CLEAR**LL**AB LS
LYMPHOID SCREEN REAGENT
CE MARKED ANTIBODY COMBINATION
FOR LEUKEMIA / LYMPHOMA ANALYSIS

CASEBOOK

EVERY *event matters.*

BECKMAN COULTER
Life Sciences

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INTRODUCTION

This casebook has been designed to assist in the analysis of flow cytometric immunophenotyping data generated using Beckman Coulter's ClearLLab LS Lymphoid Screen reagent on the Beckman Coulter Navios flow cytometer.

Cases with characteristic findings typical of various lymphoid and myeloid neoplasms are included, as are cases from patients with clinical and/or laboratory findings that suggest an underlying neoplastic process, but in which no immunophenotypic abnormality is identified. Specimen types include peripheral blood, bone marrow, and lymph nodes.

Each case includes a clinical vignette that describes the patient demographics and clinical history, case-specific listmode data files for reanalysis by the user of this casebook, ClearLLab LS-specific analysis protocols to be used with the listmode data, and a report showing the analysis with provided protocols. Each report includes analysis notes that highlight the immunophenotypic findings as well as potential pitfalls.

NOTE: Casebook examples are provided for illustrative purposes only, and not all categories of hematolymphoid neoplasms may be represented, nor are all possible immunophenotypic variants described or demonstrated.

BACKGROUND

Flow cytometric immunophenotyping evaluates the presence and absence of specific antigens for each individual cell present in the specimen. When taken together, these results generate an immunophenotypic profile for each cell which is either consistent with an expected population (i.e. normal) or inconsistent with an expected population (i.e. aberrant) in that sample type. When evaluating samples from patients with suspected hematolymphoid malignancies, several steps are involved [1]:

• Assessment of all cell populations in the sample.

• Assignment of each cell population to either "normal" or "aberrant".

• Detailed characterization of the aberrant population according to the presence or absence of antigens as well as increased or decreased intensity of staining by fluorochrome-labeled antibodies.

• Interpretation of the aberrant immunophenotype, incorporating where available additional information such as clinical history, histology, cytology, immunohistochemistry, and genotyping studies such as *in situ* hybridization, karyotyping, and molecular diagnostics.

CONSENSUS RECOMMENDATIONS FOR IMMUNOPHENOTYPING

Consensus recommendations for flow cytometric immunophenotyping of samples from patients with known or suspected hematolymphoid malignancies have emerged over the last two decades, and several guidelines have been published in the scientific literature.

Flow cytometric immunophenotyping has been included in the WHO classification of Tumors of Haematopoetic and Lymphoid Tissues since 2008 [2].

Medical indications and flow cytometry assay validation including pre-analytic, analytic, and post-analytic details of testing are addressed in the 2006 Bethesda International Consensus Conference recommendations [3, 4, 5] and the ICSH/ICCS practice guidelines for cell-based fluorescence assays [6, 7, 8].

ClearLLab LS LYMPHOID SCREEN REAGENT INTENDED USE

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. This 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 or Heparin), bone marrow (collected in EDTA, ACD, or Heparin) and lymph node specimens for immunophenotyping. The results should be interpreted along with additional clinical and laboratory findings. These reagents provide qualitative results for T, B and NK lineages.

ClearLLab LS LYMPHOID SCREEN REAGENT (PART NUMBER B74073)

PART NUMBER	405 nm EXCITATION		488 nm EXCITATION					638 nm EXCITATION		
	PB[1]	KrO[2]	FITC	PE	ECD	PC5.5	PC7	APC	APC-AF700[3]	APC-AF750[4]
B74073	CD3	CD45	Kappa/CD8	Lambda/CD4	CD19	CD56	CD10	CD34	CD5	CD20

1. Pacific Blue   2. Krome Orange   3. APC Alexa Fluor 700   4. APC Alexa Fluor 750

The above reagent is provided in a standardized format to be used with reagents for sample preparation and cytometer set-up, along with software for data acquisition and analysis. ClearLLab LS reagent meets recommendations for standardization as outlined by the Bethesda guidelines [2].

