EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
CLEARLLAB REAGENTS
DECISION SUMMARY

A. DEN Number:

DEN160047

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation of the ClearLLab Reagents

C. Measurand:

Cluster of Differentiation (CD) Surface Markers on white blood cells

D. Type of Test:

Immunophenotyping, qualitative, flow cytometric assay

E. Applicant:

Beckman Coulter Inc.

F. Proprietary and Established Names:

Trade Name: ClearLLab T1, T2, B1, B2, M
Common Name: ClearLLab

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.7010

2. Classification:

   Class II (Special Controls)

3. Product code:

   PWD

4. Panel:

   81-Hematology

H. Indications For Use:

1. Indications for Use

    The ClearLLab reagents are intended for in vitro diagnostic use as a panel for qualitative identification of cell populations by multiparameter immunophenotyping on an FC 500 flow cytometer. These reagents are used as an aid in the differential diagnosis of hematologically abnormal patients having, or suspected of having the following hematopoietic neoplasms: chronic leukemia, acute leukemia, non-Hodgkin lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). The reagents can be used with peripheral whole blood collected in EDTA (K2 and K3EDTA), Acid Citrate Dextrose or Heparin, bone marrow collected in K2EDTA, ACD, or Heparin, and lymph node specimens. Interpretation of the results should be confirmed by a pathologist or equivalent professional in conjunction with other clinical and laboratory findings.

    These reagents provide multiparameter, qualitative results for the Cluster of Differentiation (CD) parameters listed below:
    - ClearLLab T1: CD2, CD56, CD7, CD5, CD45
    - ClearLLab T2: CD8, CD4, CD3, CD45
    - ClearLLab B1: Kappa, Lambda, CD19, CD5, CD45
    - ClearLLab B2: CD20, CD10, CD19, CD38, CD45
    - ClearLLab M: CD7, CD13, CD34, CD33, CD45

2. Special conditions for use statement(s):

    For prescription use only.

    For *in vitro* diagnostic use.

3. Special instrument requirements:

    Beckman Coulter FC500 flow cytometer, CXP Software

I. Device Description:

The ClearLLab Reagents panel is used with the following reagents with their respective indicated uses. A description of the reagents provided is described below in Table 1.

Table 1: Components of the ClearLLab Reagents

Item	Description	Use
The T-Cell Lineage panels	Comprising T1 CD2-FITC/CD56-PE/CD7-ECD/CD5-PC5.5/CD45-PC7 and T2 CD8-FITC/CD4-PE/CD3-PC5.5/CD45-PC7 Monoclonal Antibody Reagents.	For use to identify lymphocytes that contain the specific cell surface markers associated with the T-cell lineage.
The B-Cell Lineage panels	Comprising B1 Kappa-	For use to identify lymphocytes

	FITC/Lambda-PE/CD19-ECD/CD5-PC5.5/CD45-PC7 and B2 CD20-FITC/CD10-PE/CD19-ECD/CD38-PC5.5/CD45-PC7 Monoclonal and Polyclonal Antibody Reagents.	that contain the specific cell surface markers associated with the B-cell lineage.
The Myeloid Lineage panel	Comprising M CD7-FITC/ CD13-PE/ CD34-ECD/ /CD33-PC5.5/CD45-PC7 Monoclonal Antibody Reagents.	For use to identify lymphocytes that contain the specific cell surface markers associated with the Myeloid lineage.
Accessory Reagents Required		
Flow-Check Pro Fluorospheres	Flow-Check Pro Fluorospheres are a suspension of fluorescent microspheres used for daily verification of the optical alignment and fluidics for Forward Scatter (FS) and FL1-FL4 fluorescence parameters.	Instrument Alignment Quality Control Reagent to provide instrument alignment Quality Control instructions.
Flow-Set Pro Fluorospheres	Flow-Set Pro Fluorospheres are a suspension of fluorescent microspheres used as an aid in standardizing forward scatter, side scatter, and fluorescence detectors on the FC 500 (FL1-FL5).	Auto Setup Reagent for standardization of flow cytometer light scatter and fluorescence intensity instrument settings to provide application-specific instrument target ranges for standardization.
QuickComp 4 Kit	The QuickComp 4 Kit consists of four single-color fluorescent reagents comprised of one monoclonal antibody each. Each antibody is labeled with one of four fluorochromes, three are utilized for this test: CD45-FITC, CD45-PE, and CD45-ECD.	The QuickComp 4 Kit is used to adjust color compensation settings on a flow cytometer equipped with AutoSetup software, prior to multi-color analysis with FITC, PE, and ECD conjugated monoclonal antibody reagents.
Color Compensation Reagents	Compensation reagents CD45-PC5.5 and CD45-PC7.	Used to adjust color compensation settings on a flow cytometer with AutoSetup software. Note: Color Compensation Reagents are required. It is recommended to use the reagents with normal whole blood specimens to adjust color compensation settings on a flow cytometer, prior to multi-color analysis.

IOTest 3 Fixative Solution	The IOTest 3 Fixative Solution, which has a formaldehyde base, specially developed for the fixing of leukocytes.	Leukocyte fixative solution used on all leukocytic preparations to enable leukocytic preparations to be stored for several hours without deterioration after staining with a fluorescent antibody. It is used to fix leukocytes following immunofluorescent staining with the fluorochrome-conjugated antibodies and lysis of the red blood cells.
VersaLyse Lysing Solution	Red cell lysing reagent intended for the lysis of red blood cells in the preparation of biological samples for flow cytometry analysis.	VersaLyse is intended for the lysis of red blood cells in the preparation of biological samples for flow cytometry analysis.
Accessory Reagents Recommended but Not Provided		
Immutrol Control Cells	Process controls for flow cytometry.	Quality control material assayed for lymphocyte, granulocyte and monocyte specific antigens and single platform absolute counts. The light scatter, population distribution, fluorescence intensity, and antigen density mimic those of normal whole blood.
StemTrol Control cells	An antibody-to-antigen positive control for CD34 and CD45 staining in flow cytometry studies, consisting of a preserved cell line, BK010044, that expresses CD34.	Quality control material for CD34 and CD45.

ClearLLab Immunophenotyping Panel

Lineage	FITC	PE	ECD	PC5.5	PC7
T-cell tube 1 (T1)	CD2	CD56	CD7	CD5	CD45
T-cell tube 2 (T2)	CD8	CD4		CD3	CD45
B-cell tube 1 (B1)	Kappa	Lambda	CD19	CD5	CD45
B-cell tube 2 (B2)	CD20	CD10	CD19	CD38	CD45
Myeloid (M)	CD7	CD13	CD34	CD33	CD45

J. Standards/Guidance Documents Referenced:

CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures, A Statistical Approach

CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory

CLSI H26-A2, Validation, Verification, and Quality Assurance of Automated Hematology Analyzers

CLSI EP17-A2, Protocols for Determination of Limits of Detection and Limits of Quantitation

CLSI EP25-A, Evaluation of Stability on In Vitro Diagnostic Reagents

CLSI EP09-A3 Measurement Procedure Comparison and Bias Estimation Using Patient Samples

CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance

CLSI H43-A2, Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells

K. Test Principle:

Beckman Coulter's ClearLLab product is comprised of pre-cocktailed, lineage-driven combinations of the consensus Cluster of Differentiation (CD) reagents for evaluation of B and T cell neoplasia and the consensus limited CD reagents for myelomonocytic neoplasia as described in Table 3 of "2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry: Optimal Reagents and Reporting for the Flow Cytometric Diagnosis of Hematopoietic Neoplasia"[1]. The ClearLLab product is intended for use on the FC 500 flow cytometer with instrument set-up performed with Flow-Check Pro Fluorospheres, Flow-Set Pro Fluorospheres, and QuickComp color compensation reagents for alignment, voltage standardization, and compensation.

This test depends on the ability of a monoclonal or polyclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. Specific cell staining is accomplished by incubating specimens prepared for staining with the appropriate antibody reagent. The ClearLLab Reagents are composed of five panels containing four or five monoclonal or polyclonal antibody reagents, each specific for a different cell surface antigen and conjugated to a specific fluorochrome. After sample preparation, the specimens are analyzed on the flow cytometer with manual gating.

The FC 500 flow cytometer running under the control of CXP Software applies the principles of flow cytometry to analyze a whole blood, bone marrow or lymph node sample. Samples are prepared and stained with a monoclonal antibody reagents followed by lysis of red blood cells prior to introduction into the instrument. Cellular populations are identified based on the specific monoclonal and polyclonal antibodies and fluorochromes used in the different panels. Detection of fluorescent antibodies bound to cells utilizes the capability of the FC 500 flow cytometer to

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[1] 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. Wood BL, Arroz M, Barnett D, DiGiuseppe J, Greig B, Kussick SJ, Oldaker T, Shenkin M, Stone E, Wallace P. Cytometry B Clin Cytom. 2007; 72 Suppl 1:S14-22

detect fluorescence with one laser, a blue laser with a 488 nm excitation and five different detection channels (FL1-FL5).

