for all BCP-ALL MRD samples and upon evaluation of sufficient cells (> 4 million, preferably more), a sensitivity comparable to RQ-PCR (golden standard methodology) was reached.[6]

During early phases of disease treatment, the cellularity of BM patient samples is usually low and using the standard methodology requiring the direct staining of 100 µL of whole BM will not allow the acquisition of the millions of cells needed to reach similar sensitivity as obtained by RQ-PCR. Therefore, the BCP-ALL MRD SOP as developed with the Euroflow™ Consortium, consists of a new erythrocyte lysing procedure (using BulkLysis™) to lyse sufficiently large volumes of BM and resuspend the resulting leukocytes in a small volume of washing buffer suitable for staining. This new protocol allows for the staining of 10 million cells in 100 ·L of cell suspension without compromising the data quality and as such enables the earlier mentioned sensitivity of at least 10^{-5}.[6]

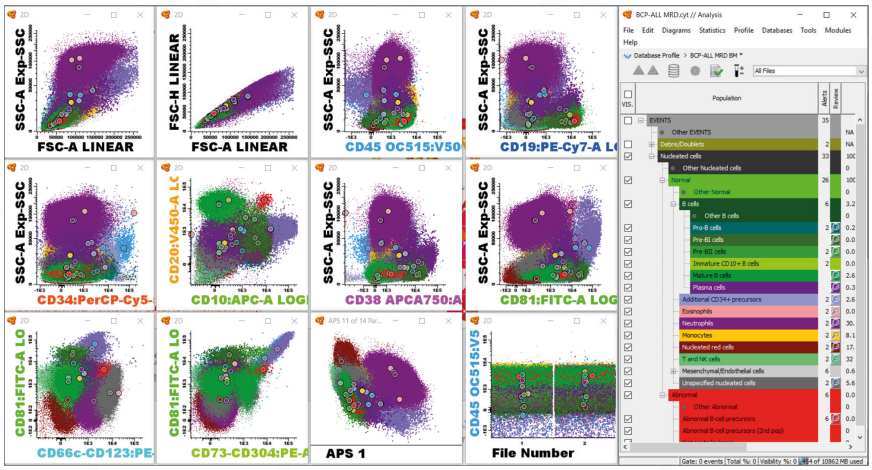
*The corresponding SOPs may be found at www.euroflow.org.

Infinicyt™ data analysis and reference databases

Delivery of timely and accurate results is important for appropriate patient care. Therefore, it is essential that flow cytometry data analysis and result reporting is accurate, objective, and specific to the clinical questions, and that the data are processed and reported rapidly. The number of immunophenotypic markers that can be evaluated in 8-color assays and the high number of cells to be acquired to achieve the desired assay sensitivity, clearly increases the complexity of data analysis.

EuroFlow™ developed a database containing representative flow cytometry data sets from normal healthy BM samples processed in different standardized centers. The database (available through Infinicyt™) when used with files which follow EuroFlow™ SOPs allows for a more automated analysis of the complete BM sample[12,13].

The BCP-ALL-MRD database is designed to be used with Infinicyt™ Automated Gating and Identification (AG&I) tool13 in order to provide a more automated analysis of the sample. Normal populations are identified comparing them with the reference database while events which differ from normal need to be confirmed by the user. All the information from the analysis and deviations from the normality ranges are automatically included in the results report (Figure 3).



In summary, the EuroFlow™ BCP-ALL MRD panel, with the SOPs and the database, is a powerful tool that helps the users to generate reproducible and objective data also when handling complex patient samples.[6,12,13]

Figure 3 *displays the result of the automatic analysis in Infinicyt ™ of a sample stained with the BCP-ALL-MRD panel. The image shows the entire sample analyzed, with the alert column warning of deviations of the populations from the normal BM database.*

CONCLUSION

The BCP-ALL MRD assay, used in combination with Infinicyt™ software, enables labs to qualitatively identify and discriminate BCP-ALL cells from normal/reactive BCP in BM samples from treated BCP-ALL patients,[6] ultimately supporting good patient outcomes.[17]