Additional information regarding ClearLLab LS Lymphoid Screen Reagent is available at: **beckman.com/clearllab-ls**

REFERENCES

1. Flow Cytometric Immunophenotyping for Hematologic Neoplasms. F.E. Craig, K.A. Foon. Blood. 2008; 111; 3941-3967.

2. Swerdlow SH, Campo E, Harris NL, Jaffe EA, Pileri SA, Stain H, Thiele J, & Vardiman JW (eds) (2008) WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon

3. 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

4. 2006 Bethesda International Consensus recommendations on the flow cytometric immunophenotypic analysis of hematolymphoid neoplasia: medical indications. Davis BH, Holden JT, Bene MC, Borowitz MJ, Braylan RC, Cornfield D, Gorczyca W, Lee R, Maiese R, Orfao A, Wells D, Wood BL, Stetler-Stevenson M. Cytometry B Clin Cytom. 2007;72 Suppl 1:S5-13

5. 2006 Bethesda International Consensus Conference on Flow Cytometric Immunophenotyping of Hematolymphoid Neoplasia. Stetler-Stevenson M, Davis B, Wood B, Braylan R. Cytometry B Clin Cytom. 2007;72 Suppl 1:S3

6. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part III - analytical issues. Tanqri S, Vall H, Kaplan D, Hoffman B, Purvis N, Porwit A, Hunsberger B, Shankey TV; ICSH/ICCS Working Group. Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):291-308. doi: 10.1002/cyto.b.21106

7. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part IV - postanalytic considerations. Barnett D, Louzao R, Gambell P, De J, Oldaker T, Hanson CA; ICSH/ICCS Working Group. Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):309-14. doi: 10.1002/cyto.b.21107

8. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part V - assay performance criteria. Wood B, Jevremovic D, Béné MC, Yan M, Jacobs P, Litwin V; ICSH/ICCS Working Group. Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):315-23. doi: 10.1002/cyto.b.21108

RELATED DOCUMENTS

•   ClearLLab LS (Lymphoid Screen) Reagent Instructions for Use, PN B74073

•   VersaLyse Lysing Solution Instructions for Use, PN A09777

