ClearLLab LS Lymphoid Screen Reagent

Fluorochrome	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700	APC-A750	Pacific Blue	Krome Orange
Specificity	Kappa / CD8	Lambda / CD4	CD19	CD56	CD10	CD34	CD5	CD20	CD3	CD45

REF **B74073 – 25 tests**

PN B74073 -AE

CE BECKMAN COULTER

For *In Vitro* Diagnostic Use

INTENDED USE

The ClearLLab LS (Lymphoid Screen) reagent is intended for *in vitro* diagnostic use as a screening panel for identification of various hematolymphoid cell populations by immunophenotyping on Navios and Navios EX Flow Cytometers. The reagent is used as an aid in the differential diagnosis of patients with signs and/or symptoms of hematolymphoid malignancies. The reagent can be used with peripheral whole blood (collected in EDTA, ACD and Heparin), bone marrow specimens (collected in EDTA, ACD and Heparin) and lymph node specimens for immunophenotyping. The results should be interpreted along with additional clinical and laboratory findings. The reagents provide qualitative results for B, T, and NK lineages.

SUMMARY AND EXPLANATION

The specific choices and combinations in the ClearLLab LS reagent are based on the guiding principles of (1) addressing the clinical indications, (2) accounting for all major lymphoid cell populations present in the specimen[17], and (3) providing sufficiently comprehensive identification of all major categories of hematopoietic cell populations in both normal and neoplastic states[1-7].

CD45 is a pan-leukocyte antigen that is differentially expressed on different leukocyte populations. In combination with Side Scatter, CD45 can be used to define discrete population gates for identification of the lineage specific antigens. The CD45 antigen is expressed on every type of hematopoietic cell except mature erythrocytes and their immediate progenitors. It has not been detected in differentiated nonhematopoietic tissue.[2-5]

T Cell Characterization:

Cell surface antigens are acquired and lost by T lymphocytes in a manner reflecting the maturational (differentiation) and/or functional state of the cell. Once acquired, the same cell may co-express some or all of these antigens for varying periods of time[9].

The CD3 antigen is normally present on the cell surface of mature thymocytes, resting and activated peripheral blood mature T lymphocytes (both inducer and suppressor/cytotoxic populations) [10-14].

The CD4 antigen is present on thymocytes and the inducer T lymphocyte population in peripheral blood. It is also expressed at low density on monocytes.[17]

The CD5 antigen is present on all mature T lymphocytes and on most thymocytes. CD5 is also present on a small population of the B lymphocyte subset (B1 cells) but is not found on granulocytes or monocytes[40].

The CD8 antigen is normally present on approximately 80% of thymocytes and approximately 30-35% of peripheral blood T lymphocytes and some natural killer cells[26-29].

The CD56 antigen is expressed on a subpopulation of lymphocytes that demonstrate natural killer (NK) activity. Virtually all of the cells capable of mediating non-TCR mediated cytotoxicity in peripheral blood express CD56[22,24]. This subpopulation consists of both natural killer cells (CD3-/CD56+) and a small subset of T cells (CD3+/CD56+)[25]. CD56 is not expressed on other T or B lymphocyte, monocyte, granulocyte or erythrocyte populations.

B Cell and Kappa and Lambda Characterization:

Cell surface antigens are acquired and lost by B lymphocytes in a manner reflecting the maturational (differentiation) and/or functional state of the cell. Once acquired, the same cell may coexpress some or all of these antigens for varying periods of time[30].

Expression of pan B cell surface antigens by B lymphocytes occurs in the following sequence:

CD19 (committed B cell progenitor/pre-pre-B cell); CD20 (early pre-B cell). Once expressed, the CD19 and CD20 antigens are coexpressed continuously throughout the remainder of B lymphocyte differentiation including the resting and the activated mature peripheral blood or lymphoid tissue B lymphocyte. Both antigens are lost at the last stage of B lymphocyte differentiation, the plasma cell[30-32].

The CD19 antigen is expressed on all B cells, including early progenitor B cells. It can also be found on follicular dendritic cells and myelomonocytic lineage progenitor cells, but is not expressed on T cells, monocytes or granulocytes.

The CD20 antigen is present on all normal B lymphocytes from peripheral blood, lymph node, spleen, tonsil and bone marrow but is absent from plasma cells. It is expressed naturally but at low density on a subset of peripheral blood T lymphocytes.

The Kappa antigen is expressed on the surface of a subpopulation of approximately 50%-66% of mature B cells in peripheral blood. This light chain is also found on the surface of a subpopulation of immature bone marrow B lymphocytes.

The Lambda antigen is expressed on the surface of a subpopulation of approximately 33%-50% of mature B cells in peripheral blood. This light chain is also found on the surface of a subpopulation of immature bone marrow B lymphocytes.

The CD10 antigen is expressed on uncommitted lymphoid precursors[34]. CD10 expression is lost as cells enter the T lineage. In the B lineage, CD10 expression is lost later in ontogeny, as cells acquire surface immunoglobulin expression. It is also expressed on activated and proliferating B cells in the germinal center, and on neutrophils as well as bone marrow stromal cells. Additionally, it is expressed on a number of other cells of epithelial origin[35-39].

The CD34 antigen is expressed on hematopoietic progenitor cells of all lineages as well as the pluripotent stem cells. CD34 antigen expression is highest on the most primitive stem cells and is gradually lost as lineage committed progenitors differentiate. The CD34 antigen is also present on capillary endothelial cells and on bone marrow stromal cells[38,39].

PRINCIPLES OF TEST

This test depends on the ability of monoclonal or polyclonal antibody to bind to discrete antigenic determinants that are expressed on the surface of cells. Specific cell staining is accomplished by incubating specimens prepared for staining with the appropriate antibody reagent. The ClearLLab LS reagent is provided in a 12 antibody-10 fluorochrome format and specific for different cell surface antigens.

Red blood cells are lysed with the VersaLyse Lysing Solution prior to cell staining. White blood cells are analyzed by flow cytometry using an appropriate combination of subpopulation gates.

REAGENT CONTENTS

ClearLLab LS kit contains the following:

- 25 tests of the LS Tube in a dry format (i.e. a single tube for a single test)

STATEMENT OF WARNINGS

SDS Safety Data Sheet is available at **beckman.com/techdocs**.

1. The ClearLLab LS reagent contains less than 0.1% Sodium Azide. Sodium Azide under acid conditions yields Hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while discarding. These precautions are recommended to avoid deposits in metal piping in which explosive conditions may develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipette by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use reagent beyond the expiration date on the label.
5. Use Good Laboratory Practices (GLP) when handling reagent.
6. Do not store the tubes in the refrigerator; do not freeze/thaw the tubes.
7. Minimize exposure of reagent to light during storage or incubation.
8. Discard used or unused reagent tubes and packaging materials as per applicable regulations.
9. Review all plots before reporting results
10. Ensure the latest revision of the Instructions for USE (IFU) is used. It can be downloaded from ***beckmancoulter.com/ifu***

STORAGE CONDITIONS AND STABILITY

Store the reagent tubes between 18 - 30 °C, in a dry place. Minimize exposure to light. Refer to the label for the date of expiration of the reagent. After removing the required tubes, reseal the pouch containing the remainder of the tubes with the desiccant bag inside. Once the pouch is opened, use the reagents within 90 days.

EVIDENCE OF DETERIORATION

Any damage to the tube may indicate product deterioration and the product should not be used.

REAGENT PREPARATION

No preparation is necessary. The ClearLLab LS reagent is ready to use in a dry unitized format.

SPECIMEN COLLECTION

- Each flow cytometric analysis requires 100 µL of whole blood, bone marrow or single lymphoid cell suspension.
- Avoid contamination of the tops and sides of the reagent tubes with blood, or incomplete lysis may occur.
- Staining may be performed on specimens with white blood cell counts in the range of 2-20 x 10^3 cells/µL.
- White blood cell counts exceeding 20 x 10^3 cells/µL require dilution of the sample prior to staining.
- White blood cell counts below 2 x 10^3 cells/µL require concentration of the sample prior to staining.
- Whole blood may be collected using EDTA, Heparin or ACD anticoagulants as appropriate for the specimen.
- Bone marrow may be collected using EDTA, Heparin or ACD anticoagulants as appropriate for the specimen.
- For detailed information on the collection of whole blood by venipuncture and interfering conditions, refer to "Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (H3), Approved Edition" published by the Clinical and Laboratory Standards Institute.