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Ordering information:

Product	Reference	Format	Size
BCP-ALL-MRD kit	CYT-BCP-ALL-MRD	Lyophilized	20 test

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BD-770422 V1.0 092022

Individuals need standards

Approved by EuroFlow

BD OneFlow™ Solution

on BD FACSCanto™ II and BD FACSLyric™ Flow Cytometers

BD

BD OneFlow™ Solution

Built on the research and validation work of the EuroFlow™ Consortium on the characterization of hematological malignancies for improved diagnostic outcomes,[1] the BD OneFlow™ Solution brings the standardization of leukemia and lymphoma immunophenotyping one step forward. It is a comprehensive set of reagents (BD OneFlow LST, B-CLPD T1, PCST, PCD, and ALOT), setup beads, protocols, and assay templates to reproducibly set up the flow cytometer and stain, acquire, and analyze patient specimens for immunophenotyping of normal and aberrant cell populations. The BD OneFlow solution improves efficiency by providing a standardized and simplified methodology, increasing reliability and enabling accuracy and confidence in results.[2,3]

The EuroFlow Consortium designed multicolor antibody panels to fully characterize the cell populations in a patient specimen using immunophenotypic markers that are indicative of normal and abnormal cells.[1] In addition to the optimized multicolor antibody panels, the EuroFlow protocol comprises standardized procedures for cytometer setup, determination of assay settings, sample preparation and staining, sample acquisition, and data analysis.[4]

The single-tube screening panels and multi-tube classification panels fit into the EuroFlow diagnostic algorithm for the identification and classification of hematological disorders. Each tube contains a set of backbone-markers and a set of classification markers.[1]

Backbone markers are shared across a particular set of panels and are used to normalize the samples so that data files can be combined and analyzed as a single, large data file. They are markers that identify distinct cell populations in a particular cell lineage. Classification markers have been selected for their diagnostic utility in discriminating between cell types within a given lineage and in classifying the abnormal cell type in the sample.

EFFICIENCY

Optimized workflows improve efficiency

BD OneFlow™ Reagents improve laboratory efficiency by reducing the time spent for sample preparation.[2,3]

Provided in a ready-to-use, dried, single-test tube format, BD OneFlow™ Reagents allow for direct specimen staining, eliminating the need for antibody pipetting, minimizing operational mistakes and the risk for testing repetition, thus reducing manual workload.