•   Navios and Navios EX Systems

CASES

The listmode data presented in this case book were generated following the procedure detailed within the ClearLLab LS Lymphoid Screen reagent Instructions For Use (IFU) available at **beckman.com**.

Representative cases were selected from clinical trial data and were reviewed, annotated, and interpreted by Hematopathologist, Jeannine T. Holden MD MBA, and Director of Scientific Affairs for Beckman Coulter Inc.

View the protocol file and explore the listmode data files linked within the cases below.

NO IMMUNOPHENOTYPIC ABNORMALITY

Flow cytometry is a means of characterizing leukocyte populations. It can aid in the differential diagnosis of hematologically abnormal patients having, or suspected of having hematopoietic neoplasia including chronic leukemia, acute leukemia, non-Hodgkin lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). Crucial to the identification of aberrant populations in these clinical situations is the familiarity with normal cell populations present in bone marrow, whole blood and lymph node tissue samples. The following are examples of normal samples stained with ClearLLab LS reagent.

PERIPHERAL BLOOD

Case #1: Normal Whole Blood

Clinical Vignette

This 64-year-old male presents with mild lymphocytosis. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

Access Case #1 list mode data
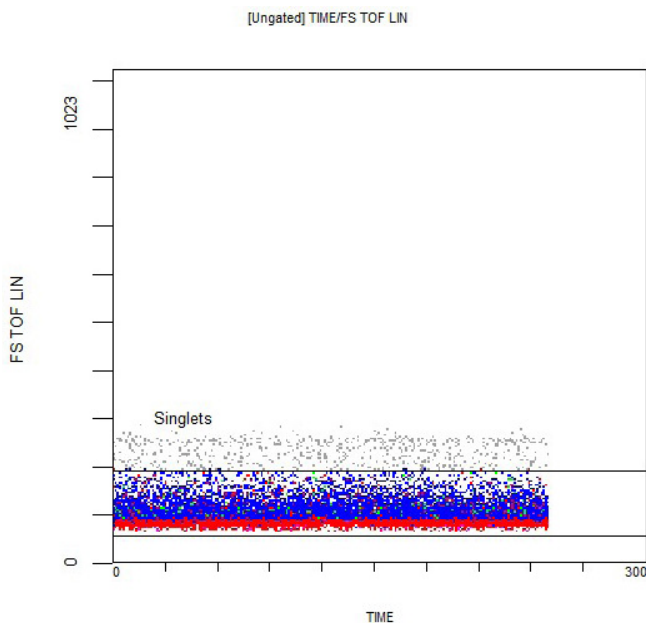
This TIME/FS Time of Flight dot plot is a helpful first look at your data. The smooth and uninterrupted acquisition of events displayed here is the expected result. A "Singlets" gate may be defined here and applied to other dot plots in order to exclude coincident events resulting from cells adhering to each other. All subsequent dot plots in this analysis employ the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.
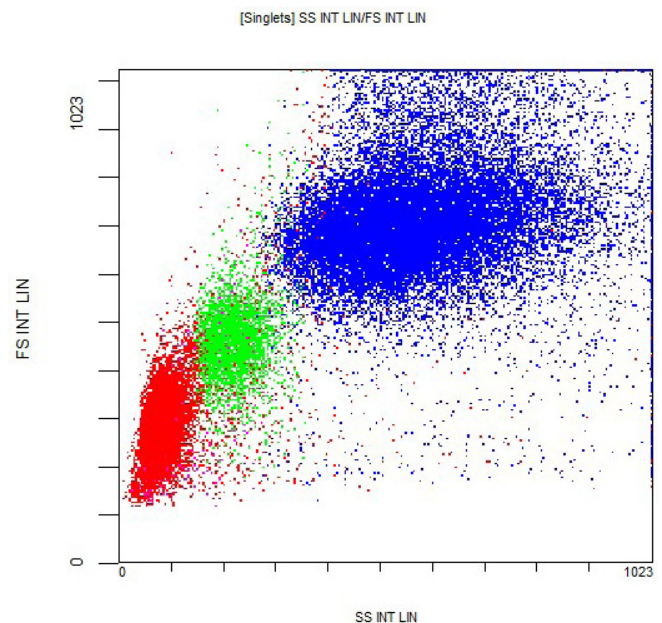
This Side Scatter/Forward Scatter dot plot demonstrates lymphocytes (red), monocytes (green), and granulocytes (blue).