PROCEDURE

MATERIALS SUPPLIED

ClearLLab LS Lymphoid Screen Reagent
REF B74073 – 25 tests

MATERIALS REQUIRED BUT NOT SUPPLIED

ClearLLab Compensation Kit PN B74074
VersaLyse Lysing Solution, PN A09777
IOTest 3 Fixative Solution, PN A07800
Blood collection tubes with anticoagulant
(K_2 or K_3 EDTA, ACD-A, and Sodium or Lithium Heparin recommended)
Heat Inactivated Fetal Calf Serum
Phosphate Buffered Saline (PBS), PN 6603369

Transfer pipettes
Pasteur pipette
Micropipettes
Vortex mixer
Navios Flow Cytometer equipped with 3 Lasers 10 colors, PN A80706
Navios EX Flow Cytometer equipped with 3 Lasers 10 colors, PN B73085AB
Flow-Check Pro Fluorospheres, PN A63493
Flow-Set Pro Fluorospheres, PN A63492
Cell counter or Hemocytometer
Cotton tip applicators

Preparation of Reagents
PBS/2% Fetal Calf Serum (FCS) Wash Buffer:

a. Prepare a solution of PBS with 2% heat inactivated FCS for use as the wash buffer (1:50 v/v FCS/PBS).

0.1% Formaldehyde PBS Resuspension Buffer:

a. Prepare a sufficient volume of 0.1% formaldehyde PBS resuspension buffer by diluting 12.5 µL of the 10X IOTest 3 Fixative Solution in 1 mL of PBS

SPECIMEN PREPARATION
Both blood and bone marrow specimens are pre-lysed and pre-washed prior to sample staining to avoid plasma/serum protein interferences. Optimal washing efficiency is obtained when three sequential washing steps including lysis are performed before staining.

NOTE: If the single cell suspensions prepared from lymphoid tissues have visible red blood cells, process the sample as per the procedure below. Otherwise, follow your laboratory procedure for washing and continue to Step 4 of the Staining procedure.

CAUTION: Failure to follow the washing instructions (volumes and wash cycles) may cause erroneous results.

STAINING PROCEDURE
1. Add 100 µL specimen in an empty 12 x 75 mm tube, then add 3 mL of PBS/2% FCS Wash Buffer.
2. Mix by inversion and centrifuge at 300 x g for 5 minutes.
3. Remove the supernatant by aspiration.

   CAUTION: Failure to ensure supernatant is completely removed may result in poor lysis in step 4.

4. Add 1.0 mL VersaLyse Lysing solution to the tube.
5. Vortex the sample immediately for 3-6 seconds and incubate at 18-25°C for at least 20 minutes.
6. Add 2.0 mL of PBS/2% Fetal Calf Serum (FCS) Wash Buffer to the lysed sample.
7. Centrifuge at 300 x g for 5 minutes and remove the supernatant by aspiration.
8. Add 3.0 mL of PBS/2% FCS Wash Buffer to the cell pellet.
9. Mix by inversion and centrifuge at 300 x g for 5 minutes.
10. Remove the supernatant by aspiration.
11. Re-suspend the pellet in 100 µL of PBS/2% FCS Wash buffer to obtain WBC count in the range of 2,000 – 20,000 cells/µL.
12. Transfer the 100µL of the washed sample to the dry reagent tube and vortex vigorously for 6-8 seconds. Incubate at 18-25°C for 15 minutes.
13. Add 3 mL PBS/2% FCS Wash Buffer to the sample and centrifuge at 300 x g for 5 minutes.
14. Remove the supernatant by aspiration.
15. Re-suspend the sample in 500 µL of the 0.1% Formaldehyde PBS Re-suspension Buffer. The sample is now ready for acquisition.

NOTE: The prepared whole blood and bone marrow sample can be stored at 18 - 25 ºC in the dark for up to 4 hours before acquisition, and at 2 - 8 ºC in the dark for up to 24 hours before acquisition. Prepared Lymph Node samples should be analyzed immediately.

QUALITY CONTROL

1. Ensure the flow cytometer is properly aligned and standardized for light scatters and fluorescence intensities according to manufacturer's recommendations (refer to Navios or Navios EX Instructions for Use manuals for specific instructions for setting PMT voltages).
   a. Run Flow-Check Pro Fluorospheres to verify instrument alignment (refer to the product Instructions for Use).
   b. Flow Set Pro target ranges specific for the ClearLLab LS test are provided on the Flow Set Pro assay sheet, and the daily AutoSetup is to standardize the instrument voltages and Gains to the assigned targets. (Refer to the product Instructions for Use and the Table of Application Target Settings).
2. The fluorochromes fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin -Texas Red-X (ECD), phycoerythrin-Cy5.5 (PC5.5), phycoerythrin-Cy7 (PC7), Allophycocyanin (APC), Allophycocyanin-Alexa Fluor 700 (APC-A700), Allophycocyanin-Alexa Fluor 750 (APC-A750), Pacific Blue and Krome Orange, have different emission spectrum, but they do have spillover due to spectral overlap, which must be corrected by compensation.
   a. Initial compensation levels can be established with a normal blood specimen or CYTO-COMP Cells with the use of the ClearLLab Compensation kit (PN B74074) using full matrix compensation, such as the AutoSetup program in the Navios or Navios EX Software. Refer to the ClearLLab Compensation kit IFU (PN B74074) for details.
   b. Before patient samples are analyzed, a normal blood specimen stained with the ClearLLab LS should be used to verify compensation and antibody reactivity. Manual adjustment to initial compensation values can be made during verification prior to the patient specimen testing, if necessary.

FLOW CYTOMETRY ACQUISITION / ANALYSIS PROCEDURE

CAUTION: If the laser on the flow cytometer is misaligned, inappropriate filters are present, or the gates improperly set, results may be erroneous.

1. A discriminator of 80 on Forward Scatter (FS) is recommended but shall be adjusted as per the requirement of the samples.
2. Set compensation using the ClearLLab Compensation Kit on the Navios or Navios EX Flow Cytometer.

3. Create appropriate analysis protocols to define the population gates and the series of dual parameter histograms for analysis of the reagent specificities.
4. Follow the steps below as a recommended gating strategy for the reagent. Additional plots and gates may be created to assess the cell populations as needed.
   A. A two parameter FS TOF Lin versus Time dot plot gated on Ungated is created to identify the "Singlets" population.
   B. A FS INT Lin vs. SS INT Lin two-parameter dot plot gated on Singlets may also be collected to assess the prepared sample scatter characteristics.

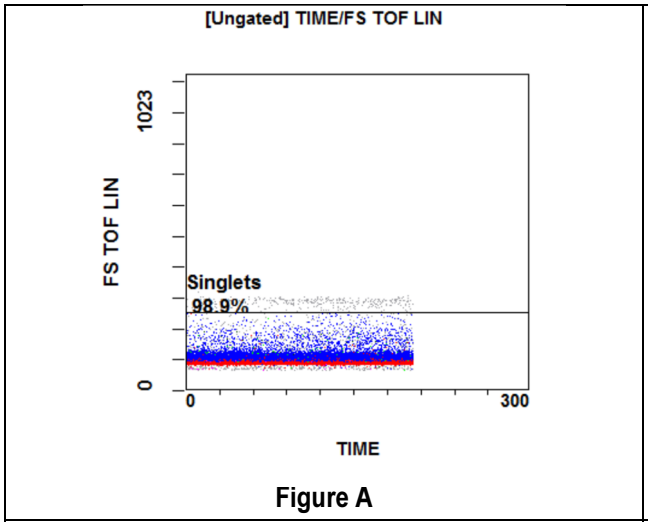


Figure A

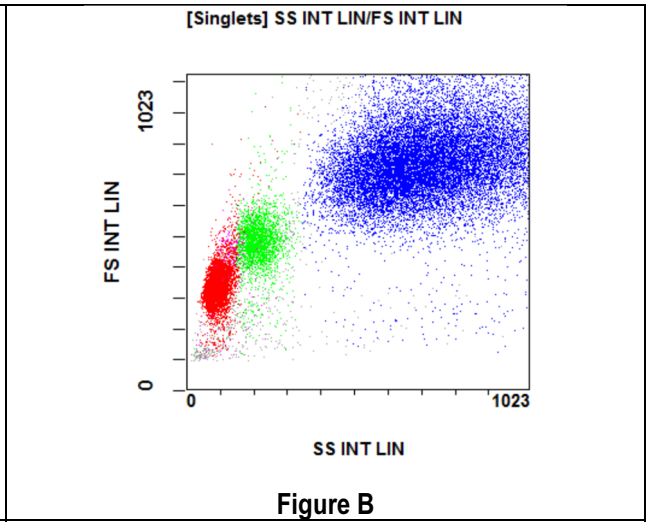


Figure B

   c. A two-parameter CD45-Krome Orange (KrO) (FL10 LOG) vs. SS Lin dot plot gated on Singlets is created to identify lymphocytes (Gate "Lymphs"), $CD45^{dim}/SS^{Low}$ cells (Gate "Blasts"). Additional gates for Monocytes ("Monos") and Granulocytes ("Grans") may be created, if desired. Create a gate (CD45+) to encompass all white blood cell events and collect a minimum of 100,000 CD45+ events from SSC – CD45 plot with the stop criteria.
   d. A two-parameter CD3-Pacific Blue (PBE) (FL9 LOG) vs. SS Lin dot plot gated on CD45+ is created to identify the CD3+ cells.

