Standardized Flow Cytometry System to Aid in the Diagnosis of Chronic Lymphocytic Leukemia

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Abstract

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia found in adults, and represents about 30% of all new leukemia diagnoses in the Western world. As with other hematological cancers, flow cytometry is a key laboratory test that aids in the diagnosis of CLL. Despite the global use of flow cytometry for this application, there have been technical and diagnostic challenges due to a lack of standardization, which has resulted in variable results and placed a high burden upon the expertise of the user. The EuroFlow™ Consortium developed an early standardized flow cytometry approach for the screening and classification of leukemia and lymphoma. In this application note, we introduce the BD OneFlow™ system that builds upon and advances the standardized EuroFlow approach. Here we focus on the BD OneFlow™ LST (Lymphoid Screening Tube) and BD OneFlow™ B-CLPD T1 (B-cell Chronic Lymphoproliferative Diseases Tube 1), which aid in the diagnosis of CLL when used with the BD FACSCanto™ II flow cytometer and supporting reagents, protocol and BD FACSDiva™ software. Data from a multisite clinical validation study showed 100% agreement (101 of 101 patient specimens) between the EuroFlow comparator (reference) method and the BD OneFlow investigational (test) method in classifying patients as having CLL or other B-CLPDs. Furthermore, we show that the BD OneFlow system results were highly concordant (95.8% agreement) with the final diagnosis derived from patient history and other laboratory findings. In summary, the BD OneFlow system with LST and B-CLPD T1 provides a highly standardized, clinically-validated flow cytometry solution for the classification of CLL from other B-CLPDs, thus supporting a reproducible, efficient and accurate diagnosis of CLL.

BD

The role of flow cytometry in the diagnosis of CLL

CLL is the most common form of leukemia found in adults in Western countries, and worldwide accounts for approximately 191,000 cases and 61,000 deaths per year.[1] The incidence increases rapidly with age with a median age of 70 years at diagnosis. Although the median survival is about 10 years, the clinical course is very heterogeneous. Some patients never require treatment and essentially live a normal lifespan, whereas others have an advanced course of the disease and require early treatment.

Lymphocytosis detected during a routine physical examination is often the first indication of CLL. In the laboratory follow-up of lymphocytosis, flow cytometry immunophenotyping is a key technology (Figure 1) used in conjunction with patient history and other laboratory findings to reach a final diagnosis.