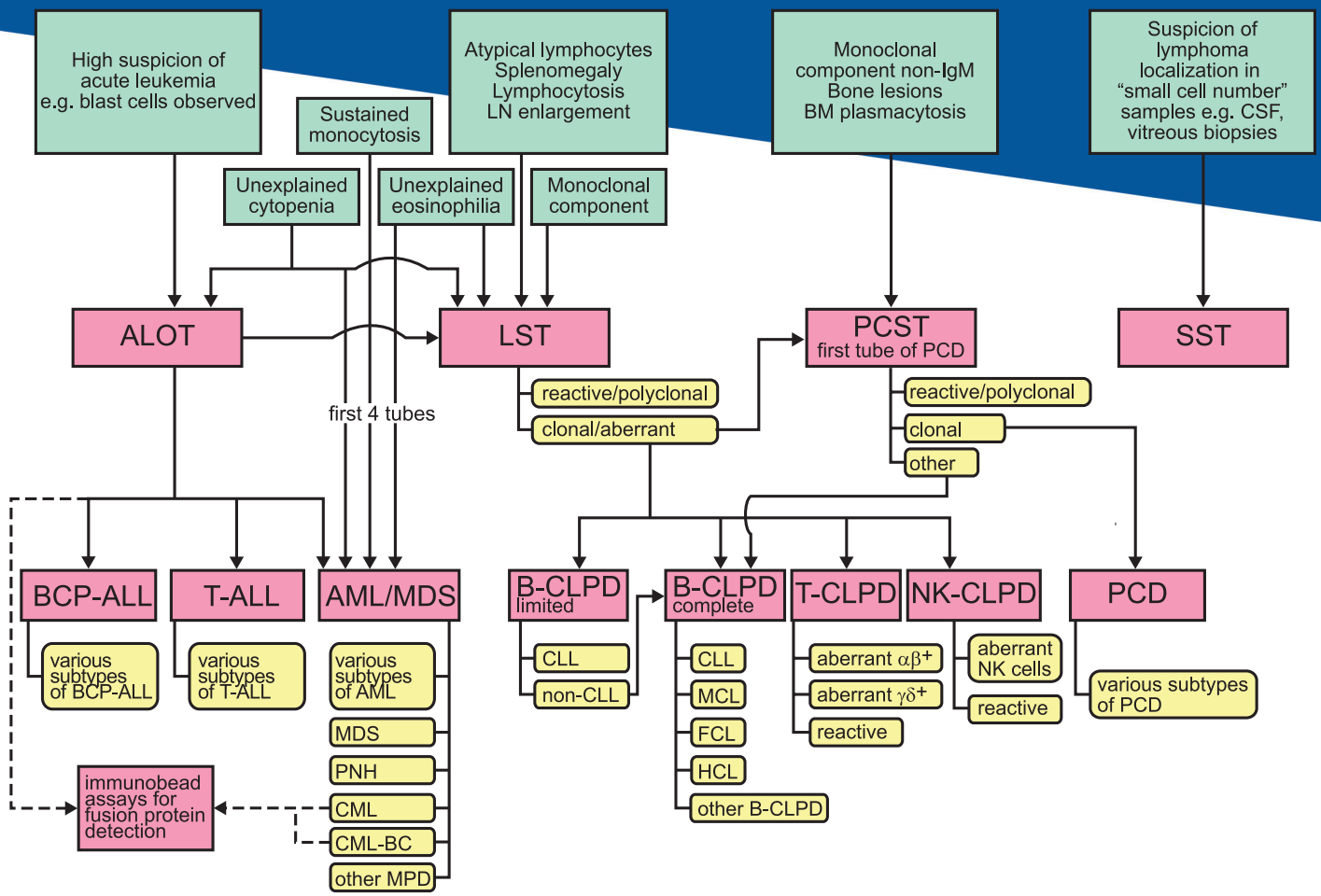
BD OneFlow™ Instrument setup and ready-to-use single-dose compensation beads simplify instrument standardization and reduce technical burden and training needs.

COMPLIANCE

Complete CE/IVD system enables compliancy

The BD OneFlow™ Solution is CE marked to the European In Vitro Diagnostic Medical Device Directive 98/79/EC. The BD OneFlow™ solution also helps laboratories in their accreditation process to comply with EN ISO 15189 standard ("Medical laboratories – Requirements for quality and competence").

EuroFlow™ Strategy for immunophenotypic characterization of hematological malignancies



J.J.M. van Dongen et al. LEUKEMIA 2012; 26: 1908-1975. This diagram has been provided courtesy of EuroFlow Consortium.

ACCURACY

Standardization improves data accuracy

Standardization of processes supports the quality of results and ultimately support the diagnosis and treatment of patients. Built on the standards defined by the EuroFlow™ Consortium, the predefined, disease-specific 8-color reagent panels provide high diagnostic utility delivering accurate and reproducible results.

"The (EuroFlow) LST detected aberrant B-, T- or NK-cells immunophenotypes in 149/150 (99.4%) of B-CLPD and in 78/83 (94%) of T/NK-CLPD with an overall frequency of 97.4%."*[1]

"An unprecedented orientation efficiency of 98.3% for non-ambiguous lineage cases was shown for the (EuroFlow) ALOT combination with a series of 483 newly diagnosed acute leukemia cases, tested prospectively at different centers."[1]

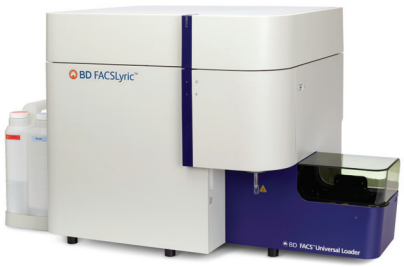
*B-cell chronic lymphoproliferative diseases

Workflow

BD OneFlow™ Reagents can be run on both the BD FACSLyric™ and BD FACSCanto™ II Flow Cytometers for equivalent results.