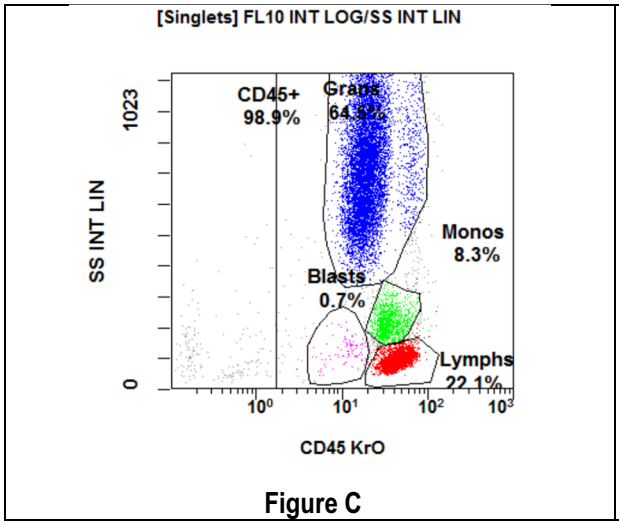


Figure C

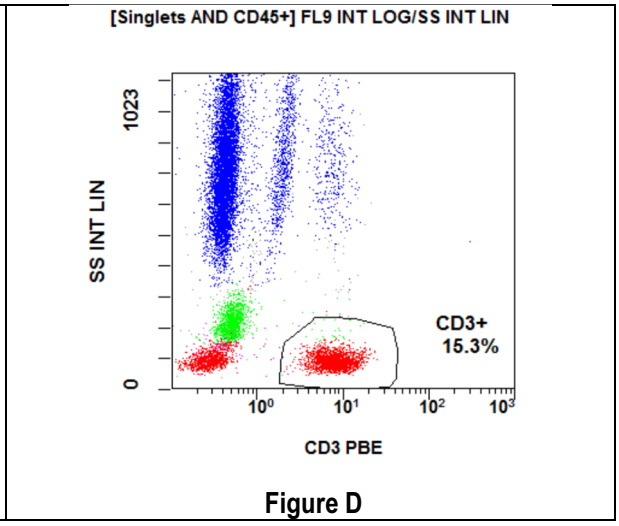


Figure D

   e. A two-parameter CD19-ECD (FL3 LOG) vs. SS Lin dot plot gated on CD45+ is created to identify the CD19+ lymphocytes.
   f. A CD19 ECD (FL3 LOG) vs. CD3 PBE (FL9 LOG) two-parameter dot plot is created, gated on "Lymphs" to identify the CD3+ (T cells) and the CD19+ (B cells).

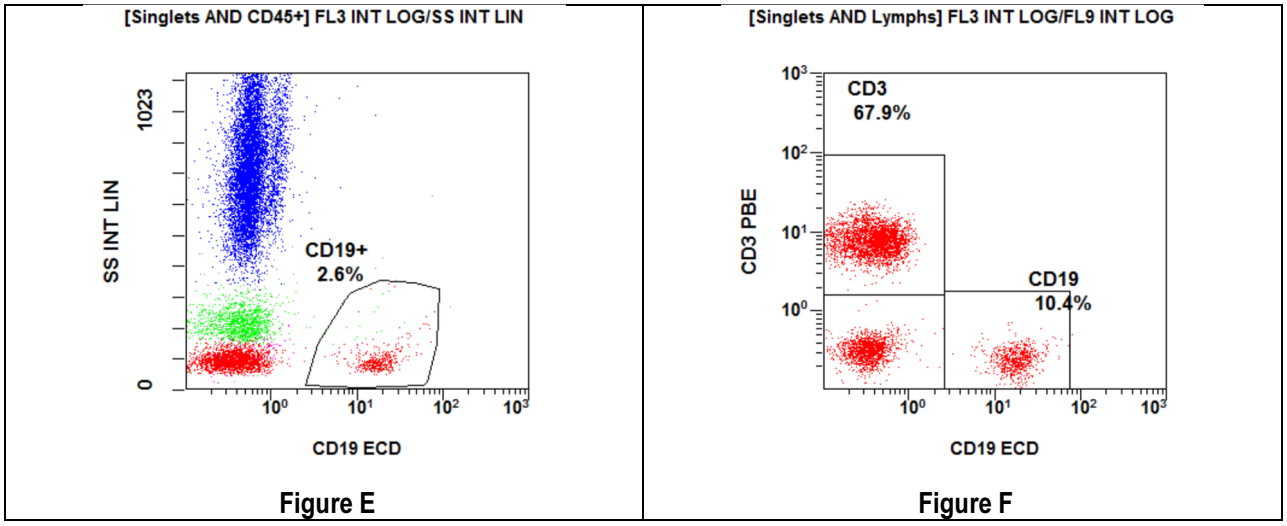


Figure E **Figure F**

g. Create a CD8/Kappa-FITC (FL1 LOG) vs. CD4/Lambda-PE (FL2 LOG) two-parameter dot plot and apply the gate CD3 onto this plot. CD4+ and CD8+ T cells can be identified from this plot.
h. Create a CD8/Kappa-FITC (FL1 LOG) vs. CD4/Lambda-PE (FL2 LOG) two-parameter dot plot and apply the gate CD19 onto this plot. Kappa+ and Lambda+ B cells can be identified from this plot.

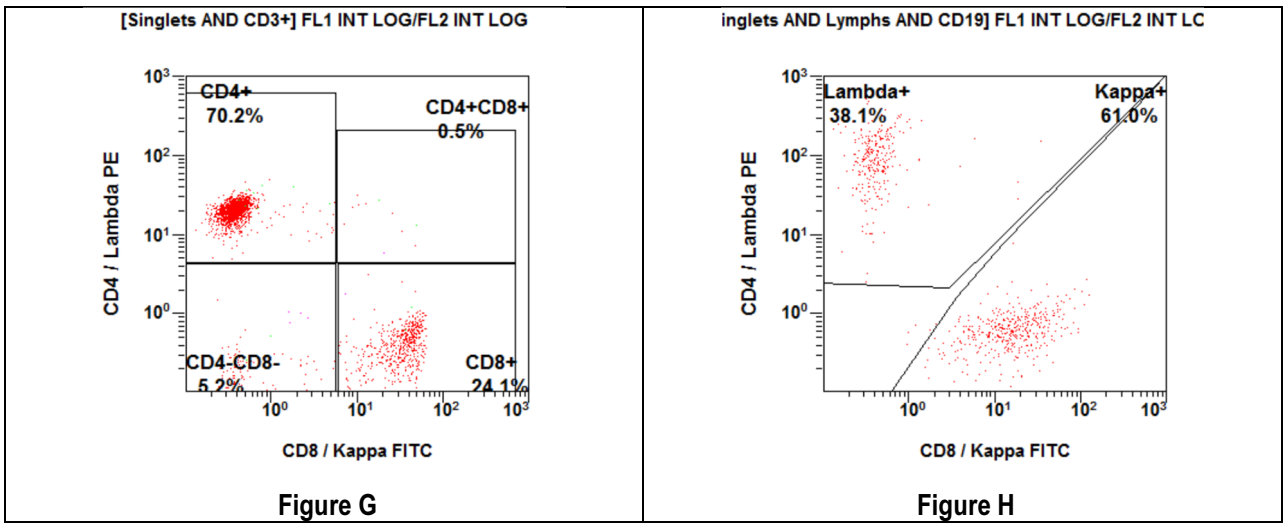


Figure G **Figure H**

i. Create a CD5-APC A700 (FL7 LOG) vs. CD3-PBE (FL9 LOG) two-parameter dot plot and apply the gate “Lymphs” onto this plot. This plot shows the CD3+CD5+ T lymphocytes.
j. Create a CD3-PBE (FL9 LOG) vs. CD56-PC5.5 (FL4 LOG) two-parameter dot plot and apply the gate “Lymphs” onto this plot. NK and NK-T cells can be identified from this plot.