The CXP SYSTEM application software (K030828) provides automated instrument setup of light scatter and fluorescence intensity standardization, color compensation, and verification when used with the quality control reagents. The CXP Software has an Auto-Set Panel which automatically standardizes the cytometer, adjusts compensation settings, passes cytometer settings to designated test protocols, and verifies cytometer setup and antibody performance. Compensation settings are determined using CD45-FITC, CD45-PE, and CD45-ECD supplied in the QuickComp 4 Kit and CD45-PC5.5 and CD45-PC7 single color reagents. The existing FC 500 CXP software will support the ClearLLab reagents for use on the FC 500 flow cytometer without any further modifications from the original clearance in K030828.

Sample Preparation & Analysis

Whole Blood (WB) and Bone Marrow (BM) preparation:
Specimens are prepared manually with three pre-wash steps using phosphate buffered saline (PBS) containing 2% Heat Inactivated Fetal Calf Serum (HI-FCS). The remaining white blood cell pellet is then resuspended in PBS containing HI-FCS. The washed specimen is stained with the ClearLLab 5-Color Reagents. VersaLyse Lysing Solution for lysing red blood cells and the IOTest 3 Fixative Solution are used for red blood cell lysis and fixation prior to analysis.

Lymph Node preparation:
Lymph node specimens require disaggregation of the tissue into a single cell suspension. Single cell suspensions prepared from lymphoid tissues may not require washing prior to staining if the specimen was washed during the disaggregation process. If washing steps were not performed for removal of residual soluble proteins, or if the cells were re-suspended in a buffer containing human serum or serum proteins, then pre-washing is necessary.

Sample analysis:
White blood cells (WBCs) are analyzed on an FC 500 flow cytometer. Data are analyzed using a sequential gating strategy. The total WBC gate is defined in the first histogram (Side Scatter vs. CD45-PC7) by the inclusion of all CD45+ events with low, medium and high Side Scatter (SSC). A second histogram defines specific leukocyte subsets such as lymphocytes, monocytes, and granulocytes. The low SSC/bright CD45+ population identifies lymphocytes. Applicable markers are then displayed in subsequent histograms gated on lymphocytes. This process is repeated for mid SSC/medium CD45+ populations to identify monocytes and for high SSC/medium CD45+ to identify granulocytes.

L. **Interpretation of Results**

Flow cytometric immunophenotyping is multiparametric and relies on simultaneous identification and characterization of both normal and candidate malignant cell populations. Normal cells, which vary in type and relative numbers depending on the sample type, patient age, and clinical setting, display highly conserved and reproducible patterns of cell surface marker expression and light scatter. These normal internal control patterns may serve as both process controls for sample type, sample degradation, sample preparation and staining, data acquisition, and data analysis as well as references against which candidate malignant populations may be compared. Candidate malignant populations may display aberrant loss of a marker, aberrant gain of a marker, or aberrant over- or under-expression of a marker. Any population that does not conform to the normal internal control pattern is evaluated for

malignancy. Interpretation of these patterns requires expert knowledge and training in the technique. It is not possible to predict the phenotype of a particular patient's hematolymphoid malignancy and apply fixed templates for samples for detection of specific phenotypes. Even well-characterized diseases such as chronic lymphocytic leukemia may display case-to-case immunophenotypic variability and it is therefore the role of the expert interpreter to assess the significance of findings and to interpret them together with clinical history, morphological assessment, and other ancillary techniques in order to arrive at a final diagnosis. The device labeling states that interpretation of specimens should be performed by a pathologist or equivalent professional who has the appropriate training. Finally, a small number of rare samples contain no or very few normal cells and require careful evaluation and correlation with other sources of information such as clinical history and other laboratory findings to obtain the appropriate final diagnosis.

M. Performance Characteristics:

All results provided below met the manufacturer's pre-specified acceptance criteria.

1. <u>Analytical Performance:</u>

   a. Precision and Reproducibility: Precision studies were conducted according to the methodology presented in *CLSI EP05-A3*, *Evaluation of Precision Performance of Quantitative Measurement Procedures; Approved Guideline – Third Edition*.

      i. *Precision (repeatability):* Thirty-eight specimens across the three specimen types were evaluated. For Whole Blood (WB) and Bone Marrow (BM), testing was conducted at three sites, with each of the three sites using a different anticoagulant according to their current clinical testing procedures. For lymph node specimens (LN), testing was conducted at two sites. Since LN specimens are not collected using an anticoagulant, this was not a variable for lymph node specimen analysis.

         A minimum of one specimen for each specimen type and lineage were collected at each site. Lineage is defined by the tubes used, where T lineage is comprised of T1 and T2 tubes, B lineage is comprised of B1 and B2 tubes, and M lineage is comprised of Myeloid tube. Specimens were selected to cover the markers in each lineage tube regardless of the disease. The same specimen was analyzed twice each day with one time point in the morning and one time point in the afternoon. Six sample preparations per time point were made for a total of 12 replicates per specimen per lineage tube.

         A qualitative approach was applied to repeatability assessment where the presence or absence of an abnormal phenotype was reported. An analysis of the % abnormal population, if present, or pre-selected lineage-specific normal populations was assessed for repeatability and reproducibility. The specimens were tested in 12 replicates. The expected result was to obtain the same number of positive or negatives for all replicates. The total actual number positive or negative was divided by the total expected number positive or negative to calculate the % positive or % negative results. The qualitative results for both abnormal and normal populations met 100% agreement for all sites and anticoagulants for expected and actual results with respect to the presence or absence of an abnormal phenotype.

Pre-selected Lineage-Specific Normal Population

ClearLLab Lineage Tube	Cells	Markers	Population of cells Identified
T1 CD2/CD56/CD7/CD5/CD45	T	CD45+ Bright, Low SS CD2+CD5+CD7+ (Partial) CD56- (Few +)	N#1
	NK	CD45+ Bright, Low SS CD2+CD7+CD56+CD5-	N#2
T2 CD8/CD4/CD3/CD45	T Helper	CD45+ Bright, Low SS CD3+CD4+CD8-	N#3
	T Suppressor	CD45+ Bright, Low SS CD3+CD4-CD8+	N#4
B1 KAPPA/LAMBDA/CD19/CD5/CD45	B	CD45+ Bright, Low SS CD19+ CD5-Kappa+ and Lambda+	N#5
B2 CD20/CD10/CD19/CD38/CD45	B	CD45+ Bright, Low SS CD19+CD20+ CD38 Dim/Variable CD10-	N#6
	Mature Neutrophils	CD45+ Dim, High SS CD10+	N#7
M CD7/CD13/CD34/CD33/CD45	Mature Neutrophils	CD45+ Dim, High SS CD7-CD13+CD34-CD33+	N#8
	Monocytes	CD45+ Med, Med SS CD7-CD13+ CD34-CD33+ Bright	N#9

	Total Samples		38
Gender	**Male**		22
	Female		16
Age	**Child (2−12)**	N	1
		Mean	3
	Adolescent (12−21)	N	0
		Mean	0
	Adult (21−65)	N	19
		Mean	53
	Senior (66−89)	N	18
		Mean	77

		Specimen Type	
Site	Anticoagulant	WB	BM
Site 1	ACD	6	6
Site 2	K2EDTA	4	5
Site 3	Heparin	6	7
	Total Samples	16	18

Repeatability for Site 1 using the ClearLLab Immunophenotyping Panel

Site 1 Sample	Population of Cells Identified	Specimen Type	N	Lineage Tube	Mean (%)	Repeatability		Between Runs		Within Specimen	
						SD	%CV	SD	%CV	SD	%CV
7	ABN#1	LN	12	B1	63.40	1.30	2.04	0.78	1.23	1.51	2.39
	ABN#2		12	B1	14.99	1.13	7.55	0.38	2.56	1.20	7.98
10	N#5	WB	12	B1	2.03	0.14	6.74	0	0	0.14	6.74
	ABN		12	B1	61.96	1.32	2.13	0	0	1.32	2.13
13	ABN#1	LN	12	B1	69.32	1.25	1.80	1.96	2.83	2.33	3.36
	ABN#2	LN	12	B2	85.97	0.70	0.81	1.82	2.1	1.95	2.26
14	N#6	BM	12	B2	7.56	0.45	5.92	0.22	2.92	0.50	6.60
	N#7		12	B2	29.15	0.96	3.30	0.58	2.00	1.13	3.86
17	ABN	WB	12	B1	87.49	0.53	0.60	0.86	0.98	1.01	1.15
18	ABN	WB	12	B2	98.10	0.16	0.17	0.02	0.02	0.16	0.17
19	ABN	BM	12	B1	86.24	0.66	0.77	0	0	0.66	0.77
	N#6		12	B2	83.02	0.46	0.55	0.33	0.39	0.56	0.68
	N#7		12	B2	19.56	0.85	4.32	1.08	5.51	1.37	7.01
11	N#1	WB	12	T1	71.28	0.65	0.91	0.23	0.32	0.69	0.97
	N#2		12	T1	11.17	0.51	4.59	0.74	6.64	0.90	8.07
	N#3		12	T2	35.72	0.44	1.23	0.09	0.24	0.45	1.25
	N#4		12	T2	32.75	0.61	1.85	0	0	0.61	1.85
12	N#1	BM	12	T1	76.44	0.83	1.09	2.74	3.58	2.86	3.74
	N#2		12	T1	8.84	0.70	7.90	0	0	0.70	7.90
	N#3		12	T2	41.61	1.24	2.99	1.17	2.82	.71	4.11
	N#4		12	T2	38.51	1.31	3.39	1.89	4.92	2.30	5.97
15	N#1	BM	12	T1	81.19	0.83	1.02	0	0	0.83	1.02
	N#2		12	T1	16.92	0.67	3.96	0	0	0.67	3.96
5	ABN	WB	12	M	84.26	1.01	1.20	2.82	3.35	3.00	3.56
8	ABN	BM	12	M	78.78	1.14	1.44	2.36	3.00	2.62	3.33
9	ABN	BM	12	M	7.06	0.41	5.81	0.71	10.10	0.82	11.65
16	N#8	WB	12	M	47.31	1.98	4.18	1.65	3.49	2.58	5.45
	N#9		12	M	3.69	0.32	8.54	0.25	6.86	0.40	10.95