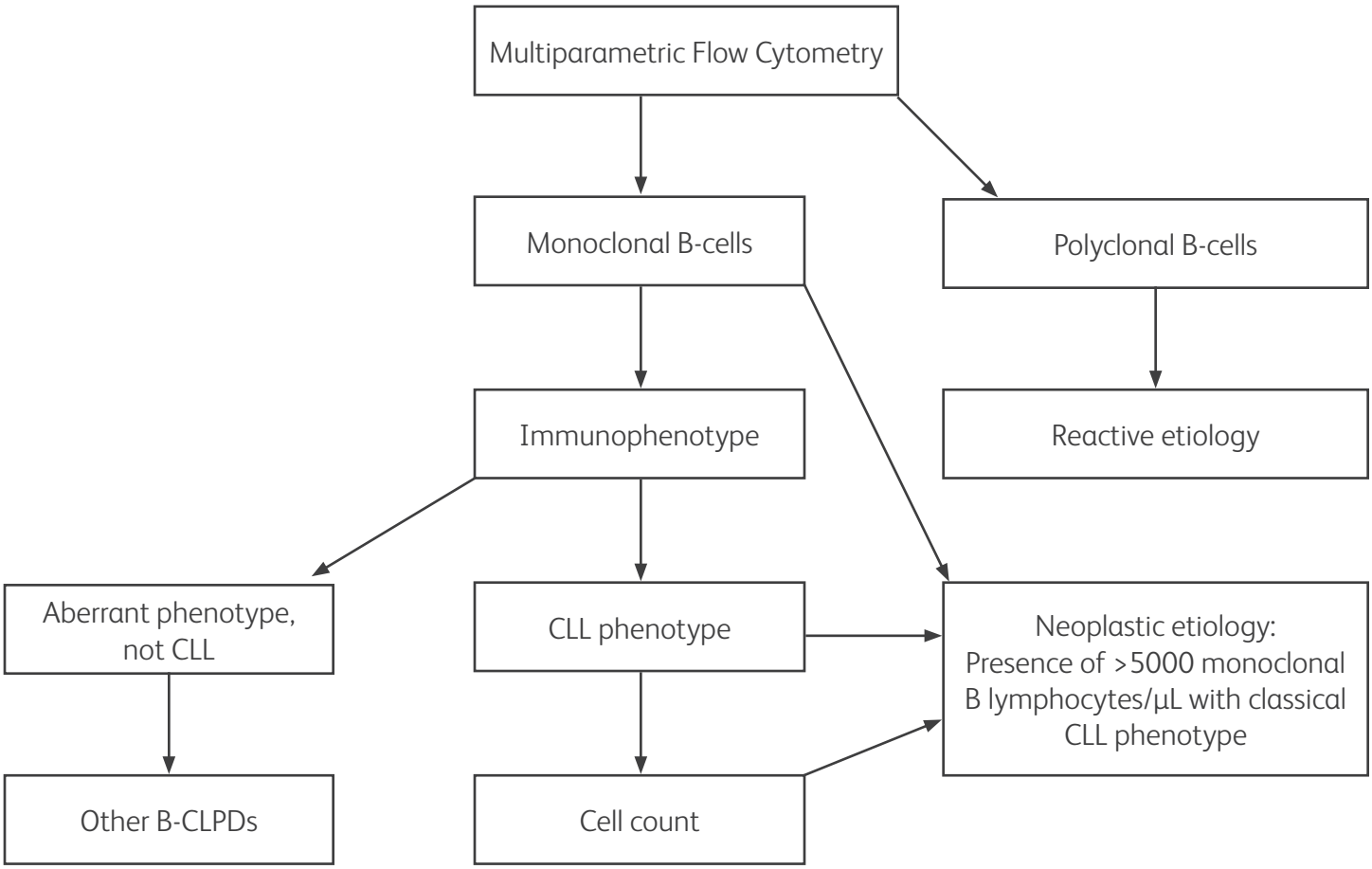

Figure 1. Role of flow cytometry as an aid in the diagnosis of CLL
Flow cytometry determines the clonality of the B-cell lymphocytosis and defines the immunophenotype of the abnormal population, supporting the diagnosis of CLL versus other B-CLPDs.

International guidelines have established the diagnostic criteria for CLL as the finding of an absolute count of ≥5000 monoclonal B cells/µL in peripheral blood expressing the classic CLL surface markers by flow cytometry (CD5, CD19, CD20 and CD23) and identification of characteristic atypical blood cells by morphological examination.[3,4,5] Testing of bone-marrow aspiration and biopsy specimens with flow cytometry is not a standard requirement in CLL diagnosis, although in some cases it may be needed to assess complications. Patients with an absolute count of ≤5000/µL clonal B cells with the CLL-phenotype are diagnosed with monoclonal B-cell lymphocytosis (MBL). This is considered a pre-malignant condition, and patients with higher cell counts (>5,000 clonal B cells/ µL)[6] are typically monitored, with about 1-2% of these individuals progressing to CLL annually.[7] Flow cytometry with appropriate antibody panels (for example, EuroFlow B-CLPD T2, T3 and T4) can additionally be used for the diagnosis of patients with other B-CLPDs, including mantle cell lymphoma, marginal zone lymphoma (MCL), follicular lymphoma, diffuse large B-cell lymphoma and hairy cell leukemia.