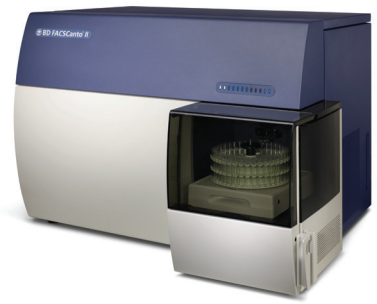
Periodic Setup

Instrument Setup and QC



BD FACSLyric

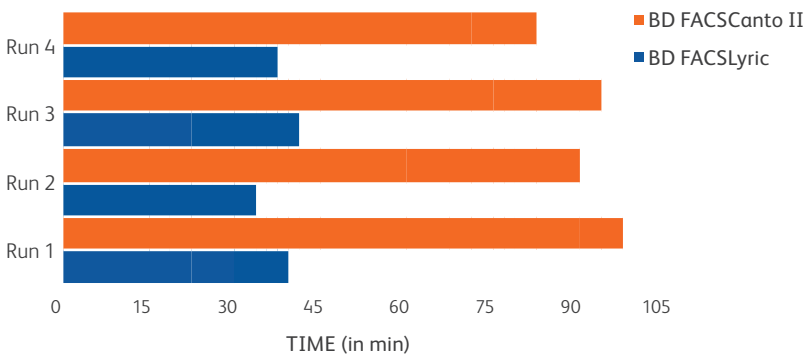
- Characterization QC with BD™ CS&T Beads (6 months or as needed)
- Reference settings update with BD™ FC Beads 7-Color and 5-Color Kits (Every 60 days)



BD FACSCanto II (monthly)

- Performance check with BD FACSDiva™ CS&T IVD Bead
- PMT voltage adjustment with BD OneFlow™ Setup Beads
- FSC and SSC adjustments with lysed wash blood
- Compensation with BD™ FC Beads 8-Color Kit for BD OneFlow Assays

Instrument Setup Time for Operator



BD FACSLyric Instrument Benefits*

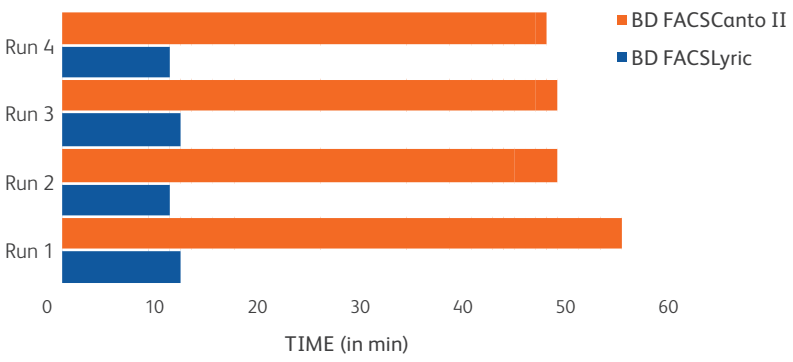
- **69%** annual reduction in periodic instrument setup and QC time
- **95%** annual reduction in instrument set-up hands-on time to less than **30** minutes annually

*Relative to BD OneFlow on BD FACSCanto II based upon internal testing. BD FACSLyric warmup time is not included as it is pre-programmable and does not impact operator time. Individual lab performance will vary.