Repeatability for Site 2 using the ClearLLab Immunophenotyping Panel

Site 2 Sample	Population of Cells Identified	Specimen Type	N	Lineage Tube	Mean (%)	Repeatability		Between Runs		Within Specimen	
						SD	%CV	SD	%CV	SD	%CV
7	N#5	LN	12	B1	25.04	0.71	2.84	0.63	2.53	0.95	3.80
	N#6		12	B2	25.99	0.50	1.92	0.91	3.49	1.04	3.99
	N#7		12	B2	17.14	1.40	8.18	0.60	3.47	1.52	8.89
9	N#5	BM	12	B1	22.40	0.72	3.21	0.19	0.83	0.74	3.31
	N#6		12	B2	4.41	0.27	6.14	0	0	0.27	6.14
	N#7		12	B2	28.04	0.28	1.01	0.29	1.03	0.41	1.45
11	N#5	WB	12	B1	17.63	0.27	1.51	0.10	0.56	0.28	1.61
	N#6		12	B2	18.37	0.32	1.72	0.19	1.02	0.37	2.00
	N#7		12	B2	73.47	0.69	0.93	0.30	0.41	0.75	1.02

Site 2 Sample	Population of Cells Identified	Specimen Type	N	Lineage Tube	Mean (%)	Repeatability SD	Repeatability %CV	Between Runs SD	Between Runs %CV	Within Specimen SD	Within Specimen %CV
12	N#5	LN	12	B1	25.91	0.49	1.90	0.76	2.95	0.91	3.51
	N#6		12	B2	26.36	0.40	1.50	0.60	2.28	0.72	2.73
	N#7		12	B2	5.05	0.38	7.61	0	0	0.38	7.61
1	N#1	BM	12	T1	70.34	0.80	1.14	0.50	0.71	0.94	1.34
	N#2		12	T1	9.83	0.33	3.38	0.55	5.60	0.64	6.54
	N#3		12	T2	33.86	0.51	1.51	0.60	1.77	0.79	2.33
	N#4		12	T2	37.25	0.86	2.30	0	0	0.86	2.30
4	N#1	BM	12	T1	70.37	0.73	1.03	0.48	0.68	0.87	1.24
	N#2		12	T1	8.66	0.57	6.59	0.62	7.15	0.84	9.72
	N#3		12	T2	46.81	0.51	1.10	0.61	1.30	0.80	1.70
	N#4		12	T2	27.61	0.75	2.73	0	0	0.75	2.73
10	ABN	WB	12	T1	7.38	0.16	2.12	0	0	0.16	2.12
3	ABN	BM	12	M	58.53	0.40	0.69	0.17	0.30	0.44	0.75
5	ABN	WB	12	M	3.04	0.15	4.78	0.13	4.34	0.20	6.46
6	ABN	WB	12	M	62.27	0.48	0.77	0.09	0.15	0.49	0.79
8	N#8	BM	12	M	70.44	0.47	0.66	0.37	0.53	0.59	0.84
	N#9		12	M	6.86	0.16	2.27	0.09	1.28	0.18	2.61

Repeatability for Site 3 using the ClearLLab Immunophenotyping Panel

Site 3 Sample	Population of Cells Identified	Specimen Type	N	Lineage Tube	Mean (%)	Repeatability SD	Repeatability %CV	Between Runs SD	Between Runs %CV	Within Specimen SD	Within Specimen %CV
1	ABN	BM	12	B1	33.95	0.93	2.73	1.68	4.94	1.92	5.65
	N#6		12	B2	98.82	0.11	0.11	0.10	0.11	0.15	0.15
	N#7		12	B2	28.17	0.68	2.43	1.02	3.63	1.23	4.37
6	ABN	WB	12	B1	97.16	0.10	0.11	0.04	0.04	0.11	0.12
	N#6		12	B2	99.89	0.02	0.02	0.01	0.01	0.02	0.02
	N#7		12	B2	4.18	0.12	2.85	0	0	0.12	2.85
11	N#5	BM	12	B1	18.40	0.50	2.70	0.37	2.03	0.62	3.37
	N#6		12	B2	78.88	1.82	2.31	4.26	5.40	4.63	5.87
	N#7		12	B2	46.24	0.61	1.31	0.09	0.20	0.61	1.33
12	ABN	BM	12	B1	53.80	0.66	1.23	0.41	0.77	0.78	1.45
	N#6		12	B2	91.62	0.53	0.58	0	0	0.53	0.58
	N#7		12	B2	15.42	0.37	2.43	0.18	1.16	0.42	2.69
15	N#5	WB	12	B1	10.83	0.39	3.59	0	0	0.39	3.59
	N#6		12	B2	99.20	0.21	0.21	0.12	0.12	0.24	0.24
	N#7		12	B2	35.81	1.28	3.58	1.80	5.02	2.21	6.17
2	ABN	WB	12	T1	74.75	1.46	1.95	1.61	2.16	2.18	2.91
3	N#1	BM	12	T1	86.56	0.32	0.37	0	0	0.32	0.37
	N#2		12	T1	1.45	0.09	6.40	0	0	0.09	6.40
	N#3		12	T2	60.52	0.42	0.69	0	0	0.42	0.69
	N#4		12	T2	19.56	0.28	1.41	0	0	0.28	1.41
8	N#1	WB	12	T1	67.56	1.49	2.20	0	0	1.49	2.20
	N#2		12	T1	10.00	0.17	1.69	0.13	1.30	0.21	2.13
	N#3		12	T2	41.41	0.67	1.63	0.18	0.44	0.70	1.68
	N#4		12	T2	20.77	0.49	2.38	0	0	0.49	2.38
9	N#1	BM	12	T1	69.73	1.27	1.83	0.36	0.51	1.32	1.90
	N#2		12	T1	14.46	0.54	3.74	0.49	3.39	0.73	5.05
	N#3		12	T2	43.97	1.01	2.30	0.12	0.28	1.02	2.31
	N#4		12	T2	28.49	0.97	3.41	0	0	0.97	3.41
4	ABN	WB	12	M	60.71	1.36	2.24	0	0	1.36	2.24
5	N#8	BM	12	M	74.08	0.95	1.28	1.14	1.54	1.48	2.00
	N#9		12	M	7.98	0.17	2.10	0.11	1.38	0.20	2.52
7	ABN	BM	12	M	34.34	0.48	1.40	2.17	6.32	2.22	6.48

Site 3	Population of Cells Identified	Specimen Type	N	Lineage Tube	Mean (%)	Repeatability		Between Runs		Within Specimen	
Sample						SD	%CV	SD	%CV	SD	%CV
10	ABN	WB	12	M	42.42	1.30	3.06	0	0	1.30	3.06

Population of Cells
ABN: abnormal population identified in each sample; some samples have more than one abnormal population identified
N: normal population identified based on pre-selected lineage-specific populations (see Table above)

	Positive (abnormal phenotypes)	Negative (normal phenotypes)	Total	% positive (actual/expected)	% negative (actual/expected)
Site 1	11	17	28	100	100
Site 2	4	22	26	100	100
Site 3	7	26	33	100	100

ii. *Operator-to-operator and instrument-to-instrument imprecision:* Seven whole blood specimens with or without abnormal phenotypes to cover all CD markers were evaluated. The same specimen was prepared and analyzed twice in one day by three operators. Each operator made three sample preparations per time point and analyzed the samples on two FC 500 flow cytometers that had successfully passed quality control for a total of 12 determinations per operator. Considering three operators, a total of 36 acquisitions per specimen per ClearLLab reagent tube were collected. A qualitative approach was applied to the assessment of imprecision where the presence or absence of an abnormal phenotype was reported. In all cases, the operators were blinded to the patient's diagnosis. The qualitative results for both abnormal and normal populations met 100% agreement for all operators and instruments for expected and actual results with respect to the presence or absence of an abnormal phenotype. The demographics of the seven samples included six men and one woman between the ages of 21−65 years old.