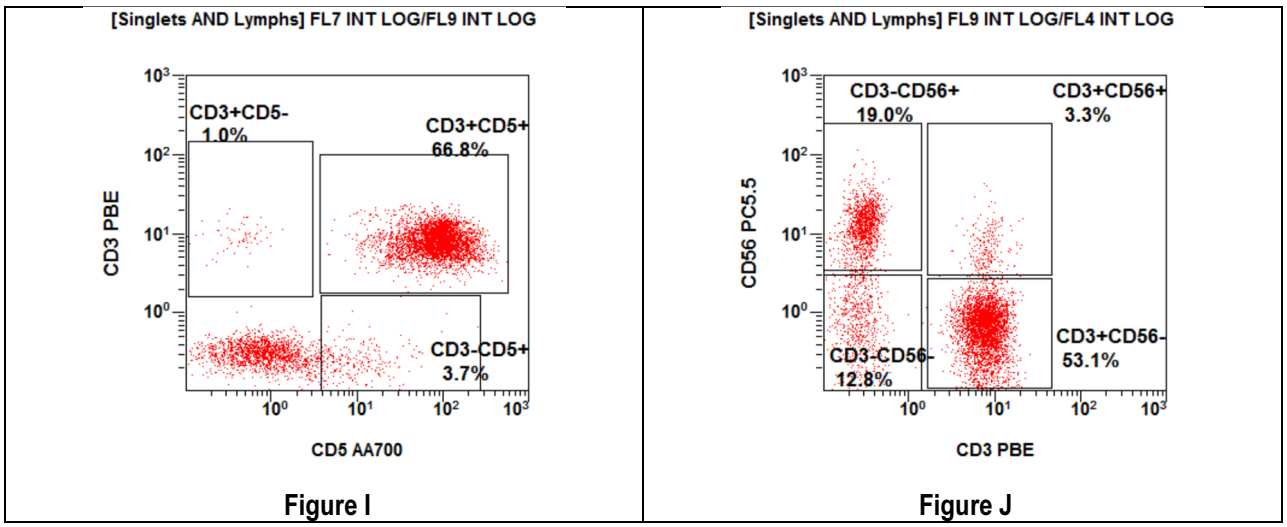


Figure I **Figure J**

k. Create a CD20-APC A750 (FL8 LOG) vs. CD10-PC7 (FL5 LOG) two-parameter dot plot and apply the gate “Lymphs” onto this plot. Maturing B cells can be identified from this plot.
l. Create a CD20-APC A750 (FL8 LOG) vs. CD19-ECD (FL3 LOG) two-parameter dot plot and apply the gate “Lymphs” onto this plot. Mature B cells can be identified from this plot.

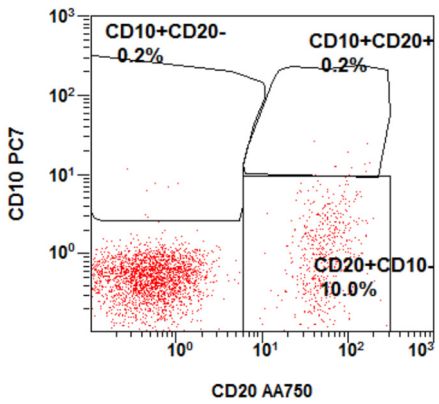


Figure K

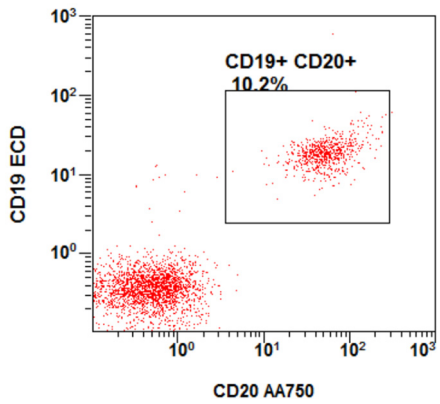


Figure L

m. Create a CD34-APC (FL6 LOG) vs. CD10-PC7 (FL5 LOG) two-parameter dot plot and apply the gate “Lymphs” onto this plot. Immature Blasts can be identified in this plot.
n. Create a CD19-ECD (FL3 LOG) vs. CD10-PC7 (FL5 LOG) two-parameter dot plot and apply the gate “Lymphs” to this plot. Follicular B cells can be identified in this plot.

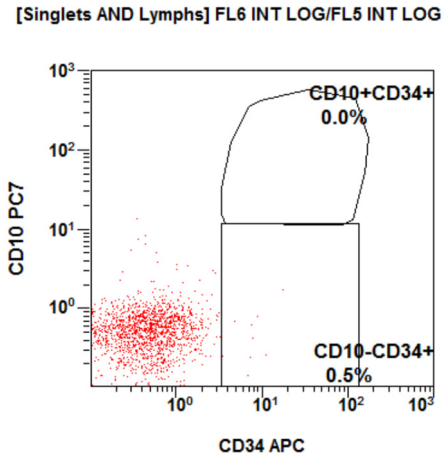


Figure M

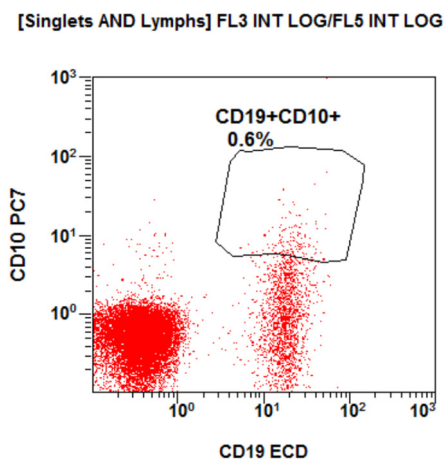


Figure N

o. Create a CD5-APC A700 (FL7 LOG) vs. CD19-ECD (FL3 LOG) two-parameter dot plot and apply the gate “Lymphs” onto this plot. This plot shows the CD19+CD5+ lymphocytes.

NOTE: Use the negatively stained populations for placement of the positive region boundaries in all plots with the exception of CD5-APC-A700 vs CD19-ECD. CD5 is conjugated to a bright fluorophore APC-A700 and this appear to show a variable expression on the B cells. To identify the CD5+CD19+ B cells in normal whole blood specimens, it is recommended to draw a gate with the positioning just below the CD5+ T cell population on APC-A700 channel. This will enable correct identification of CD5+ B cells. The figure below shows the normal expression of CD5+CD19+ cells on peripheral blood (Figure O_1), and abnormal expression of CD5+CD19+ cells (Figure O_2).

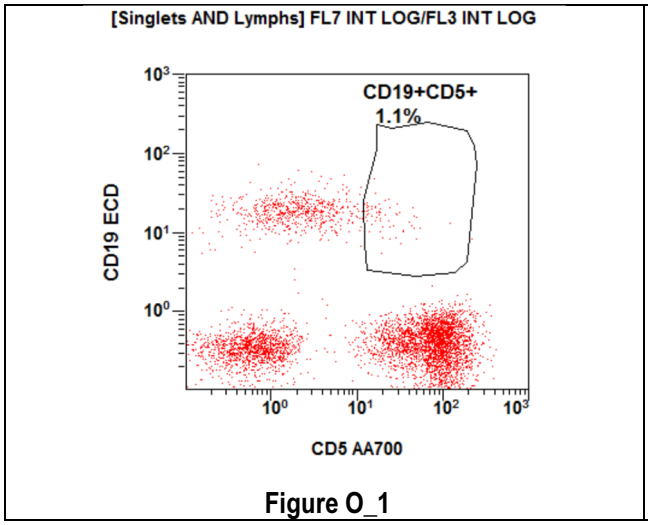


Figure O_1

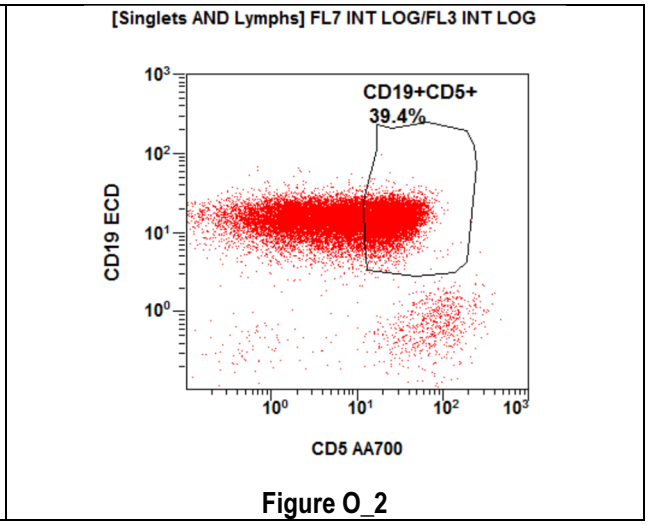


Figure O_2

p. Create two-parameter dot plots each with the specificities of CD56, CD10, CD34, CD5 and CD20 versus SS INT Lin and gated on the “CD45+” to see the staining on all cell populations (Lymphs, Blasts, Monos and Grans). These plots help users identify multiple phenotypes expressing corresponding markers.