Daily Workflow

Startup	Daily Setup and QC	Sample Preparation	Acquisition and Analysis
BD FACSLyric			
▶ Automatic Startup	▶ Performance QC with BD CS&T Beads ▶ Assay and Tube Settings Setup with BD CS&T Beads	▶ Standardized sample preparation with BD OneFlow Reagents	▶ Sample acquisition and analysis with predefined templates and reports
BD FACSCanto II			
▶ Instrument Start Up ▶ Fluidics Start Up	▶ Performance QC with BD FACSDiva CS&T IVD Beads ▶ PMT voltage confirmation with OneFlow Setup Beads	▶ Standardized sample preparation with BD OneFlow Reagents	▶ Sample acquisition and analysis with predefined templates and reports

Daily Setup Time for Operator



BD FACSLyric Instrument Benefits*

- **74%** reduction in manual daily startup and setup steps
- **76%** reduction in daily startup and setup time

BD OneFlow™ LST
Lymphoid Screening Tube

20 tests/box

BD OneFlow LST

The BD OneFlow™ LST (Lymphoid Screening Tube) is intended for flowcytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T, and NK lineages in peripheral blood, bone marrow, and lymph nodes, as an aid in the diagnosis of hematological disorders.

In chronic lymphoproliferative disorders (CLPD), clonogenic events lead to the expansion and accumulation of mature-appearing lymphocytes, which carry a proliferative and/or survival advantage over their normal counterparts.[1] Thus, the detection of phenotypically aberrant and clonal mature lymphocytes is critical to the diagnosis of CLPD.

"The (EuroFlow) LST detected aberrant B-, T-, or NK-cells immunophenotypes in 149/150 (99.4%) of B-CLPD and in 78/83 (94%) of T/NK-CLPD with an overall frequency of 97.4%."[1]

Laser								
Format	BD Horizon™ V450	BD Horizon™ V500-C	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
Marker	CD20 CD4	CD45	CD8 Igλ	CD56 Igκ	CD5	CD19 TCRγδ-1	CD3	CD38

The antibodies in the BD OneFlow™ LST were chosen for their ability to separate normal lymphocytes into their major subpopulations.

- CD45 identifies mature lymphocytes and B cell precursors.
- CD3 identifies T cells.
- CD3 can also be used to identify B cells and NK cells by exclusion.
- Anti-TCRγ/δ-1, CD5, CD4, and CD8 can separate T cells into a number of subpopulations.
- CD19 and CD20 identify B cells, and together with CD45 can separate B cells into mature B lymphocytes (CD19+, CD20hi, CD45hi) and B-cell precursors (CD19+, CD20-/lo, CD45lo). CD19 and CD20 are also used to identify NK cells by exclusion.
- Anti-Kappa and Anti-Lambda can identify normal and clonally expanded populations of B cells expressing Igκ or Igλ on the surface membrane, respectively.
- CD38 identifies plasma cells and B cell precursors. In addition, it is informative in the evaluation of a wide variety of lymphoid malignancies. CD38 can also aid in the identification of NK cells.
- CD56 identifies NK cells.

BD OneFlow™ B-CLPD T1

B Cell Lymphoproliferative Disorders – Tube 1

20 tests/box

BD OneFlow B-CLPD T1

The BD OneFlow B-CLPD T1 (B cell Chronic Lymphoproliferative Diseases Tube 1) shall be used for specimens with B-lineage populations needing further investigation in combination with the BD OneFlow LST (Lymphoid Screening Tube). The BD OneFlow B-CLPD T1 is intended for flow-cytometric immunophenotyping of B cells in peripheral blood and bone marrow as an aid in the diagnosis of chronic lymphocytic leukemia (CLL) and other B cell chronic lymphoproliferative diseases.

Laser								
Format	BD Horizon™ V450	BD Horizon™ V500-C	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
Marker	CD20	CD45	CD23	CD10	CD79b	CD19	CD200	CD43

The antibodies in BD OneFlow™ B-CLPD T1 were chosen to work in conjunction with the antibodies in BD OneFlow™ LST to distinguish CLL from other B cell chronic lymphoproliferative diseases in patient specimens.