Results for ClearLLab for all Operators and Instruments Combined

Site 2	Lineage	N	Mean (%)	Repeatability		Between Runs		Between Instruments		Between Operators		Within Specimen	
Sample				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
2	B1	36	88.44	0.45	0.51	0	0	0	0	0.94	1.06	1.04	1.18
	B2	36	88.45	0.39	0.44	0.15	0.18	0	0	0.82	0.92	0.92	1.04
4	B1	36	9.63	0.65	6.77	0.47	4.93	0	0	0.57	5.91	0.99	10.25
	B2	36	9.93	0.39	3.93	0	0	0.13	1.36	0	0	0.41	4.16
	B2	36	66.59	1.76	2.64	1.18	1.78	0	0	0	0	2.12	3.18
5	B1	36	85.30	0.30	0.35	0.17	0.20	0	0	0.14	0.16	0.37	0.44
	B2	36	85.08	0.26	0.31	0.12	0.14	0.07	0.08	0	0	0.29	0.35
7	B1	36	10.43	0.37	3.52	0	0	0	0	0	0	0.37	3.52
	B2	36	10.43	0.28	2.71	0.32	3.05	0	0	0	0	0.43	4.08
	B2	36	59.39	1.04	1.75	0.79	1.32	0	0	0.71	1.20	1.49	2.50
1	T1	36	96.70	0.11	0.11	0	0	0	0	0.12	0.12	0.16	0.16
	T2	36	97.30	0.09	0.09	0	0	0.15	0.15	0	0	0.17	0.18
3	T1	36	73.39	0.53	0.72	0.11	0.15	0	0	0.69	0.95	0.88	1.20
	T1	36	14.18	0.51	3.61	0	0	0.31	2.21	0	0	0.60	4.23
	T2	36	52.38	0.62	1.18	0.11	0.20	0.43	0.82	0	0	0.76	1.45
	T2	36	19.07	0.36	1.87	0	0	0.11	0.59	0.36	1.86	0.52	2.71
1	M	36	96.38	0.12	0.12	0.09	0.09	0	0	0.05	0.05	0.16	0.16
3	M	36	68.65	0.93	1.35	0.45	0.66	0	0	0.85	1.23	1.34	1.95

Site 2 Sample	Lineage	N	Mean (%)	Repeatability		Between Runs		Between Instruments		Between Operators		Within Specimen	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
	M	36	7.81	0.27	3.45	0	0	0.23	2.94	0.19	2.42	0.40	5.13
5	M	36	64.05	0.41	0.64	0.31	0.48	0.43	0.67	0	0	0.67	1.04

	Positive (abnormal phenotypes)	Negative (normal phenotypes)	Total	% positive (actual/expected)	% negative (actual/expected)
Site 1 LHSC	40	60	100	100	100

iii. *Site-to-Site Reproducibility with Control Material*: One level of control material (IMMUNO-TROL Cells (IT)) was prepared in duplicate with the ClearLLab reagents and analyzed twice each day for 20 days at four sites with each site using one FC 500 flow cytometer, generating a total of 80 replicates per ClearLLab reagent tube per site for an overall total of 320 replicates per ClearLLab reagent lineage tube. Eight replicates were excluded due to QC failures. An additional nine replicates were excluded from the CD34 analysis in the Myeloid Lineage tube due to CD34 pipetting and washing error, resulting in a total exclusion of 17 replicates from the overall study. To assess the precision of percent positive parameter measured for CD34-ECD marker the experiments were performed using one level of control product (Immuno-Trol) with Stem-Trol Control Cells (a preserved cell line that expresses CD34, BK010044) added.

Reproducibility with Control Material

Lineage	Pop. **	Marker	N	Mean (%)	Repeatability		Between Runs		Between Days		Between Sites		Reproducibility	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
B-cell Lineage Tube 1	LY+	CD19+ Kappa+ CD19+LY	312	57.38	1.60	2.78	1.92	3.35	1.08	1.88	1.34	2.33	3.03	5.29
		CD19+ Lambda+ CD19+LY	312	40.29	1.50	3.71	1.67	4.16	0	0	0.48	1.20	2.30	5.70
		CD19+	312	14.23	0.37	2.63	0.39	2.73	0	0	0.67	4.74	0.86	6.07
		CD5+	312	71.83	0.61	0.85	0.70	0.98	0.79	1.10	2.05	2.86	2.39	3.33
B-cell Lineage Tube 2	GR+	CD10+	312	99.96	0.02	0.02	0.07	0.07	0.01	0.01	0.03	0.03	0.08	0.08
	LY+	CD19+	312	14.36	0.37	2.58	0.29	2.04	0.16	1.14	0.66	4.58	0.83	5.75
		CD20+	312	14.82	0.43	2.92	0.51	3.47	0.60	4.07	1.22	8.23	1.52	10.24
	MO+	CD38+	312	94.57	0.66	0.70	0.91	0.96	0.78	0.83	4.92	5.20	5.11	5.40
T-cell Lineage Tube 1	LY+	CD2+	312	81.75	0.65	0.79	0.78	0.95	0.66	0.81	1.13	1.38	1.66	2.03
		CD5+	312	72.71	0.63	0.87	0.79	1.09	0.32	0.44	0.96	1.32	1.73	1.97
		CD56+	312	13.76	0.46	3.32	0.97	7.07	0	0	0.18	1.31	1.09	7.92
		CD7+	312	74.59	0.65	0.87	0.77	1.03	0.73	0.97	1.73	2.33	2.13	2.86
T-cell Lineage Tube 2	LY+	CD3+CD4+	312	45.47	0.55	1.22	0.49	1.07	0.19	0.42	0.37	0.82	0.85	1.87
		CD3+CD8+	312	22.67	0.43	1.89	0.26	1.16	0.19	0.82	0.29	1.27	0.61	2.68
		CD3+	312	71.14	0.54	0.76	0.64	0.90	0.23	0.32	0.62	0.87	1.07	1.50
Myeloid Lineage	GR+	CD13+	312	99.92	0.40	0.40	0	0	0.27	0.27	0.16	0.16	0.50	0.50
	LY+	CD7+	312	73.91	0.70	0.95	1.74	2.35	2.22	3.01	4.17	5.64	5.08	6.88
	MO+	CD33+	312	95.53	0.89	0.93	0.82	0.85	2.11	2.20	3.82	4.00	4.53	4.74
	Stem Trol+	CD34+	303	11.77	0.78	6.65	0.97	8.23	0.55	4.70	1.59	13.53	2.09	17.80*

**Population measured (Pop.)

LY+: Lymphocyte; GR+: Granulocyte; MO+: Monocyte; StemTrol: StemTrol cells

*Stemtrol Cells for CD34 measurements: The repeatability of the CD34 measurement included two additional unique variability sources. One of them was the variability contributed by the pipetting of the Stem-Trol reagent needed for staining. The other source was associated with an additional washing and decanting step. These additional sources of variability contribute to the increased imprecision.

iv. **ClearLLab Reagents Lot-to-Lot Reproducibility:** Lot-to-lot variability was evaluated using normal whole blood specimens. For the CD34 marker, which is not expressed in normal specimens, a specific cell line (Stem-Trol) was spiked in the specimens. Three normal whole blood specimens were tested in triplicate with three lots of each ClearLLab reagent. Mean Fluorescence Intensity (MFI) differences of the conjugates and percent positive cell recovery for each cell lineage population were evaluated between lots and all demonstrated acceptable lot to lot variability performance.

Normal population analyzed in each ClearLLab reagent

ClearLLab Reagents	Cells	Markers
T1 Lineage Tube CD2/CD56/CD7/CD5/CD45	NK	CD45bright CD2+CD7+ CD5-CD56+
	T	CD45bright CD2+CD7+ CD5+CD56-
T2 Lineage Tube CD8/CD4/-/CD3/CD45	T Helper	CD45bright CD3+CD4+
	T Suppressor	CD45bright CD3+CD8+
B1 Lineage Tube Kappa/Lambda/CD19/CD5/CD45	B#1	CD45bright CD19+CD5-Kappa+
	B#2	CD45bright CD19+CD5-Lambda+
B2 Lineage Tube CD20/CD10/CD19/CD38/CD45	B#3	CD45bright CD19+CD20+ CD38dim/variable CD10-
	Mature Granulocytes	CD10+
M Lineage Tube CD7/CD13/CD34/CD33/CD45	Mature Neutrophils	CD45+ Dim, High SS CD7-CD13+CD34-CD33+
	Monocytes	CD45+ Med, Med SS CD7-CD13+ CD34-CD33+ Bright

Normal population % positive recovery on Sample 1

Sample 1	N	Mean	Repeatability		Between Lots		Within Sample	
			SD	%CV	SD	%CV	SD	%CV
NK	9	2.58	0.13	5.16	0.00	0.00	0.13	5.16
T	9	95.79	0.11	0.12	0.00	0.00	0.11	0.12
T Helper	9	46.68	0.25	0.54	0.00	0.00	0.25	0.54
T Suppressor	9	41.14	0.36	0.88	0.00	0.00	0.36	0.88
B #1	9	62.91	0.46	0.73	0.50	0.79	0.68	1.08
B #2	9	37.09	0.47	1.28	0.46	1.23	0.66	1.77
B #3	9	66.24	0.72	1.08	0.36	0.54	0.80	1.21
Mature Granulocytes	9	56.40	1.61	2.86	0.60	1.06	1.72	3.05
Mature Neutrophils	9	58.70	1.35	2.30	0.00	0.00	1.35	2.30
Monocytes	9	2.49	0.05	1.84	0.04	1.72	0.06	2.52