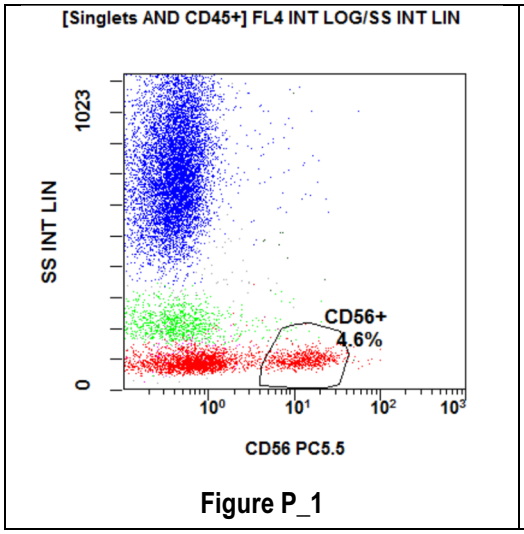


Figure P_1

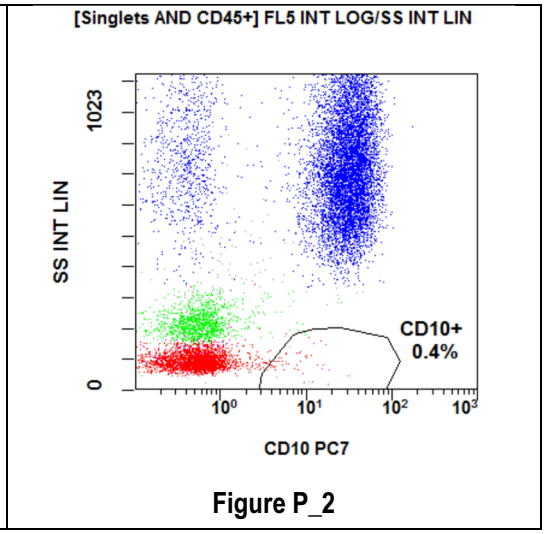


Figure P_2

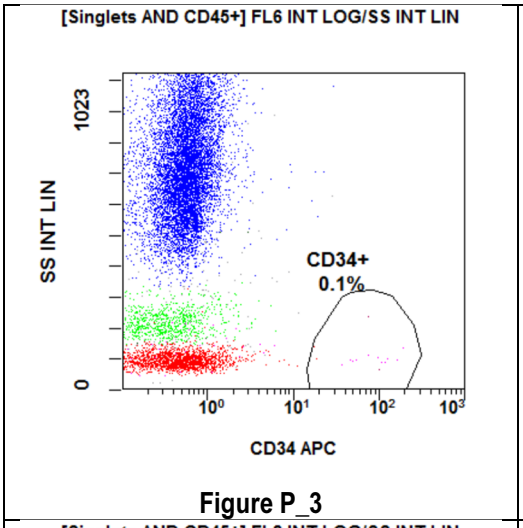


Figure P_3

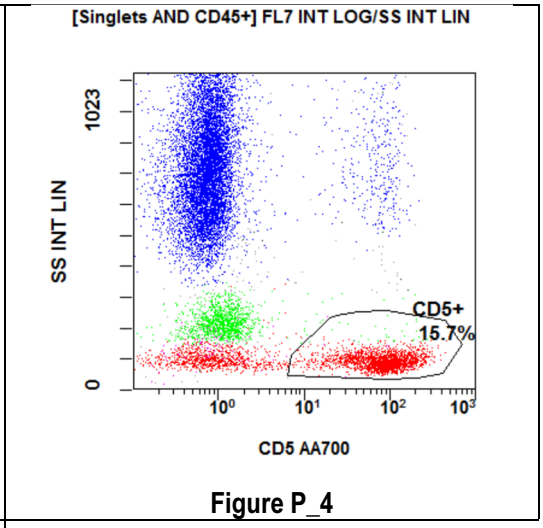


Figure P_4

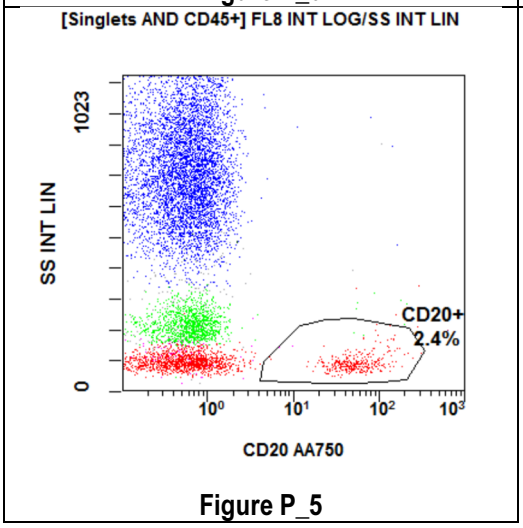


Figure P_5

q. Create additional plots as desired to check the staining patterns of various specificities. Use parent gate “CD45+”. Examples are provided below:

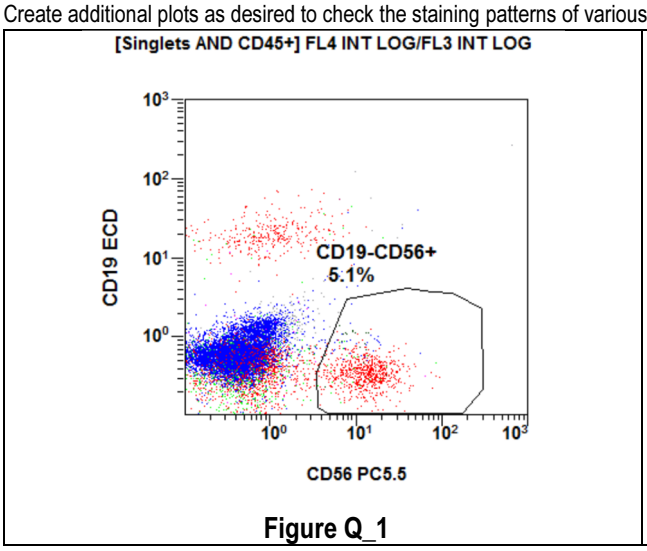


Figure Q_1

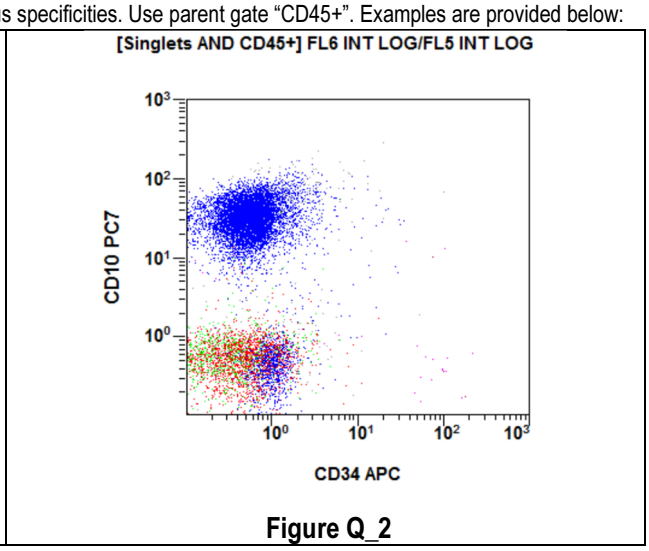


Figure Q_2

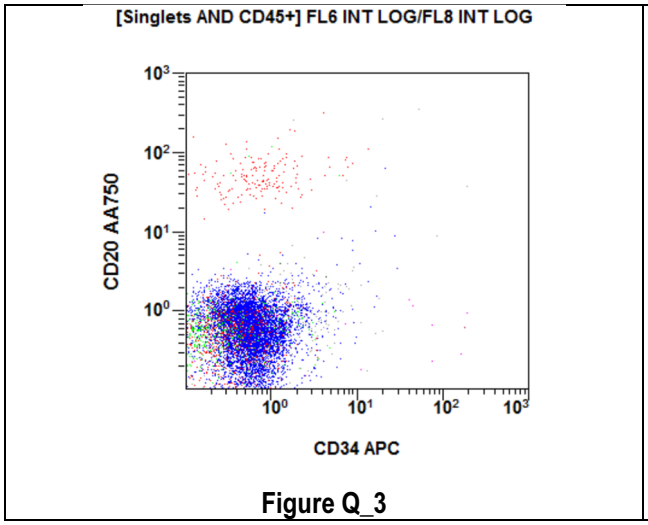


Figure Q_3

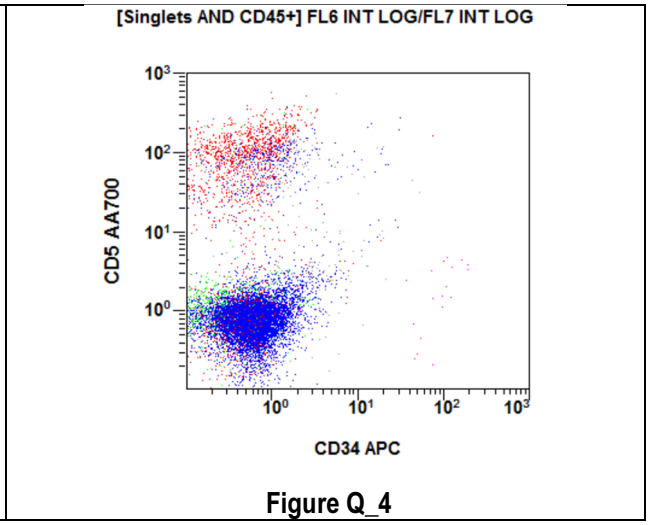
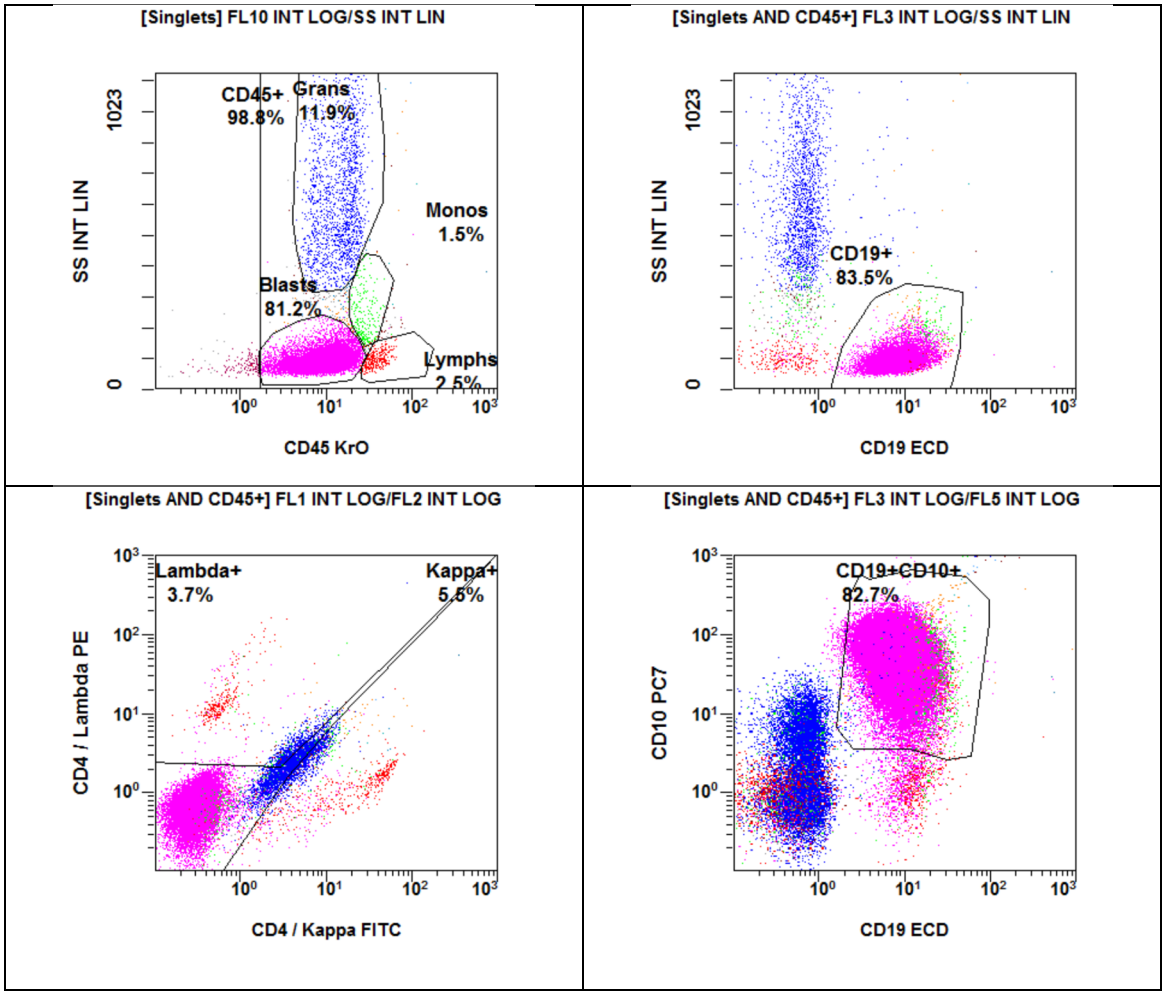


Figure Q_4

Clinical Examples

The plots below (Figure R) are examples of a bone marrow specimen with B – Acute Lymphoid Leukemia. Clear distinction of CD19 positivity on CD34+, CD10+ blasts indicates the disorder.



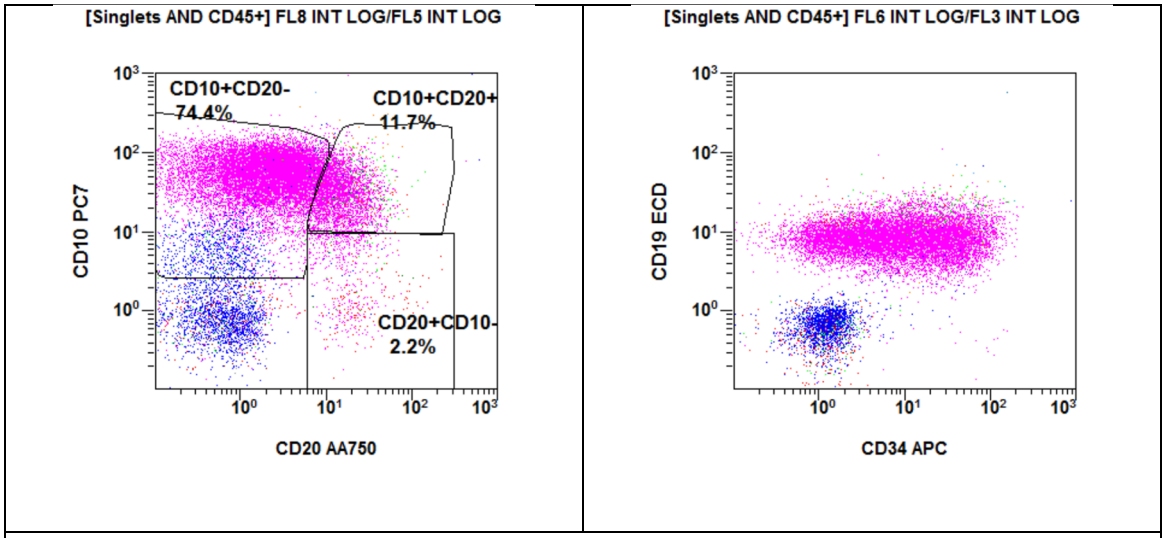
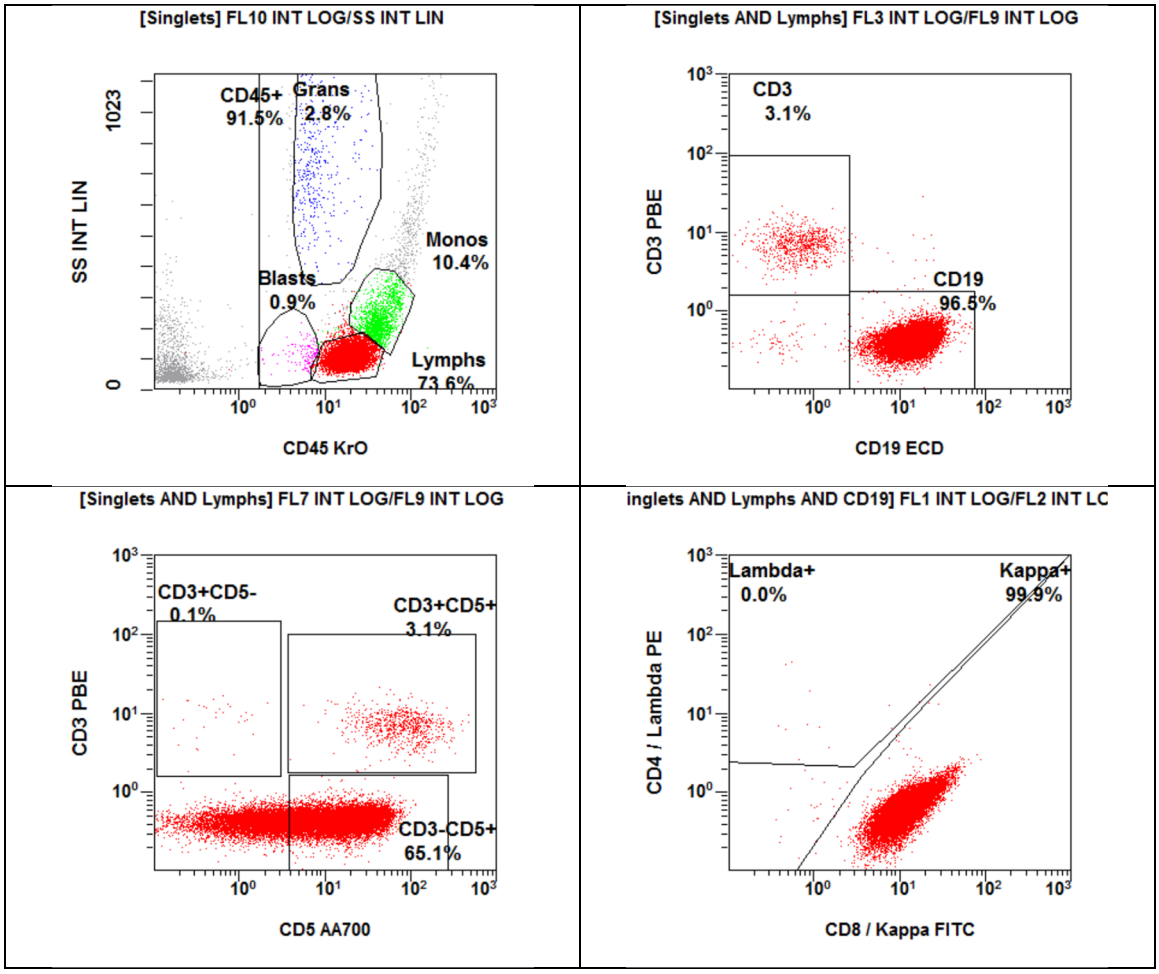