[Singlets] FL10 INT LOG/SS INT LIN
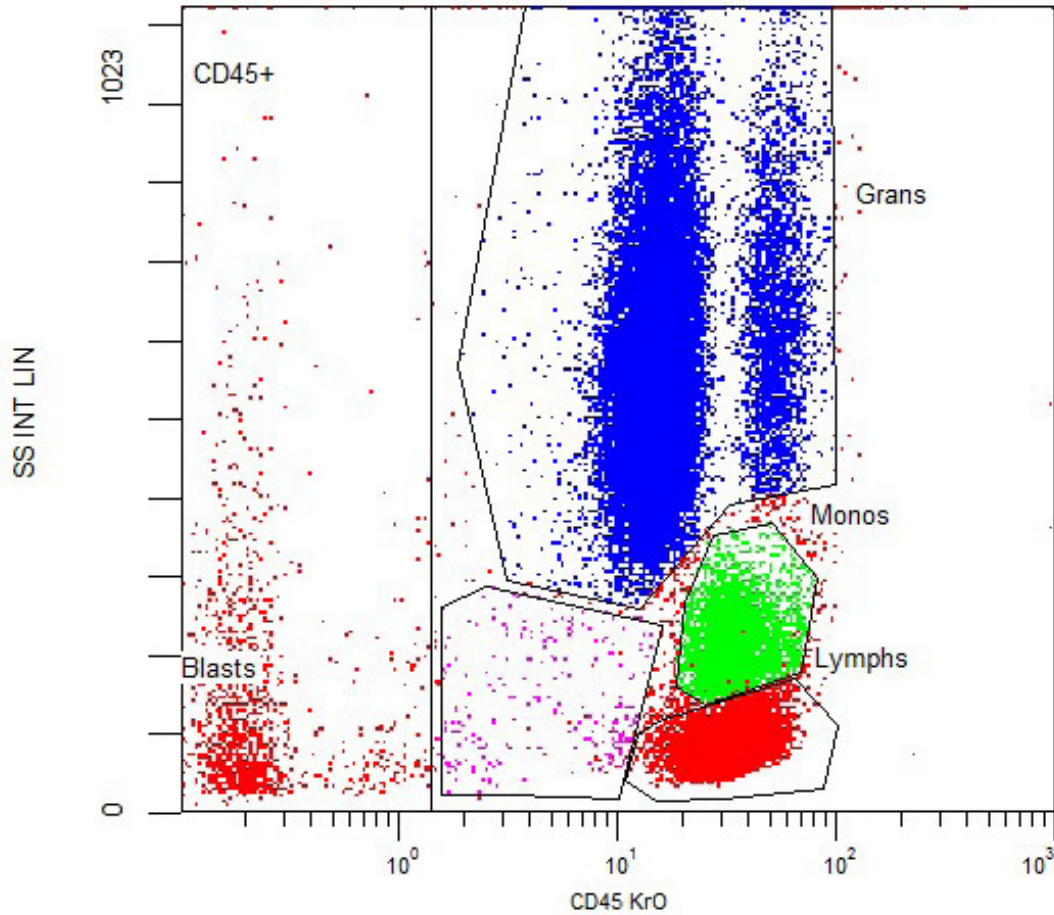
This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). The "Blast" gate contains only a few events. Note that the label for the Blast gate is not immediately adjacent to the gate, unlike normal lymphocytes, monocytes, and granulocytes, blasts and other phenotypically aberrant populations do not conform to any predictable pattern. A CD45+ gate may be used to exclude CD45 negative debris but these events should not be ignored when analyzing a case, as some aberrant populations are CD45 negative. In this case the CD45 negative events are consistent with debris.