Normal population % positive recovery on Sample 2

Sample 2	N	Mean	Repeatability		Between Lots		Within Sample	
			SD	%CV	SD	%CV	SD	%CV
NK	9	8.58	0.30	3.45	0.26	3.00	0.39	4.57
T	9	88.26	0.29	0.33	0.00	0.00	0.29	0.33
T Helper	9	70.56	0.39	0.55	0.57	0.80	0.69	0.98
T Suppressor	9	23.59	0.48	2.02	0.51	2.18	0.70	2.97
B #1	9	64.67	2.20	3.41	0.62	0.96	2.29	3.54
B #2	9	35.36	2.27	6.43	0.65	1.82	2.36	6.68
B #3	9	59.28	2.24	3.78	0.76	1.29	2.37	4.00
Mature Granulocytes	9	68.03	1.13	1.66	0.74	1.09	1.35	1.99
Mature Neutrophils	9	70.37	2.31	3.28	0.00	0.00	2.31	3.28
Monocytes	9	4.30	0.32	7.37	0.00	0.00	0.32	7.37

Normal population % positive recovery on Sample 3

Sample 3	N	Mean	Repeatability		Between Lots		Within Sample	
			SD	%CV	SD	%CV	SD	%CV
NK	9	13.30	0.26	1.93	0.23	1.76	0.35	2.61
T	9	80.68	0.31	0.38	0.00	0.00	0.31	0.38
T Helper	9	64.99	0.35	0.54	0.50	0.77	0.61	0.94
T Suppressor	9	29.63	0.28	0.96	0.37	1.26	0.47	1.59
B #1	9	57.65	1.64	2.85	0.00	0.00	1.64	2.85
B #2	9	42.20	1.68	3.98	0.00	0.00	1.68	3.98
B #3	9	72.65	1.48	2.04	1.14	1.57	1.87	2.57
Mature Granulocytes	9	63.57	1.55	2.44	0.43	0.67	1.61	2.53
Mature Neutrophils	9	58.29	1.05	1.80	0.00	0.00	1.05	1.80
Monocytes	9	5.02	0.21	4.19	0.05	0.97	0.22	4.30

     v. **Reagents Required as listed in Table 1 in Section I above:** All tests performed for the reagents listed in Table 1 above for reproducibility and stability were acceptable.

b. Linearity/Reportable Range:

Instrument Linearity : A study was performed for instrument linearity to evaluate the linearity and dynamic range of the FC500 flow cytometer's acquisition system, from the photomultiplier tubes (PMT) to the electronics, independent of the application and gating methodology. The FC 500 flow cytometer's acquisition system demonstrated linear results independent of the application and gating methodology.

c. Detection Limit: The study was used to establish the detection capability, i.e., the ability to differentiate between abnormal and normal populations, of the ClearLLab reagents.

    i. Limit of Blank (LoB):

The LoB was determined by testing five replicates of three normal specimens run with two lots of each ClearLLab reagent (B1, B2, T1, T2 and M). The highest LoB

observed from the two lots was 0.27% population of abnormal cells.

ii. <u>Limit of Detection (LoD):</u>

The LoD was determined using one representative clinical specimen collected in K2 or K3 EDTA for each ClearLLab reagent. Each clinical specimen was spiked into a normal specimen to target low levels of abnormal population as a percentage of the total percentage of leukocytes in the sample. The percentages used were 2%, 1% and 0.5% of abnormal cells in the total population of white cells measured.

The Limit of Detection defined as 1% population of abnormal cells for the ClearLLab application is therefore confirmed.

d. Analytical Specificity:

i. <u>Interfering Substances:</u>

Not applicable. Adequate washing with phosphate buffered saline (PBS) before staining with reagents removes residual plasma and interfering substances.

ii. <u>Assay Carryover:</u>

Carryover studies were conducted on three FC 500 flow cytometry systems according to the methodology presented in *CLSI H26-A2: Validation, Verification, and Quality Assurance of Automated Hematology analyzers; Approved Standard-Second Edition, Section 5.7* with regard to high or low value sample order. Two studies were conducted to evaluate specimen and reagent carryover.

<u>Specimen Carryover</u>

Three samples with high WBC counts (HTv1, HTv2, HTv3) were prepared from one clinical whole blood specimen containing a high level of WBCs. The samples were prepared to target 20,000 cells /µL WBCs. CD34 marker analysis was performed using a CD34+ clinical whole blood specimen.

Three samples with low WBC counts (LTv1, LTv2, LTv3) were prepared by spiking a diluted (1:10,000) whole blood specimen with a red blood cell pool which has been depleted of white blood cells and diluted 4:1 with Immuno-Trol storage buffer. This resulted in a contrived specimen that had a red blood cell concentration similar to whole blood with very low concentrations of white blood cells.

The reagent selected for specimen carryover was the ClearLLab M reagent. M reagent was chosen as a representative reagent since it contains all the different types of fluorochrome conjugates used in the device (FITC, PE, ECD, PC5.5 and PC7) as well as markers that allow identification and assessment of recovery of all leukocyte populations (lymphocytes, monocytes and granulocytes). Testing with M reagent also assesses the impact of lysing and fixative reagents on integrity of cell membranes for all relevant cell subsets and the affinity of various conjugated antibodies that is representative of all ClearLLab reagents.

Three HTv samples (HTv1, HTv2, HTv3) were analyzed consecutively followed immediately by the three LTv samples (LTv1, LTv2, LTv3). This was repeated three

times on three FC 500 flow cytometers for a total of nine specimen carryover runs. No significant carryover was detected as determined by percent carryover for specimen and reagent calculated from the analysis of the averages of the three runs on each flow cytometer for LTv1, LTv3, and HTv3. The specimen carryover is less than 1% for all markers.

<u>Reagent Carryover</u>

High concentration samples (HTv1, HTv2, HTv3): Normal whole blood specimens or clinical specimen (for CD34 measurement) stained with ClearLLab reagents were used as the high concentration samples.

Low concentration samples (LTv1, LTv2, LTv3): The same normal blood specimen or clinical whole blood as in the high concentration sample was processed in triplicate using antibody dilution buffer (RD1), in place of antibody reagent.

All the ClearLLab reagents (B1, B2, T1, T2 and M reagents) were selected for the reagent carryover studies. The potential for carryover of these products and the subsequent impact on performance were assessed for FS, SS, and FL1-FL5, in all seven of the detection channels.

The testing consisted of a high concentration sample analyzed consecutively three times (HTv1, HTv2, HTv3) followed immediately by testing the low concentration sample three times (LTv1, LTv2, LTv3). This run was repeated three times on three FC 500 flow cytometers for a total of nine reagent carryover runs.

The reagent carryover is less than 1% for all markers demonstrating acceptable scatter and fluorescent carryover performance.

e. Traceability and Stability (Reagent and Specimen):

i. <u>Traceability:</u>

For monoclonal antibodies, Leukocyte Typing Workshop information for each antibody clone was provided. Each clone identity is controlled and is traced back to the original source. Polyclonal antibodies for identification of Kappa or Lambda cell markers are supported by reference literature.

#	Marker	Clone/Ig Chain (species)	Leukocyte Typing International Workshop*	Reactivity
1	CD2	39C1.5 IgG2a (rat)	Second	T cells and most of the NK cells
2	CD3	UCHT1 IgG1 (mouse)	First	Mature T cell (cytoplasmic expression in immature T cells)
3	CD4	SFCI12T4D11 IgG1 (mouse)	First	Helper / inducer T cells, monocytes, immature myeloid

4	CD5	BL1a IgG2a (mouse)	Third	Thymocytes, mature T cells, subpopulation of B cells
5	CD7	8H8.1 IgG2a (mouse)	Second	T cells, NK cells, subpopulation of immature myeloid cells
6	CD8	SFCI21Thy2D3 (T8) IgG1 (mouse)	Second	Cytotoxic / suppressor T cells, subpopulation of NK cells
7	CD10	ALB1 IgG1 (mouse)	Third	Common acute leukemia antigen (CALLA), lymphatic precursor cells, neutrophils, subpopulation of mature B cells
8	CD13	SJ1D1 366 IgG1 (mouse)	Third	Mature and immature myeloid cells
9	CD19	J3-119 IgG1 (mouse)	Fourth	Precursor and mature B cells
10	CD20	B9E9 (HRC20) IgG2a (mouse)	Fifth	B cells and a subpopulation of B precursor cells
11	CD33	D3HL60.251 IgG1 (mouse)	Fifth	Monocytes, myeloid precursor cells, weak on neutrophils
12	CD34	581 IgG1 (mouse)	Fifth	Myeloid and lymphoid precursor cells
13	CD38	LS198-4-3 IgG1 (mouse)	Fifth	Activated lymphocytes, subpopulation of B cells, plasma cells
14	CD45	J.33 IgG1 (mouse)	Third	All leukocytes
15	CD56	N901/NKH-1 IgG1 (mouse)	Second	NK cell subset and activated T-cells
16	Kappa	N/A: Polyclonal	N/A	Subpopulation of immature B Lymphocytes, subpopulation of mature B cells
17	Lambda	N/A: Polyclonal	N/A	Subpopulation of immature B Lymphocytes, subpopulation of mature B cells