- CD45, CD19, and CD20 are present in both BD OneFlow™ LST and BD OneFlow B-CLPD T1 and serve as backbone markers, allowing for the direct comparison of specimens stained using the two tubes.
- CD23, CD200, CD79b, CD43, and CD10 are classification markers and, together with CD5 and CD38 from BD OneFlow LST, allow for specimens to be classified as CLL or as other B cell chronic lymphoproliferative diseases.
- Anti-Kappa and Anti-Lambda, present in BD OneFlow LST, assess the clonality of the B cell population.

BD OneFlow™ PCST
Plasma Cell Screening Tube

10 tests/box

Plasma cell disorders are a group of diseases most often characterized as having a clonal (neoplastic) population of plasma cells in the bone marrow (BM).[1] The cells may secrete a clonal immunoglobulin that can be detected in the circulation. These disorders comprise several distinct diseases, including multiple myeloma and monoclonal gammopathy of undetermined significance.

BD OneFlow PCST

The BD OneFlow PCST (Plasma Cell Screening Tube) is intended for flow-cytometric immunophenotyping of normal polyclonal and aberrant plasma cell populations in bone marrow as an aid in the diagnosis of hematological disorders.

BD OneFlow™ PCST consists of two single-use tubes containing fluorochrome-conjugated antibodies in an optimized dried formulation. BD OneFlow™ PCST (S) contains antibodies that recognize markers on the surface of cells, and BD OneFlow™ PCST (C) contains antibodies that recognize Igκ and Igλ in the cytoplasm of cells after fixing and permeabilizing them.

Laser								
Format	BD Horizon™ V450	BD Horizon™ V500-C	FITC	PE	PerCP -Cy™ 5.5	PE-Cy™ 7	APC	APC-H7
Marker	CD45 (S)	CD138 (S)	CD38 (S)	CD56 (S)	β2-Microglob. (S)	CD19 (S)	Cylgκ (C)	Cylgλ (C)

The antibodies in the BD OneFlow™ PCST tube were chosen for their ability to identify and characterize plasma cells.

- CD38, CD138, CD45, and CD19 are backbone markers used to identify plasma cells.
- CD56 and β2-Microglobulin are classification markers used to identify aberrant plasma cell populations.
- Anti-Kappa and Anti-Lambda are used to assess the clonality of the plasma cells.
- CD19, Anti-Kappa, and Anti-Lambda are also used to identify and characterize mature B cells.

BD OneFlow™ PCD
Plasma Cell Disorders Tube

10 tests/box

BD OneFlow PCD

The BD OneFlow PCD (Plasma Cell Disorders) tube, when run in parallel with the BD OneFlow PCST (Plasma Cell Screening Tube), is intended for flow-cytometric immunophenotyping of normal and aberrant plasma cells in bone marrow as an aid in the diagnosis of multiple myeloma and other plasma cell disorders.

Laser								
Format	BD Horizon™ V450	BD Horizon™ V500-C	FITC	PE	PerCP-Cy™5.5	PE-Cy™7	APC	APC-H7
Marker	CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81

The antibodies in the BD OneFlow PCD tube were chosen for their ability to identify plasma cells.

- CD38, CD138, CD45, and CD19 are backbone markers used to identify plasma cells.
- CD27, CD28, CD117, and CD81 are classification markers used to identify aberrant plasma cell populations.

BD OneFlow™ ALOT
Acute Leukemia Orientation Tube

10 tests/box

BD OneFlow ALOT

The BD OneFlow™ ALOT (Acute Leukemia Orientation Tube) is intended for flow-cytometric immunophenotyping of aberrant immature populations of hematopoietic cells (lymphoid and non-lymphoid lineage) in bone marrow and peripheral blood as an aid in the diagnosis of acute lymphoblastic leukemia and non-lymphoid acute leukemia.