Note the mild degree of overlap in this case between apparent monocytes and lymphocytes. The gates should be adjusted such that they conform to the naturally occurring separations among the populations, but where no separation is observed an estimate based on experience may be used.
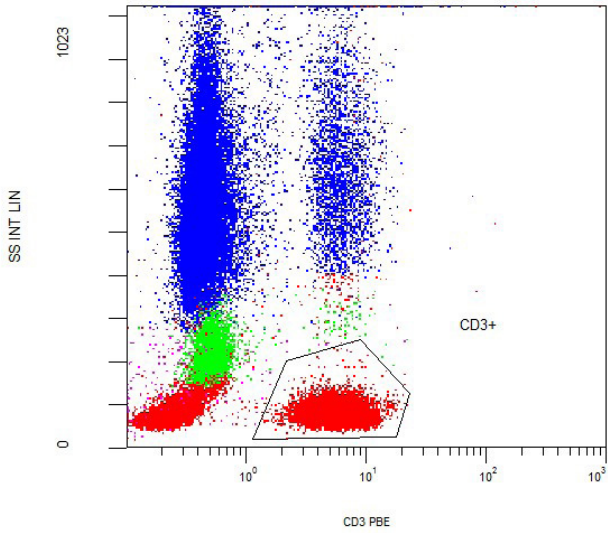
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.
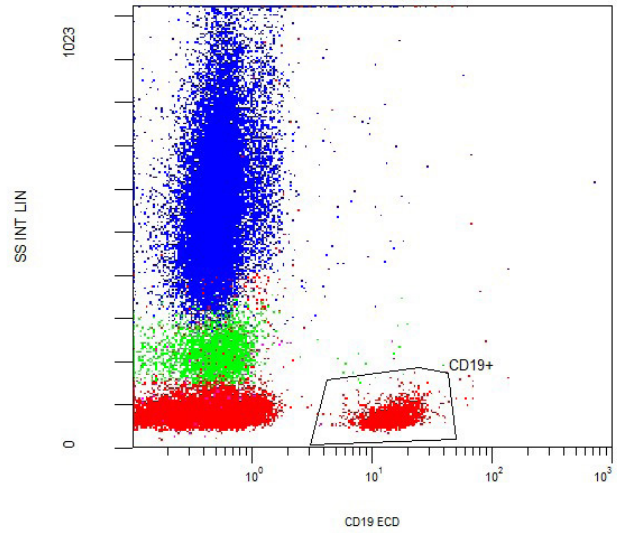
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only.
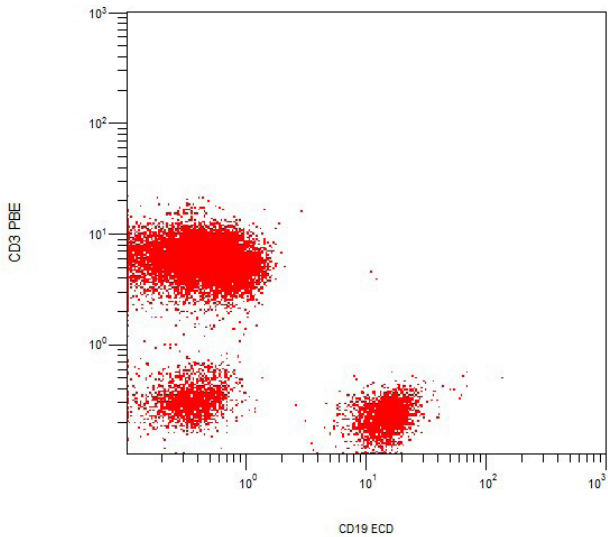
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present.
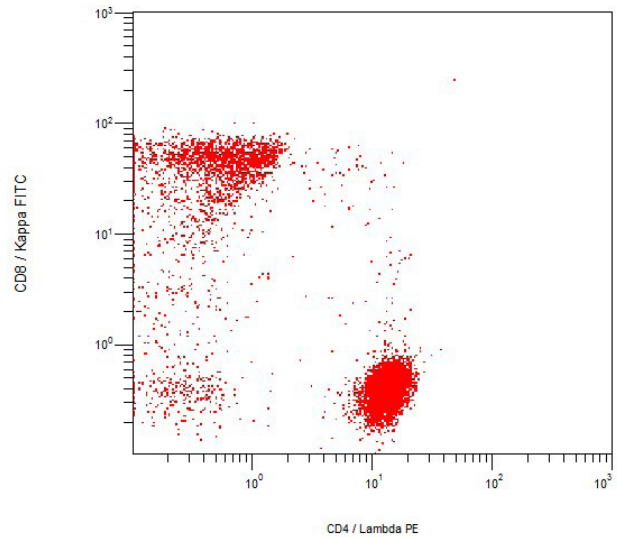
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes. A small population of CD4 and CD8 dual negative T lymphocytes is noted.