*Human Leukocyte Differentiation antigens Workshops		CD2 assigned	Number of CDs assigned
I First	Paris 1982[2]	CD1-CDw15	15
II Second	Boston 1984[3]	CD16-CDw26	11
III Third	Oxford 1987[4]	CD27-CD45	19
IV Fourth	Vienna 1989[5]	CD46-CDw78	33
V Fifth	Boston 1993[6]	CD79-CDw109	31

ii. <u>Reagent Stability:</u>

Testing of reagent stability was based on *CLSI EP-25A (Evaluation of Stability of In vitro Diagnostic Reagents, Approved Guideline*. Three lots of each ClearLLab reagent were evaluated for real-time open- and closed-vial stability. This study evaluated recovery and percent positive cells for each marker of the different lineage reagents (T1/T cell Lineage Markers (TL) 1, T2/TL2, B1/B cell Lineage markers (BL) 1, B2/BL2, M/Myeloid Lineage markers). Closed-vial stability testing was performed with stabilized control cells: Immuno-Trol and Immuno-Trol spiked with Stem-Trol to assess CD34 marker expression. Immuno-Trol Control Cells were used as a standardized assay system to enable traceability throughout the length of the study. Open-vial stability testing was performed with normal whole blood samples spiked with Stem-Trol cell line to assess the CD34+ cell population. Open-vial testing was performed at six time points with ClearLLab reagents. Percent difference from Time zero percent positive are measured for each marker of the different lineages tubes. The open- and closed-vial stability claims at refrigerated temperature (2–8°C) conditions are 90 and 365 days respectively.

iii. <u>Whole Blood and Bone Marrow Specimen Stability:</u>

A total of 45 clinical specimens consisting of 10 bone marrow specimens at each of three sites per anticoagulant (n = 30) and five whole blood specimens at each of three sites per anticoagulant (n = 15) were collected with different anticoagulants. Specimens provided from Site 1 were collected in ACD, from Site 2 were collected in Heparin, and from Site 3 were collected in K2EDTA. Each specimen was stained with the ClearLLab reagents for the B-cell Lineage Tube 1 and 2, T-cell Lineage Tube 1 and 2 and Myeloid Lineage reagent panels. Specimens were held at room temperature for the indicated time and prepared and analyzed at the following target time points post specimen collection: T0, T28, and T52 hours. The study examined the combined effect

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[2] Bernard AR, Boumsell L, Dausset J, *et al*. eds. Leucocyte Typing: Human Leucocyte Differentiation Antigens Detected by Monoclonal antibodies. Berlin: Springer-Verlag, 1984.
[3] Reinherz EL, Haynes BF, Nadler L, Bernstein ID, eds. Leukocyte Typing II. New York: Springer-Verlag, 1985.
[4] McMichael AJ, Beverley PCL, Cobbold S, *et al*. eds. Leucocyte Typing III. White Cell Differentiation Antigens. Oxford: Oxford University Press, 1987.
[5] Knapp W, Dorken B, Gilks W *et al*., eds. Leucocyte Typing IV. Oxford: Oxford University Press, 1989.
[6] Schlossman SF, Boumsell L, Gilks W, *et al*. eds. Leucocyte Typing V: White cell differentiation antigens. Oxford: Oxford University Press, 1995.

of specimen and prepared sample stability drift of ClearLLab reagents' CD marker panel percent positive recoveries. The results at room temperature (RT, 20−25°C) are presented in the table below:

Anticoagulant	Whole Blood		Bone Marrow	
	Specimen stability C(RT)	Prepared Sample stability	Specimen stability C(RT)	Prepared Sample stability
EDTA	24 hours	< 3 hours	24 hours	< 3 hours
ACD	48 hours	< 3 hours	48 hours	< 3 hours
Heparin	48 hours	< 3 hours	48 hours	< 3 hours

C(RT): Condition (Room Temperature 20−25°C)

iv. <u>Specimen Stability (Lymph Node)</u>:

A total of twelve clinical specimens consisting of four lymph node specimens per site were collected at three external sites. The prepared sample stability was assessed for the applicable markers CD19+, CD19+Kappa+, CD19+Lambda+, CD5+, CD20+, CD19+CD38+, CD7+, CD2+, CD56+, CD3+CD4+,CD3+CD8+, and CD3+. These lymphocyte-specific CD markers are detected on lymph node specimen types. Specimens were prepared and analyzed in single replicate on a total of three FC 500 flow cytometers. Specimens were stained with the ClearLLab Reagents, analyzed within 3 hours of preparation, stored at 2–8°C protected from light, and reanalyzed within 24 hours of preparation. Analysis was performed at the following target time points post preparation: T0 and T24 hours. The study examined the combined effect of specimen and prepared sample stability drift of ClearLLab reagents' CD marker panel percent positive recoveries. The data supports the use of prepared samples from lymph node specimens up to 24 hours post specimen collection under refrigerated (2–8°C) conditions. Current standard lab practice allows use of specimens without a time restriction if cell viability permits. Labeling will reflect continuation of this practice.

f. Matrix comparison: Testing of anticoagulant equivalency was based on *CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition.*

<u>Whole Blood Samples:</u> The study evaluated ClearLLab reagents' (B1, B2, T1, T2, and M) parameter recovery for normal whole blood specimens collected in different anticoagulants (K2EDTA, K3EDTA, Heparin, and ACD). Forty six normal whole blood samples were collected in four different anticoagulants and processed. One replicate per donor and per anticoagulant was analyzed. Direct comparison of parameter recovery of the percent positive for each marker of the different lineage reagents for the same specimens collected into four different anticoagulants was performed. One lot of each of the five panels of ClearLLab reagents B1, B2, T1, T2 and M were used. CD34 marker analysis was performed using normal whole blood specimens spiked with Stem-Trol Control Cells. The performance of ClearLLab reagents with whole blood specimens collected in K2EDTA, ACD, Heparin and K3EDTA anticoagulants was demonstrated to be equivalent.

Whole Blood and Bone Marrow: A total of 204 specimens including 35 whole blood specimens with normal hematology CBC/Diff and 169 specimens with abnormal hematology CBC/Diff were tested. This multi-center anticoagulant study for whole blood and bone marrow was conducted across four sites covering hematopoietic malignancies as well as those with hematological abnormalities but no malignancy. Peripheral blood and bone marrow collected in K2EDTA were analyzed within 24 hours. Specimens collected in ACD and Sodium Heparin were analyzed within 48 hours. Specimens were also stained with reference/comparator reagents containing previously FDA cleared CD marker assays. Markers with a direct comparator were used to determine the performance between anticoagulants in bone marrow and whole blood. Equivalent performance was demonstrated between different anticoagulants for both whole blood and bone marrow specimens.

2. Clinical Performance:

A multi-center, retrospective study was conducted at four sites comparing the diagnostic accuracy of the ClearLLab reagents to detect the presence or absence of an abnormal phenotype to the clinical outcome of "malignant" or "non-malignant" based on the clinical sites' final patient diagnosis. Patients with abnormal B-cell, T-cell, Natural Killer (NK) cell, and myeloid cell populations were tested. Residual samples were tested from a diverse population of patients covering hematopoietic malignancies as well as those with hematological abnormalities but no malignancy. This study included whole blood, bone marrow and lymph node specimen types. Specimen types tested reflected the distribution of the diseases and /or clinical indications encountered in the population of patients that are routinely encountered in evaluations of patients suspected of having hematopoietic neoplasia by flow cytometry. Consecutive specimens evaluated for flow cytometric immunophenotyping of leukemia and lymphoma and meeting the target disease categories were enrolled at these sites.

The study was designed to combine data from four sites to get an adequate representation of diseases. The specimen mix of the abnormal specimens consisted of peripheral blood (approximately 60%), bone marrow (approximately 30%) and lymph nodes (approximately 10%) for both hematopoietic malignant and nonmalignant categories. The samples consisted of a distribution of approximately 50% hematologically abnormal with no malignancy and approximately 50% malignant.

Clinical sites provided final diagnosis ("malignant" or "non-malignant") for all subjects and all specimen types based on the clinical testing performed at the respective sites regardless of the specimen type sent to the flow cytometry laboratory for testing as per WHO guidelines.[7] Two qualified flow experts evaluated the ClearLLab flow cytometry results independently of each other and were blinded to the final diagnosis. Each flow expert was asked to evaluate the ClearLLab results for the presence or absence of abnormal cell populations and provide the percentage of abnormal population as well as the phenotype associated with the abnormal population. The result was designated as "malignant" if an abnormal population was identified, and "non-malignant" if an abnormal population was not identified.