BD OneFlow™ ALOT consists of two single-use tubes containing fluorochrome-conjugated antibodies in an optimized dried formulation. The BD OneFlow™ ALOT (S) tube contains antibodies that recognize markers on the surface of cells, and the BD OneFlow™ ALOT (C) tube contains antibodies that recognize antigens in the cytoplasm of cells after fixing and permeabilizing them.

Acute leukemias are a heterogeneous group of diseases characterized as having a clonal (neoplastic) population of immature hematopoietic cells in the peripheral blood (PB) or bone marrow (BM).[1]

There are two major classes of acute leukemias: lymphoid precursor leukemias and acute myeloid leukemias (AML). The lymphoid precursor leukemias are divided into B cell and T cell precursor lymphoblastic leukemias (BCP-ALL and T-ALL, respectively).

In addition, a small number of neoplasms do not fit into any of these categories because they either show no clear expression of markers indicative of a particular lineage or they express markers specific to more than one lineage. They include acute undifferentiated leukemia (AUL) and mixed phenotype acute leukemia (MPAL).

Laser								
Format	BD Horizon™ V450	BD Horizon™ V500-C	FITC	PE	PerCP -Cy 5.5	PE-Cy7	APC	APC-H7
Marker	cyCD3 (C)	CD45 (S)	cyMPO (C)	cyCD79a (C)	CD34 (S)	CD19 (S)	CD7 (S)	CD3 (S)

The antibodies in the BD OneFlow ALOT were chosen for their ability to identify and characterize aberrant immature populations of hematopoietic cells.

- CD45, CD34, and CD19 are the backbone markers for the BCP-ALL panel.
- CD45, cytoplasmic CD3 (cyCD3), and CD3 are the backbone markers for the T-ALL panel.
- CD45 and CD34 are the backbone markers for the AML panel.
- CD34 and negative or dim expression of CD45 ($CD45^{neg/dim}$) are markers for immature cells.
- Cytoplasmic myeloperoxidase (cyMPO) is a myeloid lineage marker.
- cyCD3 and CD7 are T cell lineage markers.
- CD3 is used as a maturity marker for T cells.
- CD19 and cytoplasmic CD79a (cyCD79a) are B cell lineage markers

BD OneFlow™ setup on the BD FACSCanto II

BD FACSDiva™ CS&T IVD Beads

- Standardize setup and monitoring for consistent performance.

BD OneFlow™ Setup Beads

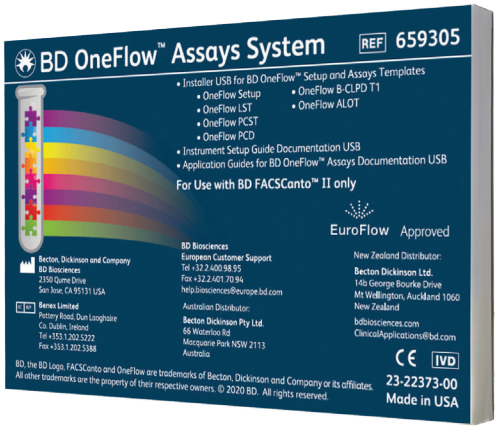
- Ensure data accuracy and reproducibility by providing assay-specific target values, as per EuroFlow™ SOPs.[4]
- Enable lab efficiency by minimizing the technical burden and training needs.[3]

BD™ FC Beads 8-Color-Kit for BD OneFlow™ Assays

- Eliminate the need for using single vial reagents with ready-to-use single-test dye-coupled beads.
- Enable lab efficiency with a simplified procedure for standardized compensation.[3]
- Support consistency of results, eliminating need for using cells for compensation.[2,3]

BD OneFlow Assays System for the BD FACSCanto II

- Standardize acquisition and analysis in BD FACSDiva™ v8.0.1, v8.02, v8.0.3, and v9.0 with predefined templates for consistency of results.