No Immunophenotypic Abnormality > Peripheral blood > Case #1: Normal Whole Blood
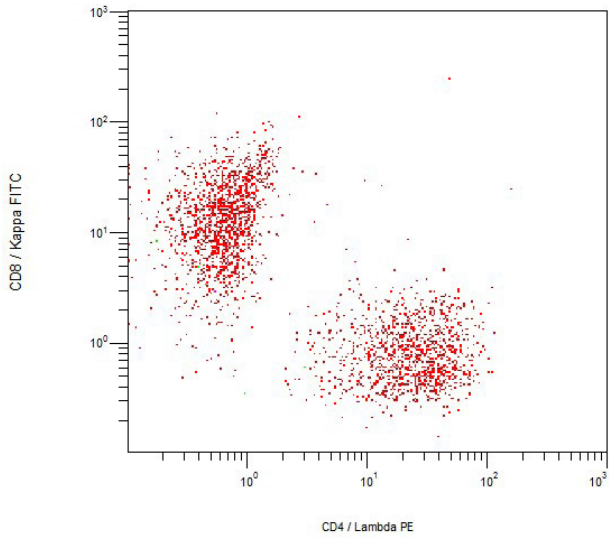
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations.
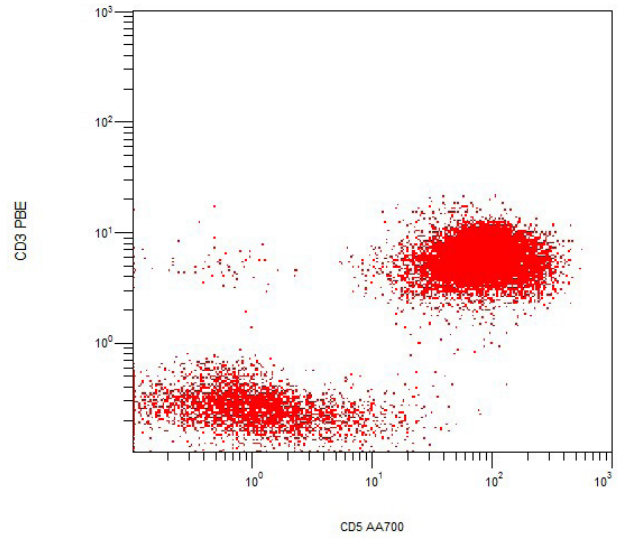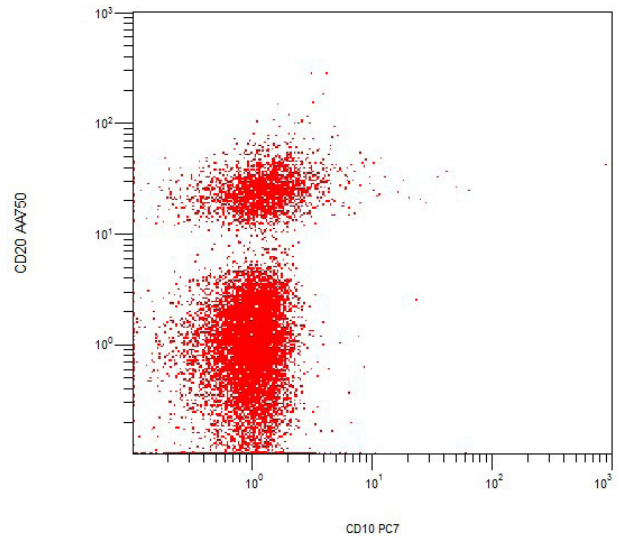
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable CD3 positive, CD5 positive T lymphocytes comprise the majority of cells. The remaining cells are a mixture of B lymphocytes and NK cells.
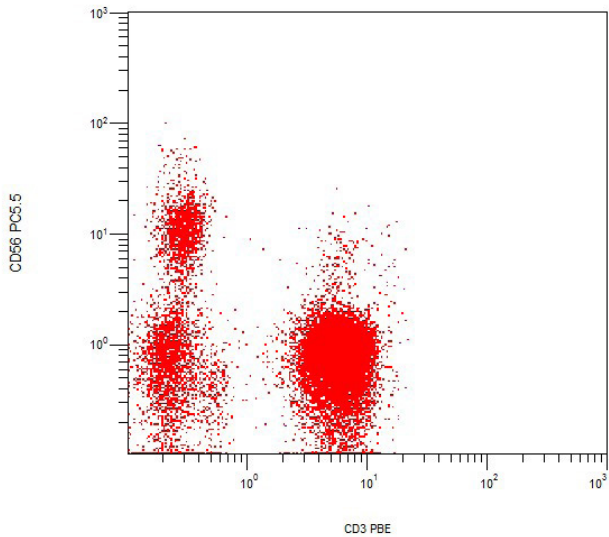
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. A small population of CD56 positive T lymphocytes is noted. The CD3 and CD56 dual negative cells are B lymphocytes.
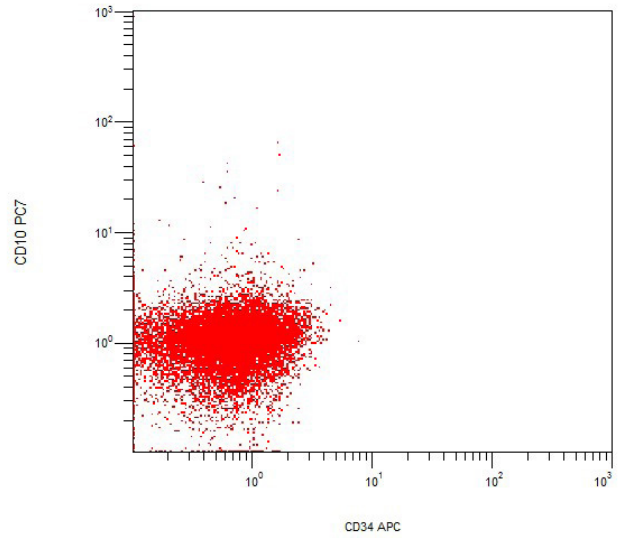
This CD10/CD20 dot plot is gated on Lymphocytes. No distinct population of B lymphocytes that co-express CD10 is noted.