Flow cytometry challenges and the need for a standardized flow cytometry system

Flow cytometry is an essential tool used in the diagnosis and monitoring of hematological cancers. Despite its broad deployment, there has been an acknowledgement of the lack of standardization in the clinical use of the technology. This lack of standardization has been broadly reflected in antibody panel design, instrument setup, protocols and data analysis. As a result, flow cytometry for hematological malignancies has remained highly dependent upon the expertise of the user and has had limited comparability of results over time and across sites.[8,9] Historically, attempts have been made to standardize testing for hematological cancers, but the efforts have largely focused on biomarker selection and matching of fluorochromes to meet biology and instrument-performance requirements. An exception to this limited approach to bring standardization to flow cytometry, has been through the efforts of the EuroFlow Consortium, an independent scientific organization of 20 clinical flow laboratories. Over a period of six years, through multiple rounds of testing, this organization identified the most relevant markers and their respective antibody clones and fluorochromes for use in multicolor panels. Further, they developed standardized instrument setup, protocols and data interpretation for the screening and classification of most hematological cancers.

BD Biosciences has developed and released the BD OneFlow system to aid in the diagnosis of hematological malignancies. This system of instrumentation, reagents, software and protocols was built on the previous efforts of the EuroFlow Consortium, and brought further standardization and validation to the clinical laboratory workflow. The BD OneFlow system offers a standardized instrument setup, including BD™ CS&T beads for instrument performance monitoring and BD OneFlow™ Setup beads supporting data reproducibility through assay-specific target values.[10] Additionally, BD™ FC beads for compensation enable a simpler, standardized process than the EuroFlow method.

BD OneFlow LST and B-CLPD T1 reagents are ready-to-use, single-test antibody cocktails based on the EuroFlow Consortium panel design. As single-use, pretitered, predispensed dried reagent tubes, they have eliminated the need for multiple, manual antibody pipetting steps and the storage of multiple liquid reagents. These improvements in reagents can increase assay reproducibility and minimize potential operational mistakes[11] including, multiple tube labeling and pipetting steps, fluorochrome compensation, cocktail preparation, and in-lab validation,[12] a concern that has become more pronounced with worldwide adoption of 8- and 10-color panels.[13] Furthermore, the BD OneFlow system provides templates for acquisition and analysis with a predefined gating strategy.

Clinical validation of the BD OneFlow system as an aid in diagnosis of CLL

Recently the BD OneFlow system using the LST in combination with B-CLPD T1 was CE marked according to the European iIn Vitro Diagnostic Medical Device Directive 98/79/EC and released for clinical flow cytometry laboratory use. This followed a multisite method comparison clinical study that validated the BD OneFlow system as an aid in the diagnosis of CLL.

That study was performed on de-identified, remnant peripheral blood and bone-marrow specimens from patients with suspected disorders of mature B-cell lymphocytes to demonstrate the equivalency (accuracy) between the BD OneFlow system and the EuroFlow liquid reagent approach. One hundred and one (101) specimens (70 whole blood and 31 bone marrow) were stained with BD OneFlow LST and BD OneFlow B-CLPD T1 dried reagents or the reference EuroFlow Consortium liquid reagents and acquired. Acquisition and analysis for both EuroFlow and BD OneFlow systems were performed on a BD FACSCanto II instrument using BD OneFlow LST and B-CLPD T1 templates in BD FACSDiva software v8.0.1.[14]

BD OneFlow workflow improvements were incorporated into the clinical study protocol, as illustrated in Table 1. The primary improvements were related to compensation, reagent preparation and specimen staining.

	BD OneFlow	EuroFlow
Instrument and Assay Setup		
Cytometer Performance QC	BD FACSDiva CS&T IVD beads (1 tube)	BD FACSDiva CS&T IVD beads (1 tube)
PMTV adjustments	BD OneFlow Setup Beads (1 tube)	SPERO™ 8 peak Rainbow Calibration Particles (1 tube)
Side scatter adjustment	Normal lyse washed blood (1 tube)	Normal lyse washed blood (1 tube)
Compensation*	BD™ FC Beads fo BD OneFlow™ (8 tubes)	Lab prepared tubes with stained cells (9 tubes)
Specimen Preparation, Acquisition and Analysis		
Reagent preparation and specimen staining*	BD OneFlow dried reagents tubes • LST: one dried tube of 12 reagents • B-CLPD T1: one dried tube of 8 reagents	Liquid reagent cocktails • LST: a cocktail of 12 liquid reagents • B-CLPD T1: a cocktail of 8 liquid reagents
Acquisition	Acquisition worksheet of BD OneFlow LST or B-CLPD T1 template on BD FACSDiva (automatic plots and sample gates)	Acquisition worksheet of BD OneFlow LST or B-CLPD T1 template on BD FACSDiva (automatic plots and sample gates)
Analysis	Acquisition worksheet of BD OneFlow LST or B-CLPD T1 template on BD FACSDiva (automatic plots and sample gates)	Acquisition worksheet of BD OneFlow LST or B-CLPD T1 template on BD FACSDiva (automatic plots and sample gates)