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[7] Swerdlow, S, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues 4th Edition. . Lyon: International Agency for Research on Cancer, 2008.

The sample size was determined based on the approach recommended by Burderer.[8] For this study the assumption was prevalence =1 (design was not prevalence driven). The target outcome for all specimen types that were identified as positive by flow cytometry was 80%±10% agreement with the clinical diagnosis and for all specimen types that were identified as negative by flow cytometry was 90%±10%. For whole blood specimens only, the target outcomes for both positive and negative was 90%±10%.

A total of 279 abnormal hematologic specimens were enrolled: 137 hematologically abnormal but no malignancy and 142 with hematolymphoid malignancy per site's final diagnosis. The demographic information regarding the 279 subjects is shown in the graphic below.

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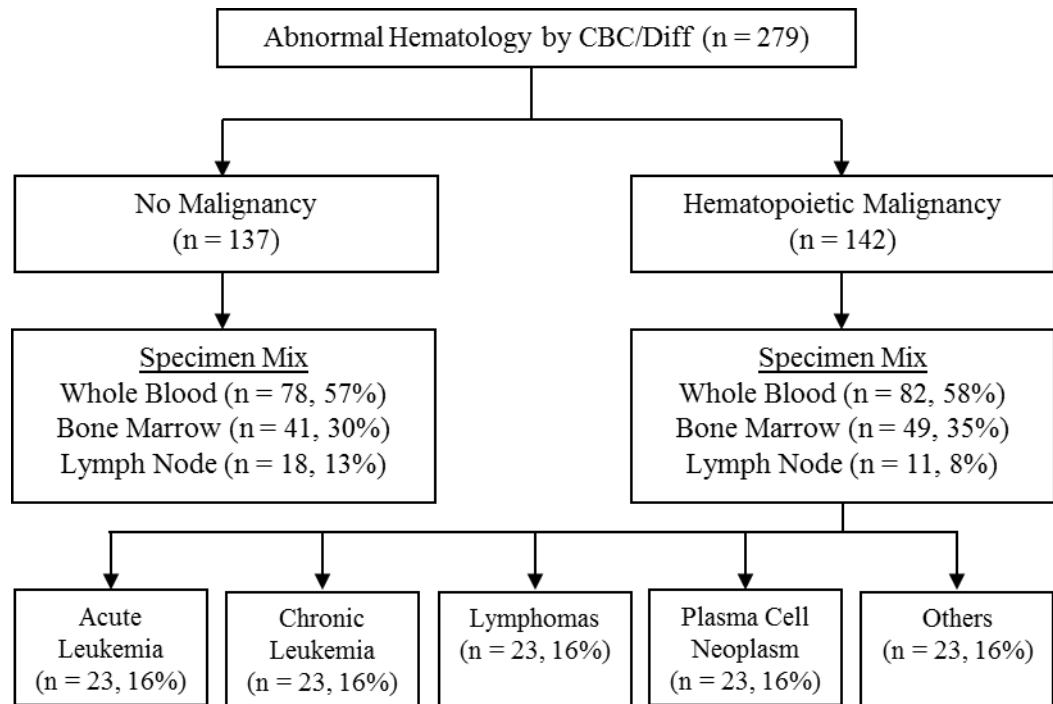
[8] Statistical methodology. I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. NM, Burderer. s.l. : Acad. Emerg. Med, 1996, Vols. 3(9): 895-900.

Number of Samples in Disease categories from each site

Sites	Category						Total Number of Samples per Site
	Abnormal Hematology -No Malignancy	Abnormal Hematology- Malignancy				Others: MDS MPN	
		Acute Leukemia	Chronic Leukemia	Lymphoma	Plasma Cell Neoplasm		
Site 1	24	4	10	19	4	1	62
Site 2	29	6	3	11	3	2	54
Site 3	42	6	15	12	0	6	81
Site 4	42	7	8	20	1	4	82
Total Number per Category	137	23	36	62	8	13	279

Statistical analysis results for sensitivity, specificity, PPV, and NPV for the two experts are presented below. The agreements between the two experts were also calculated.

Agreement for Presence (Malignant) or Absence (Non-Malignant) of Abnormal Cell Populations for All Specimen Types for Flow Expert #1

ClearLLab Reagents Population	Clinical Outcome		
	Malignant	Non-Malignant	Total
Malignant	117	8	124
Non-Malignant	25	129	154
Total	142	137	279

Agreement Statistics for All Specimen Types- Flow Expert #1

Statistics	Estimate	Lower 95%CI	Upper 95%CI
Sensitivity	82.4%	75.3%	87.8%
Specificity	94.2%	88.9%	97.0%
PPV	93.6%	87.9%	96.7%
NPV	83.8%	77.1%	88.8%

Agreement for Presence (Malignant) or Absence (Non-Malignant) of Abnormal Cell Populations for All Specimen Types for Flow Expert #2

ClearLLab Reagents Population	Clinical Outcome		
	Malignant	Non-Malignant	Total
Malignant	122	10	132
Non-Malignant	20	127	147
Total	142	137	279

Agreement Statistics for All Specimen Types- Flow Expert #2

Statistics	Estimate	Lower 95%CI	Upper 95%CI
Sensitivity	85.9%	79.2%	90.7%
Specificity	92.7%	87.1%	96.0%
PPV	92.4%	86.6%	95.8%
NPV	86.4%	79.9%	91.0%

Agreement for Presence (Malignant) or Absence (Non-Malignant) of Abnormal Cell Populations for All Specimen Types for Combined Evaluations for Flow Experts #1 And #2

ClearLLab Reagents Population	Clinical Outcome		
	Malignant	Non-Malignant	Total
Malignant	239	18	256
Non-Malignant	45	256	301
Total	284	274	558

Agreement Statistics for All Specimen Types- Combined Evaluations for Flow Experts #1 And #2

Statistics	Estimate	Lower 95%CI	Upper 95%CI
Sensitivity	84.2%	79.5%	87.9%
Specificity	93.4%	89.9%	95.8%

Agreement between Flow Experts for Presence (Malignant) or Absence (Non-Malignant) of Abnormal Cell Populations for All Specimen Types

ClearLLab Reagents	Flow Expert #1		
Flow Expert #2	Malignant	Non-Malignant	Total
Malignant	123	9	132
Non-Malignant	2	145	147
Total	125	154	279

Agreement between Flow Experts for All Specimen Types

Agreement	Estimate	Lower 95%CI	Upper 95%CI
Positive	98.4%	94.4%	99.6%
Negative	94.2%	89.3%	96.9%

There were a total of 33 discordant specimens by Flow Expert #1 of which 25 specimens were False Negative (FN) and eight were False Positive (FP). Flow Expert #2 had a total of 30 discordant specimens of which 20 were FN specimens and 10 were FP specimens. The sponsor discussed the discordant cases from both experts. The false negative results fell into specimen type limitation, flow cytometry limitation, or ClearLLab reagents limitation. These limitations occur when the phenotypically abnormal population is not found in a particular specimen, the clinical condition is not typically detected through flow cytometry, or the reagents did not have all the antibodies required to detect a particular clinical condition, respectively. For all false positive cases, one or both flow experts identified an abnormal phenotype. False positive cases were primarily due to increases in populations that could represent reactive responses to external stimuli such as viral, bacterial and cytokine stimulation where lymphoid proliferation is common.

An additional analysis was performed for the 160 hematologically abnormal whole blood specimens consisting of 78 hematologically abnormal with no malignancy and 82 with hematolymphoid malignancy from all sites combined. Statistical analysis results on sensitivity, specificity, PPV, and NPV for the two experts are presented below.

Agreement for Presence (Malignant) or Absence (Non-Malignant) of Abnormal Cell Populations for Whole Blood Specimen Types for Flow Expert #1

ClearLLab Reagents Population	Clinical Outcome		
	Malignant	Non-Malignant	Total
Malignant	73	7	80
Non-Malignant	9	71	80
Total	82	78	160

Agreement Statistics for Whole Blood Specimen Types- Flow Expert #1

Statistics	Estimate	Lower 95%CI	Upper 95%CI
Sensitivity	89.0%	80.4%	94.1%
Specificity	91.0%	82.6%	95.6%
PPV	91.3%	83.0%	95.7%
NPV	88.8%	80.0%	94.0%

Agreement for Presence (Malignant) or Absence (Non-Malignant) of Abnormal Cell Populations for Whole Blood Specimen Types for Flow Expert #2

ClearLLab Reagents Population	Clinical Outcome		
	Malignant	Non-Malignant	Total
Malignant	76	8	84
Non-Malignant	6	70	76
Total	82	78	160

Agreement Statistics for Whole Blood Specimen Types for Flow Expert #2

Statistics	Estimate	Lower 95%CI	Upper 95%CI
Sensitivity	92.7%	84.9%	96.6%
Specificity	89.7%	81.0%	94.7%
PPV	90.5%	82.3%	95.1%
NPV	92.1%	83.8%	96.3%

Agreement between Flow Experts for Whole Blood Specimen Types

Agreement	Estimate	Lower 95%CI	Upper 95%CI
Positive	98.4%	94.4%	99.6%
Negative	94.2%	89.3%	96.9%

N. Instrument Name: Beckman Coulter FC500 flow cytometer

O. System Descriptions:

1. Modes of Operation:

Flow cytometers combine fluidics, optics, and electronics subsystems to measure and analyze signals emitted when particles in a liquid stream flow through a glass cuvette at which beams of laser light are directed. The scatter and fluorescence light signals from these particles provide information about cell size, internal complexity, and relative fluorescence intensity. The Instructions for Use for Beckman Coulter FC500 flow cytometer instrument includes more details on the system components and theory of operations.