No Immunophenotypic Abnormality > Peripheral blood > Case #1: Normal Whole Blood
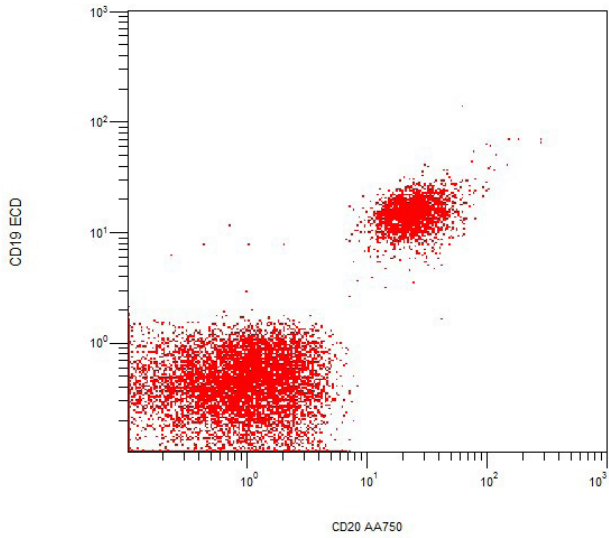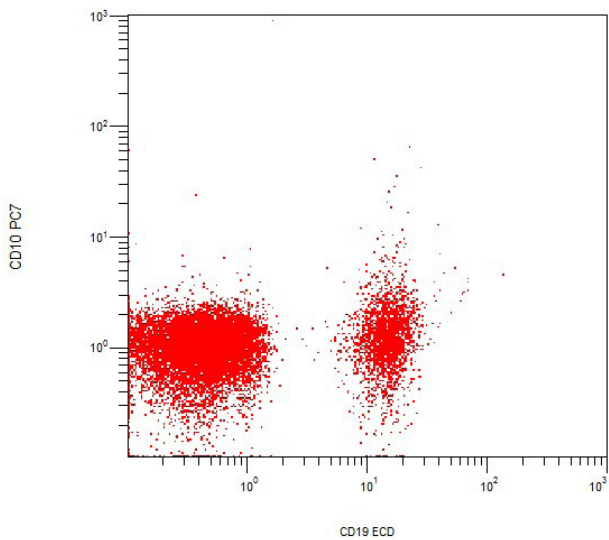
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display expected normal co-expression of CD19 and CD20.
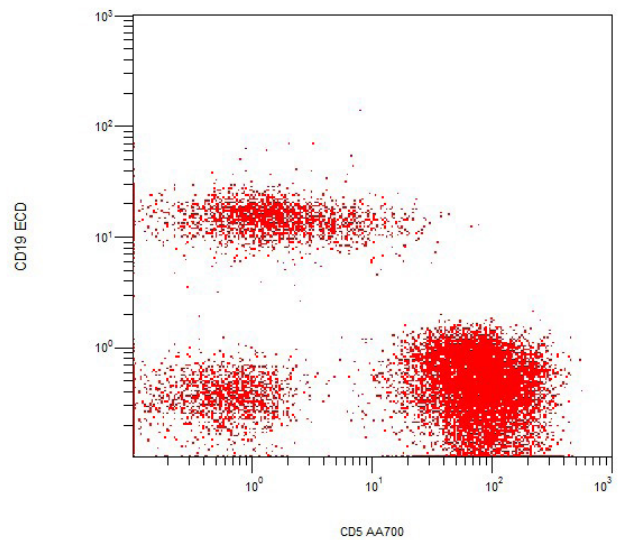
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, as expected for mature peripheral blood lymphocytes.
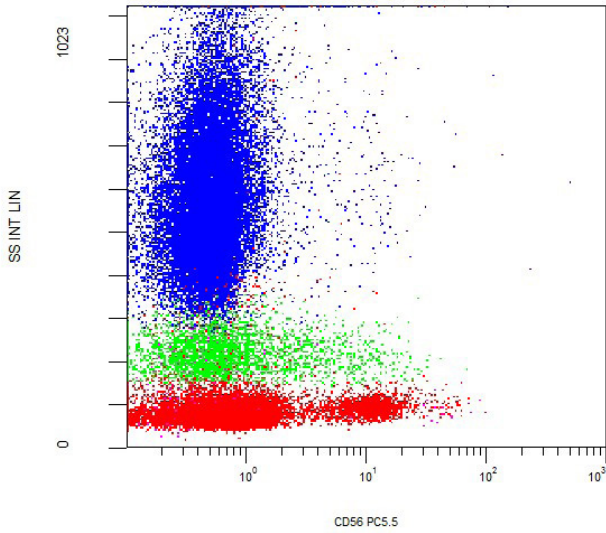
This CD19/CD10 dot plot is gated on Lymphocytes. B lymphocytes comprise the minority of lymphocytes in this peripheral blood sample, as expected.
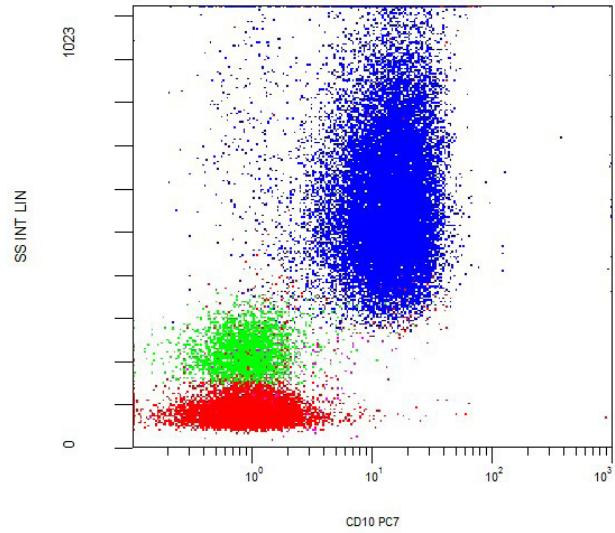
This CD5/CD19 dot plot is gated on Lymphocytes. Possible low density co-expression of CD5 and CD19 is identified on a small subset of B lymphocytes. Additional analysis of this population could be performed in order to establish or rule out immunoglobulin light chain restriction within this compartment.