Figure R: Representative plots of a B – Acute Lymphoid leukemia bone marrow specimen

The plots below (Figure S) are example of a bone marrow specimen with B – Chronic Lympho Proliferative Disorder (Chronic Lymphocytic Leukemia). Clear distinction of CD19 positivity on CD5+ together with monoclonal Kappa light chain restriction indicates the disorder.



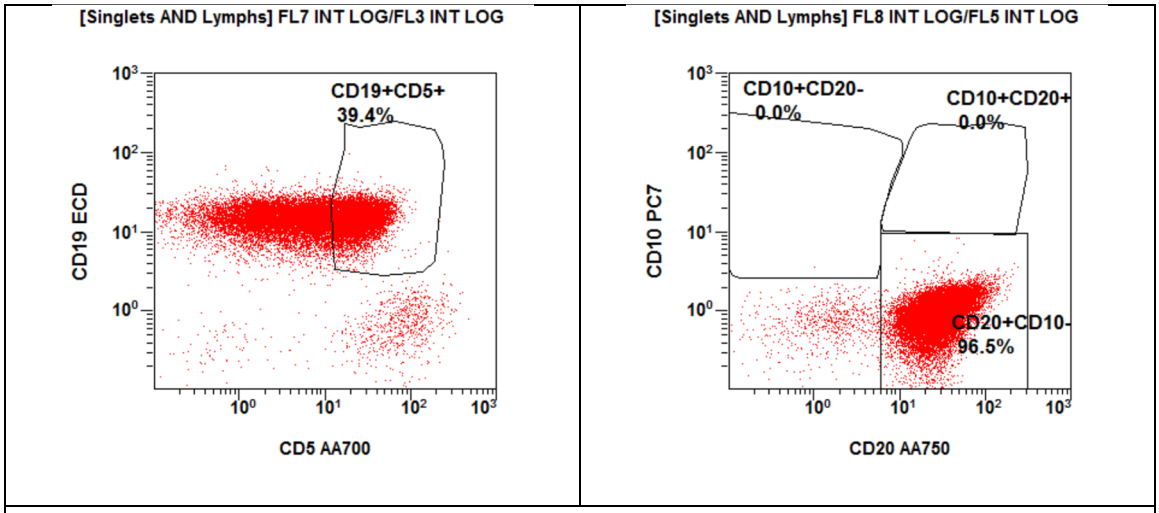


Figure S: Representative plots of a B – Chronic Lympho Proliferative Disorder (Chronic Lymphocytic Leukemia)

LIMITATIONS

1. All red blood cells may not lyse under the following conditions: nucleated red blood cells, abnormal protein concentration, or hemoglobinopathies. This may cause falsely decreased results due to unlysed or improperly lysed red blood cells being counted as leukocytes.
2. Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual in an abnormal state of health may show the same leukocyte percentages as a healthy person. Use test results in conjunction with clinical and other diagnostic data.
3. Certain patients may present special problems due to altered or very low numbers of certain cellular populations. This should be considered in the interpretation of the data.
4. Results obtained with flow cytometry may be erroneous if the laser is misaligned or the compensation or gates are improperly set.
5. Specimens from patients undergoing treatment with therapy-grade monoclonal antibodies may give varying results. This should be considered in the interpretation of the data. No study has been carried out to determine the effect of these antibodies in the screening procedure.
6. Whole blood and bone marrow specimens collected in EDTA should be stored at 18-25 °C and must be processed within 24 hours of collection.
7. Whole blood and bone marrow specimen collected with Heparin or ACD should be stored at 18-25 °C and must be processed within 48 hours of collection.
8. Prepare specimen from fresh Lymph node samples as available.
9. Prepared whole blood and bone marrow samples should be protected from light and should be analyzed within 4 hours when stored at 18-25 °C or within 24 hours when stored at 2-8 °C. Prepared Lymph Node samples should be analyzed immediately.
10. Specimens with Acute Myeloid Leukemia, Multiple Myeloma, Myelodysplastic syndrome (MDS) etc., may be detected in the screening panel.
11. Nonspecific binding may be seen with some clinical specimens due to low viability. It is strongly advised to not use partially or completely hemolyzed samples, or highly lipemic samples for processing with ClearLLab LS reagent.

PERFORMANCE CHARACTERISTICS

The performance characteristics data was collected on Navios and Navios EX Flow Cytometers with the standard filter set and running the Navios and Navios EX software.

SPECIFICITY

	Kappa-FITC	CD8-FITC	Lambda-PE	CD4-PE	CD19-ECD	CD56-PC5.5
Specificity	Kappa chain	CD8	Lambda chain	CD4	CD19	CD56
Clone	Polyclonal	B9.11	Polyclonal	13B8.2	J3-119	N901 (NKH-1)
Hybridoma	N/A	NS1 x BALB/c	N/A	NS-1 x BALB/c	NS-1 x BALB/c	NS-1 x BALB/c
Immunogen	Light polyclonal Kappa chains	Cytotoxic human T clone HLA A2	Light polyclonal Lambda chains	Human thymocytes	SKLY18 lymphoma cells	Chronic myeloid leukemia (hCML) cells
Ig Chain	$F(ab')_2$	IgG1	$F(ab')_2$	IgG1	IgG1	IgG1
Species	Rabbit	Mouse	Rabbit	Mouse	Mouse	Mouse
Source	Serum	Ascites fluid or supernatant of in vitro cultured hybridoma cells	Serum	Ascites fluid or supernatant of in vitro cultured hybridoma cells	Ascites fluid or supernatant of in vitro cultured hybridoma cells	Ascites fluid or supernatant of in vitro cultured hybridoma cells
Purification	Chromatography	Affinity chromatography	Chromatography	-	Affinity chromatography	Affinity chromatography
Fluorescence	Excites at 488 nm Emits at 525 nm	Excites at 488 nm Emits at 525 nm	Excites at 488 nm Emits at 575 nm	Excites at 488 nm Emits at 575 nm	Excites at 488 nm Emits at 613 nm	Excites at 488 nm Emits at 692 nm
Conjugation	FITC (Fluorescein Isothiocyanate)	FITC (Fluorescein Isothiocyanate)	PE (Phycoerythrin)	PE (Phycoerythrin)	ECD (Phycoerythrin - Texas Red-X)	R Phycoerythrin-Cyanine 5.5 (PC5.5)
Molar Ratio	FITC / Protein : 1.9 - 3.3	FITC / Protein: 4.5 - 5.5	PE / Protein / 0.5 - 1.5	PE / Protein: 0.5 - 1.5	ECD / Protein: 0.5 - 1.5	PC5.5 / Protein : 0.5 - 1.5

	CD10-PC7	CD34-APC	CD5-APC-A700	CD20-APC-A750	CD3-Pacific Blue	CD45-Krome Orange
Specificity	CD10	CD34	CD5	CD20	CD3	CD45
Clone	ALB1	581	BL1a	B9E9 (HRC20)	UCHT1	J.33
Hybridoma	NS-1 x BALB/c	NS0 x BALB/c	SP2/0 x BALB/c	X63 x BALB/c	NS-1 x BALB/c	NS-1 x BALB/c
Immunogen	Human Leukemia cells	Human CD34+ leukaemic cells	Lymphocytes from human thoracic duct	DAUDI Cell line (human B-lymphoblastoid)	Lignée T + IL2	Lazz 221 cell line
Ig Chain	IgG1	IgG1	IgG2a	IgG2a	IgG1	IgG1
Species	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Ascites fluid	Ascites fluid
Purification	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography
Fluorescence	Excites at 488 nm Emits at 770 nm	Excites at 633/638 nm Emits at 660 nm	Excites at 633/638 nm Emits at 720 nm	Excites at 633/638 nm Emits at 775 nm	Excites 405 nm Emits at 455 nm	Excites 405 nm Emits at 528 nm
Conjugation	R Phycoerythrin-Cyanine 7 (PC7)	Allophycocyanin (APC)	Allophycocyanin-Alexa Fluor 700 (APC-A700)	Allophycocyanin-Alexa Fluor 750 (APC-A750)	Pacific Blue	Krome Orange
Molar Ratio	PC7 / Protein : 0.5 - 1.5	APC / Protein: 0.5 - 1.5	APC-Alexa Fluor 700 / Protein: 0.5 - 1.5	APC-AlexaFluor750 / Protein: 0.5 - 1.5	Pacific Blue / Protein: 1.7 - 3.2	Krome Orange / Protein: 10.4 - 14.1

Specificity for ClearLLab LS Reagent

The antigen specificity of the CD45, CD3, CD4, CD8, CD56 and CD5 monoclonal antibodies has been previously established by the First (CD4, CD8 and CD3), Third (CD5 and CD45),and Fourth (CD56) International Workshop for Leukocyte Typing.