BD OneFlow™ setup on the BD FACSLyric

BD CS&T IVD Beads

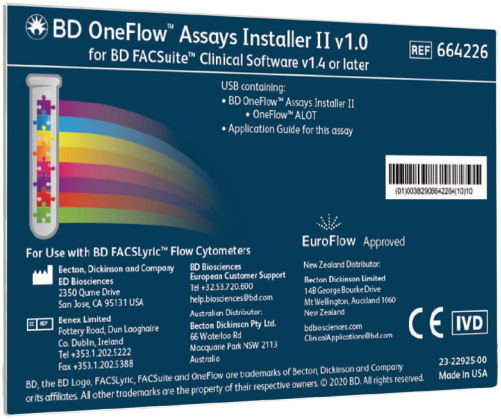
- Standardize setup and monitoring for consistent performance.
- Enables single-tube QC for daily setup and performance checks.

BD FC Beads 7-Color and 5-Color Kits

- Ready-to-use single-test dye-coupled beads for compensation every 60 days.
- No need for using single vial reagents for label-specific compensation.
- Enable lab efficiency with automated compensation.

BD OneFlow Assays Installers

- Standardize acquisition and analysis in BD FACSuite™ Clinical Application v1.4 with predefined templates for consistency of results
- Supplemental analysis reports for flexibility in examining additional cell populations
- Reports available in 25 languages



Ordering Information

Product name	Tests	Description	Reg. Status	Product number
BD OneFlow™ LST	20 tests	4 pouches/box – 5 tubes/pouch	CE-IVD	658619
BD OneFlow™ B-CLPD T1	20 tests	4 pouches/box – 5 tubes/pouch	CE-IVD	659293
BD OneFlow™ PCST	10 tests	4 pouches/box (2 S and 2 C) – 5 tubes/pouch	CE-IVD	659912
BD OneFlow™ PCD	10 tests	2 pouches/box – 5 tubes/pouch	CE-IVD	659913
BD OneFlow™ ALOT	10 tests	4 pouches/box (2 S and 2 C) – 5 tubes/pouch	CE-IVD	660228
BD FACSCanto II related products				
BD OneFlow™ Assays System Installer	–	3 USB cards: one each for assays installer, setup guide, and application guides	CE-IVD	659305
BD FACSDiva™ CS&T IVD Beads	50 tests	1 vial	CE-IVD	656046
	150 tests	3 vials of 50 tests each		656047
BD OneFlow™ Setup Beads	25 tests	1 vial + 2 MFI target value cards	CE-IVD	658620
BD™ FC Beads 8-Color Kit for BD OneFlow Assays	5 tests	8 pouches/box (1 pouch/color) - 5 tubes/pouch	CE-IVD	658621
BD FACSLyric related products				
BD OneFlow™ Assays Installer I	–	1 USB card containing BD OneFlow LST, BD OneFlow B-CLPD T1, BD OneFlow PCST, and BD OneFlow PCD assay installers and assays application guides	CE-IVD	664225
BD OneFlow™ Assays Installer II	–	1 USB card for BD OneFlow ALOT assay installer and assay application guide	CE-IVD	664226
BD™ CS&T IVD Beads	50 tests	1 vial	CE-IVD	656504
	150 tests	3 vials of 50 tests each		656505
BD™ FC Beads 7-Color Kit	5 tests	7 pouches/box (1 pouch/color) - 5 tubes/pouch	CE-IVD	656867
BD™ FC Beads 5-Color Kit	5 tests	5 pouches/box (1 pouch/color) - 5 tubes/pouch	CE-IVD	661564

Visit our website for more information on BD OneFlow™ Solution

eu.bd.com/oneflow

References

1. van Dongen, J.J., Lhermitte, L., Bottcher, S., et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia*. 2012; 26: 1908-1975.
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3. Moloney, E., Watson, H., Barge, D., et al. Efficiency and Health Economic Evaluations of BD OneFlow™ Flow Cytometry Reagents for Diagnosing Chronic Lymphoid Leukemia. *Cytometry Part B (Clinical Cytometry)*. 2019.
4. Kalina, T., Flores-Montero, J., van der Velden, V.H., et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia*. 2012; 26:1986-2010.

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