The previously cleared Instrument is indicated for use with cleared or approved in vitro diagnostic (IVD) assays for the identification and enumeration of human cell subsets that are indicated for use with the instrument.

2. Software:

FDA has reviewed the applicant's Hazard Analysis and software development processes for this line of product types:

Yes __X__ or No _____

3. Calibration and Quality Controls:

See discussion of Traceability in section N.1.f.i above

P. Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

Q. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

R. Identified Risks to Health and Identified Mitigations:

Identified Risks to Health	Identified Mitigations
Incorrect test results (false negatives or false positives)	General Controls and Special Controls (1) and (2)
Incorrect interpretation of device results by the end user	General Controls and Special Control (1), (2) and (3)
Patient harm from specimen(s) collection	General Controls and Special Control(1)

S. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	Flow cytometric immunophenotyping is an integral part of the diagnosis for hematological malignancies. Phenotypically abnormal cell populations have been documented in many hematologic neoplasms, including lymphoma, chronic lymphoid leukemias, plasma cell neoplasms, acute leukemia, mast cell disease, myelodysplastic syndromes, and myeloproliferative disorders. The 2008 World Health Organization (WHO) Classification endorses a multiparametric approach to diagnosis and outlines the morphologic, immunophenotypic, and genotypic features characteristic of each disease entity. Immunophenotyping studies are used in routine diagnosis in the vast majority of hematologic malignancies both to determine lineage in malignancy and to distinguish benign from malignant processes. Flow cytometry may provide diagnostic information, with other ancillary testing to diagnose hematological neoplasms. The safety and effectiveness of the ClearLLab reagents was demonstrated in a multicenter clinical study consisting of 279 total

	patient specimens, 137 patients presenting for flow cytometry immunophenotyping which were found to be non-malignant and 142 patient specimens found to have hematologic malignancy. The patients with hematologic malignancy included 16% acute leukemia, 25% chronic leukemia, 44% lymphoma, 6% plasma cell neoplasm and 9% Others (includes MDS, MPN and eosinophilic hyperplasia). As shown in Comparison of ClearLLab Phenotypes vs. Diagnosis of Malignancy/Non-Malignancy, the clinical trial demonstrated the performance of this reagent panel to identify abnormal cell populations indicative of hematologic malignancy and to aid in demonstrating the absence of disease. The results of two independent flow cytometry experts demonstrated sensitivity of 82% (75–88%, 95% CI) for Flow Expert #1 and 86% (79–91%) for Flow Expert #2 and a specificity of 94% (89–97%) for Flow Expert #1 and 93% (87–96%) for Flow Expert #2. The PPV of ClearLLab reagents to identify malignancy was 94% (88–97%) and 92% (87–96%), respectively for each Flow Expert. Agreement between experts was 98% for positive specimens and 94% for negative specimens.
Summary of Risk(s)	There is minimal potential risk associated with use of this device given the combination of required general controls and special controls. The recognized risks for these assays are 1) possible erroneous results (false positive or false negative), 2) incorrect interpretation of device results by the pathologist or equivalent professional, and 3) related to patient harm from specimen(s) collection.
Summary of Other Factors	With respect to risk of potential harm to the patient while obtaining specimen(s), all flow cytometry studies are conducted ex-vivo and are independent of the assay configuration. The only intervention is blood drawing and/or obtaining tissues (bone marrow, lymph node) by well-established biopsy procedures.
Conclusions Do the probable benefits outweigh the probable risks?	Yes, the probable benefits of this device outweigh the probable risks, given the combination of required general controls and special controls established for this device.

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 864.7010. FDA believes that the special controls, along with the applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PWD
Device Type: Flow Cytometric Test System for Hematopoietic Neoplasms
Class: II (special controls)
Regulation: 21 CFR 864.7010

a. *Identification*.

A flow cytometric test system for hematopeietic neoplasms is a device that consists of reagents for immunophenotyping of human cells in relation to the level of expression, antigen density, and distribution of specific cellular markers. These reagents are used as an aid in the differential diagnosis or monitoring of hematologically abnormal patients having or suspected of having hematopoietic neoplasms. The results should be interpreted by a pathologist or equivalent professional in conjunction with other clinical and laboratory findings.

b. *Classification*. Class II (special controls). A flow cytometric test system for hematopoietic neoplasms must comply with the following special controls:

1. Premarket notification submissions must include the following information:

   i. The indications for use must indicate the clinical hematopoetic neoplasms for which the assay was designed and validated, for example, chronic leukemia or lymphoma.

   ii. A detailed device description including the following:
       (A) A detailed description of all test components, all required reagents, and all instrumentation and equipment, including illustrations or photographs of nonstandard equipment or methods.
       (B) Detailed documentation of the device software including, but not limited to, standalone software applications and hardware-based devices that incorporate software.
       (C) A detailed description of methodology and assay procedure.
       (D) A description of appropriate internal and external quality control materials that are recommended or provided. The description must identify those control elements that are incorporated into the testing procedure, if applicable.
       (E) Detailed specifications for sample collection, processing, and storage.
       (F) Detailed specification of the criteria for test results interpretation and reporting including pre-established templates.
       (G) If applicable, based on the output of the results, a description of the specific number of events to collect, result outputs, and analytical sensitivity of the assay that will be reported.

   iii. Information that demonstrates the performance characteristics of the test, including:
       (A) Device performance data from either a method comparison study comparing the specific lymphocyte cell markers to a predicate device or data collected through a clinical study demonstrating clinical validity using well-characterized clinical specimens. Samples must be representative of the intended use population of the device including hematologic neoplasms and the specific sample types for which the test is indicated for use.
       (B) If applicable, device performance data from a clinical study demonstrating clinical validity for parameters not established in a predicate device of this generic type using well-characterized prospectively obtained clinical specimens including all hematologic neoplasms and the specific sample types for which the device is indicated for use.

(C)Device precision data using clinical samples to evaluate the within-lot, between-lot, within-run, between run, site-to-site and total variation using a minimum of three sites, of which at least two sites must be external sites. Results shall be reported as the standard deviation and percentage coefficient of variation for each level tested.

(D) Reproducibility data generated using a minimum of three lots of reagents to evaluate mean fluorescence intensity and variability of the recovery of the different markers and/or cell populations.

(E) Data from specimen and reagent carryover testing performed using well-established methods (e.g., CLSI H26-A2).

(F) Specimen and prepared sample stability data established for each specimen matrix in the anticoagulant combinations and storage/use conditions that will be indicated.

(G) A study testing anticoagulant equivalency in all claimed specimen type/anticoagulant combinations using clinical specimens that are representative of the intended use population of the device.

(H) Analytic sensitivity data using a dilution panel created from clinical samples.

(I) Analytical specificity data, including interference and cross-contamination.

(J) Device stability data, including real-time stability of reagents under various storage times and temperatures.

(K) For devices that include polyclonal antibodies, Fluorescence Minus One (FMO) studies to evaluate non-specific binding for all polyclonal antibodies. Each FMO tube is compared to reagent reference to demonstrate that no additional population appears when one marker is absent. Pre-specified acceptance criteria must be provided and followed.

(L) For devices indicated for use as-a semi-quantitative test, linearity data using a dilution panel created from clinical samples.

(M) For devices indicated for use as a semi-quantitative test, clinically relevant analytical sensitivity data, including limit of blank, limit of detection, and limit of quantification.

iv. Identification of risk mitigation elements used by the device, including a detailed description of all additional procedures, methods, and practices incorporated into the instructions for use that mitigate risks associated with testing the device.

2. The 21 CFR 809.10 compliant labeling must include the following:

i. The intended use statement in the 21 CFR 809.10(a)(2) and 21 CFR 809.10(b)(2) compliant labeling must include a statement that the results should be interpreted by a pathologist or equivalent professional in conjunction with other clinical and laboratory findings. The intended use statement must also include information on what the device detects and measures whether the device is qualitative, semi-quantitative, and/or quantitative, the clinical indications for which the device is to be used, and the specific population(s) for which the device is intended.

ii. A detailed description of the performance studies conducted to comply with paragraph (1)(iii) and a summary of the results.

3. As part of the risk management activities performed under 21 CFR 820.30 design controls, product labeling and instruction manuals should include clear examples of all expected phenotypic patterns and gating strategies using well-defined clinical samples representative of both abnormal and normal cellular populations. These samples must be selected based upon the indications described in paragraph (1)(i) of this section.