The antigen specificity of the CD20, CD19, CD10 and CD34 monoclonal antibodies has been previously established by the Human Leukocyte Differentiation Antigen Workshops.[24]

The application of Kappa and Lambda light chain analysis for immunophenotyping B lymphopoetic malignancies has been previously described in the flow cytometric immunophenotyping for hematologic neoplasms.[24]

PERFORMANCE

CLINICAL ACCURACY

An agreement study between the ClearLLab LS on the Navios Flow Cytometer and ClearLLab Reagents (ClearLLab T1, ClearLLab T2, ClearLLab M, ClearLLab B1, ClearLLab B2) on the FC 500 flow cytometer was performed at four (4) external clinical sites. A total of 210 specimens were analyzed (102 WB, 77 BM and 31 LN specimens), and 118 of them were clinically diagnosed with hematologic malignancy, including 80 B-cell malignancies, 11 T/NK malignancies and 27 myeloid malignancies. Analysis indicates that the ClearLLab LS agreed 100% with ClearLLab Reagents in excluding the presence of an abnormal phenotype (104 specimens in total), and 100% agreement in detecting the presence of abnormality (106 specimens in total), with an overall agreement of 100%.

	Test (ClearLLab LS)		
Reference (ClearLLab Reagents)	Presence of Abnormal Phenotype	Absence of Abnormal Phenotype	**Sum**
Presence of Abnormal Phenotype	106	0	106
Absence of Abnormal Phenotype	0	104	104
Sum	106	104	**210**

Additionally, ClearLLab LS achieved 100% agreement for the designation of maturity of the abnormal populations, including 70 mature malignancies and 36 immature malignancies. Meanwhile, the agreement for the designation of lymphoid origin was 99% for both mature & immature lymphoid cases whereas the agreement was 100% when only mature lymphoid cases were considered.

	Test (ClearLLab LS)		
Reference (ClearLLab Reagents)	Mature	Immature	**Sum**
Mature	70	0	70
Immature	0	36	36
Sum	70	36	**106**

LIMIT OF DETECTION

A study was conducted in accordance with CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. Results support a limit of detection (LoD) when collecting 100,000 CD45+events as less than 1% positive populations for all CD markers on the Navios and Navios EX Flow Cytometers.

PRECISION

Within-Run Precision:

The Within-Run precision was assessed for three peripheral blood samples (one specimen per K_2EDTA, Lithium Heparin and ACD-A anticoagulant), three bone marrow samples (one specimen per K_2EDTA, Lithium Heparin and ACD-A anticoagulant), and three lymph node specimens in replicates of 10 each.

	Peripheral Blood			Bone Marrow			Lymph Node		
Marker	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
Kappa+	1.8%	1.5%	0.8%	2.8%	2.2%	*	5.7%	0.8%	3.2%
CD8+	7.5%	4.8%	1.2%	2.2%	2.3%	0.4%	4.4%	2.6%	3.5%
Lambda+	3.2%	2.5%	1.7%	2.9%	7.5%	*	1.2%	1.0%	5.2%
CD4+	1.8%	0.9%	0.8%	0.7%	3.1%	6.3%	0.6%	0.5%	0.4%
CD19	2.4%	1.3%	2.8%	4.9%	4.3%	*	4.9%	4.0%	5.9%
CD56+	2.1%	2.6%	1.6%	4.8%	5.0%	8.7%	*	6.7%	*
CD10+	*	*	*	8.4%	1.5%	5.1%	3.7%	*	*
CD34+	*	*	*	7.7%	3.0%	4.8%	*	*	*
CD5+	2.2%	1.3%	0.5%	1.2%	1.9%	3.8%	5.0%	2.5%	0.5%
CD20+	2.6%	1.3%	3.2%	6.6%	4.2%	*	4.9%	4.3%	6.8%
CD3	2.2%	1.3%	0.3%	1.2%	2.3%	2.9%	4.8%	2.5%	0.6%
CD45+ Lymphs	2.7%	2.9%	2.4%	5.0%	2.0%	6.2%	2.6%	1.5%	0.7%

* denotes CVs that were not assessed as the specimens had <1% positive cells.

The Within-Run precision was assessed for three peripheral blood samples (one specimen per K_2EDTA, Lithium Heparin and ACD-A anticoagulant), one bone marrow samples (one specimen per K_2EDTA), in replicates of 10 each on Navios Ex Flow Cytometers.

Navios Ex Flow Cytometer				
	Peripheral Blood			Bone Marrow
Marker	EDTA	ACD-A	Heparin	EDTA
Kappa+	2.80%	4.90%	5.40%	2.20%
CD8+	2.40%	4.20%	8.90%	3.80%
Lambda+	3.40%	4.20%	2.80%	3.40%
CD4+	1.50%	2.00%	3.30%	2.80%
CD19	2.20%	2.90%	4.70%	8.40%
CD56+	3.90%	4.70%	4.70%	5.50%
CD10+	1.1	1.00%	3.20%	6.90%
CD34+	*	*	*	4.50%
CD5+	0.60%	0.80%	1.00%	2.60%
CD20+	2.20%	3.10%	4.70%	4.80%
CD3	0.90%	0.70%	1.10%	1.40%
CD45+ Lymphs	0.80%	0.40%	3.60%	2.70%

Within-Laboratory Precision:
Control cells (IMMUNO-TROL Cells, PN 6607077 and Stem-Trol Control Cells, PN IM3632) were processed as per the ClearLLab LS protocol by 3 operators performing two runs per day, two repeats per run over 5 days on two Navios Flow Cytometers. The variability of the specificities for 119 data points is reported in the table below.

Sample Type	Kappa	CD8	Lambda	CD4	CD19	CD56	CD10	CD34	CD5	CD20	CD3	CD45
n	119	119	119	119	119	119	119	119	119	119	119	119
Repeatability CV	1.7%	3.3%	2.4%	2.2%	2.8%	5.8%	3.3%	6.8%	1.4%	2.4%	1.0%	0.9%
Reproducibility CV	2.1%	3.3%	3.0%	3.0%	4.6%	6.8%	3.3%	6.8%	1.8%	5.1%	1.1%	1.2%

Control cells (IMMUNO-TROL Cells, PN 6607077 and Stem-Trol Control Cells, PN IM3632) were processed as per the ClearLLab LS protocol by 3 operators performing two runs per day, two repeats per run over 5 days on two Navios Ex Flow Cytometers. The variability of the specificities for 120 data points is reported in the table below.

Marker	Kappa	CD8	Lambda	CD4	CD19	CD56	CD10	CD34	CD5	CD20	CD3
n	120	120	120	120	120	120	120	120	120	120	120
Repeatability CV	2.1% (1.15)	2.1% (0.41)	2.5% (1.04)	1.9% (0.66)	2.2% (0.26)	4.0% (0.39)	1.7% (0.92)	5.4% (0.11)	0.4% (0.32)	11.1% (0.05)	0.5% (0.38)
Reproducibility CV	5.2% (1.70)	8.4% (1.29)	9.5% (2.00)	7.7% (1.63)	10.5% (1.12)	9.9% (0.99)	9.8% (2.33)	13.5% (0.53)	1.3% (0.97)	14.8% (0.25)	1.1% (0.92)

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PRODUCT AVAILABILITY

ClearLLab LS – 25 tests
REF B74073

TRADEMARKS

For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-742-2345 (USA or Canada) or contact your local Beckman Coulter Representative.

Glossary of Symbols is available at beckman.com/techdocs (PN C05838).

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EC REP

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Revision History

Revision AC, 07/2017

Changes were made to:

- Intended Use
- Quality Control
- Materials Required
- Performance

Revision AD, 10/2018

Changes were made to:

- Add new languages

Revision AE, 08/2019

Changes were made to:

- Add new languages
- STAINING PROCEDURE
- LIMITATIONS