*denotes BD OneFlow workflow improvement

Table 1. Comparison of the BD OneFlow and EuroFlow Consortium workflows as performed in the clinical validation study.[15,16]

When compared to the EuroFlow method, the BD OneFlow LST and B-CLPD T1 system gave 100% (101 of 101) overall agreement in classifying patients as CLL (54/54 concordant) from other B-CLPDs (47/47 concordant) with a lower 95% confidence interval (CI) of the overall agreement being 97.4% (Table 2).

		EuroFlow Reference Method	
		CLL	Other B-CLPDs
BD OneFlow Test System	CLL	54	0
	Other B-CLPDs	0	47

Table 2. Concordance between the EuroFlow reference method and the BD OneFlow test method for classifying CLL from other B-CLPDs

The clinical trial results validated that the BD OneFlow test method is fully concordant with the EuroFlow reference method in distinguishing CLL from other B-CLPDs.

Figures 2 and 3 show representative examples of flow cytometry immunophenotyping profiles for a typical CLL specimen and other B-CLPD specimen as determined by both methods.

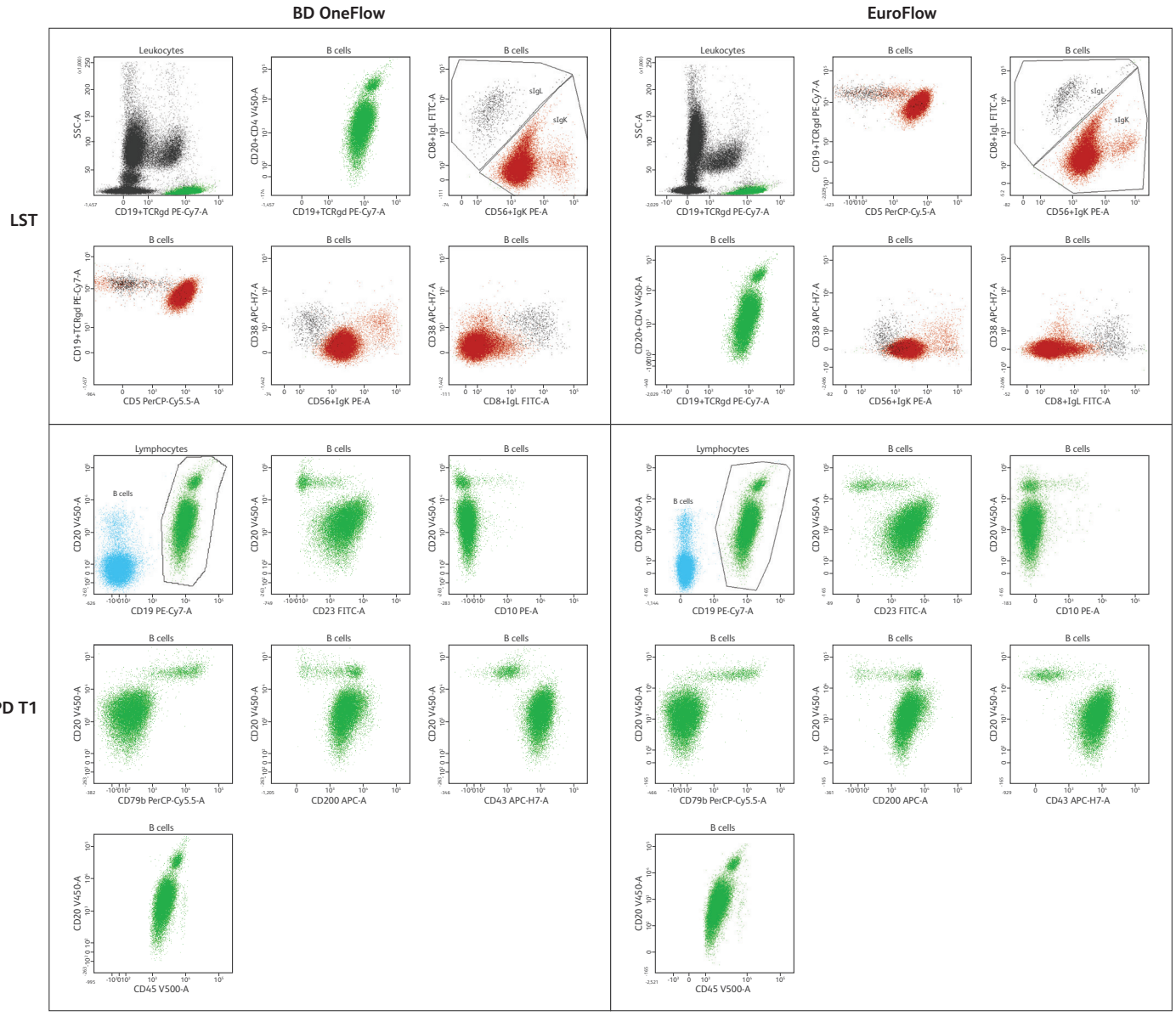

Figure 2. An example immunophenotype of a typical CLL patient specimen

This specimen was interpreted by the study site to be κ light chain restricted, CD5 positive, CD19 positive, CD20 dim, CD23 moderate, CD200 bright and CD79b dim.

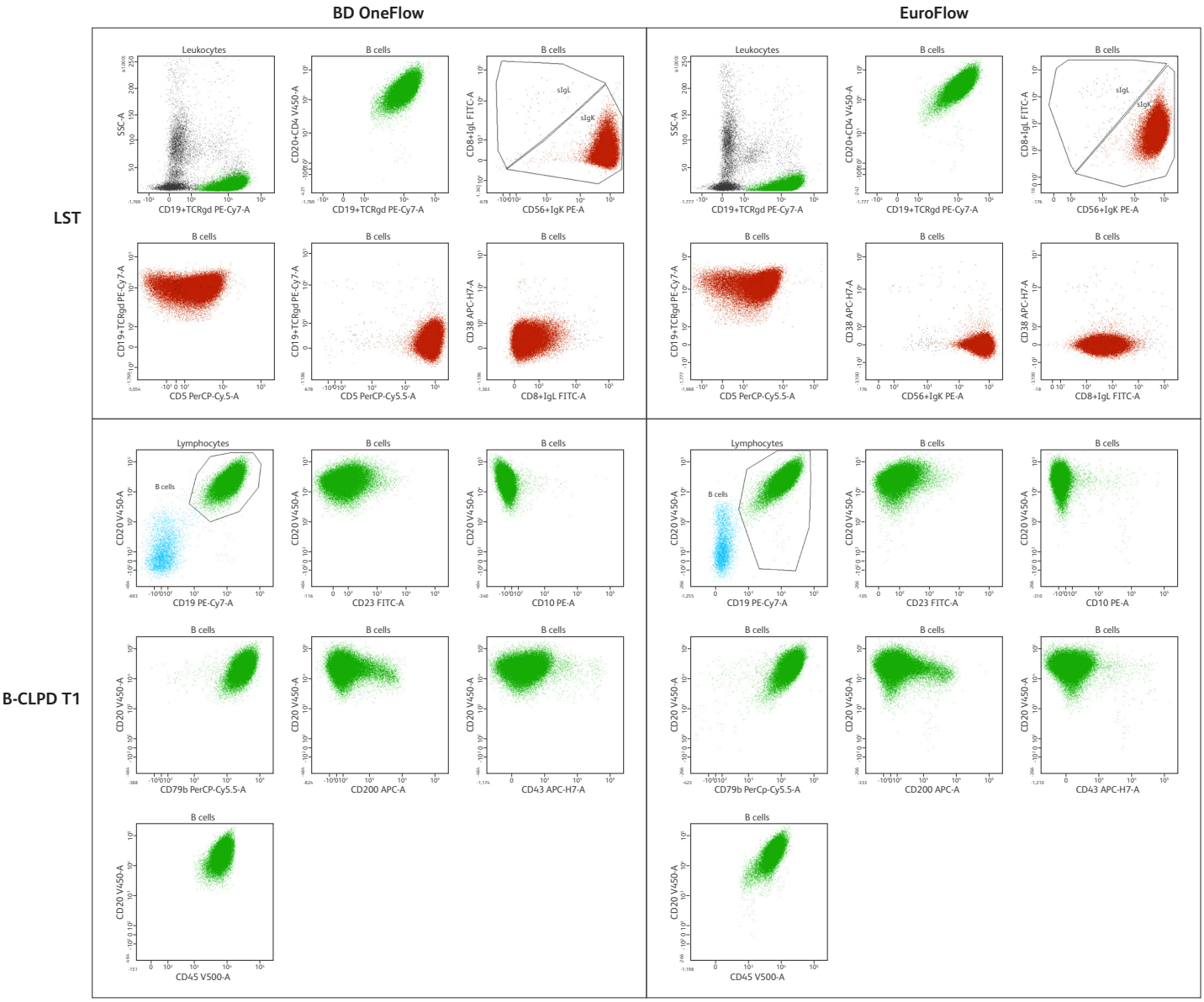

Figure 3. An example immunophenotype of other B-CLPD specimens distinguished from typical CLL
This specimen was interpreted by the study site to be κ light chain restricted, CD5 positive, CD19 positive, CD20 bright, CD23 negative, CD200 negative and CD79b bright. The final diagnosis was MCL, as determined through a composite of patient history and other laboratory tests.

As shown in Table 3, the clinical trial results for the BD OneFlow system (LST and B-CLPD T1) were compared to the diagnostic truth derived from a composite of patient history and laboratory findings.

		BD OneFlow Test Result		
		CLL (typical)	Other B-CLPDs	Total
Diagnostic Truth	CLL (typical)	49	3	52
	Other B-CLPDs	1	43	44
	Total	50	46	96

Table 3. Concordance between diagnostic Truth and BD OneFlow clinical trial results

Ninety-six (96/101) specimens gave 94.2% agreement in classifying patients as having CLL (49/52) and 97.7% agreement in identifying patients with other B-CLPDs (43/44) with an overall agreement of 95.8% agreement (92/96). An additional five specimens diagnosed as MBL (4/101) or MBL of undetermined significance (MLUS) (1/101) were excluded from the agreement analysis as the immune profile is indistinguishable from CLL and the differentiating feature of the absolute number of monoclonal B cells was not included in the clinical trial design. The results presented here demonstrate that the fully standardized and validated BD OneFlow (LST and B-CLPD T1) system is highly concordant with the diagnostic truth.

Conclusion

The BD OneFlow system comprising the BD OneFlow setup reagents, BD OneFlow LST and B-CLPD T1, BD FACSDiva software with tube-specific templates on a BD FACSCanto II is a standardized flow cytometry system to aid in the diagnosis of CLL. BD OneFlow LST and B-CLPD T1 were based upon the EuroFlow Consortium design, and through clinical validation proved equivalent to the EuroFlow system in classifying CLL from other B-CLPDs. Furthermore, the interpretation of the BD OneFlow LST in combination with B-CLPD T1 flow results was highly concordant to the diagnostic truth. As such, the BD OneFlow system overcomes many of the challenges of traditional flow cytometry by providing a standardized, reproducible workflow that reduces risks of user error, streamlines laboratory operations and increases confidence in results.

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The BD OneFlow LST and BD OneFlow B-CLPD T1 are CE Marked according to the European In Vitro Diagnostic Medical Device Directive 98/79/EC.

The BD OneFlow LST and the BD OneFlow B-CLPD T1 are not available for sale in the USA.

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23-20106-00 EU

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