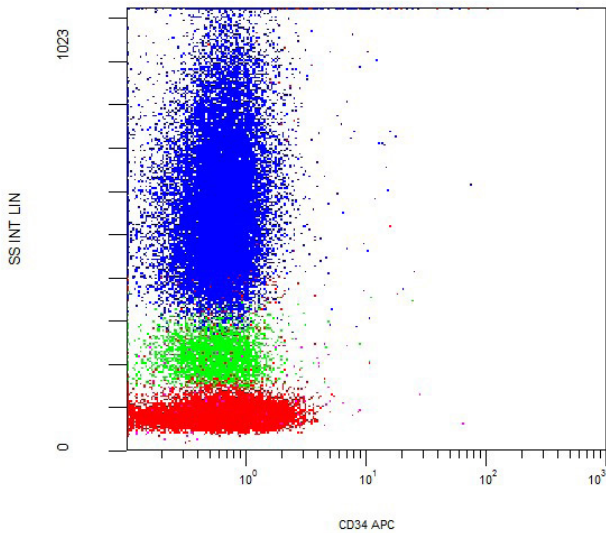No Immunophenotypic Abnormality > Peripheral blood > Case #1: Normal Whole Blood

This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells. Some co-expression of CD56 is also noted on monocytes here.
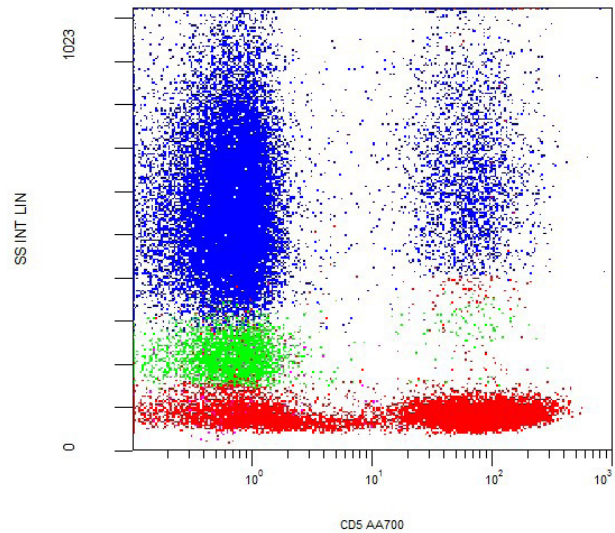
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are positive for CD10.
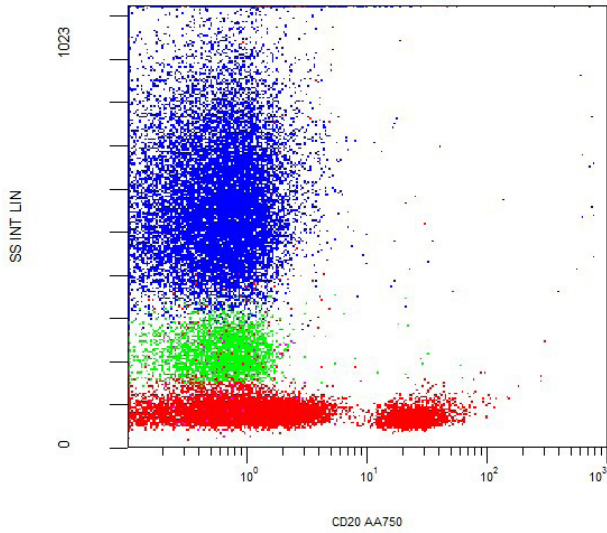
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No significant CD34 positive population is present.
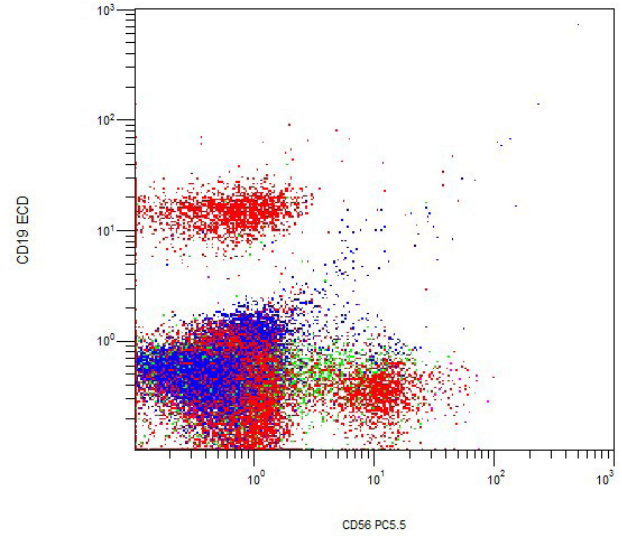
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The majority of the lymphocytes (red) express CD5, consistent with T lymphocytes.

No Immunophenotypic Abnormality > Peripheral blood > Case #1: Normal Whole Blood
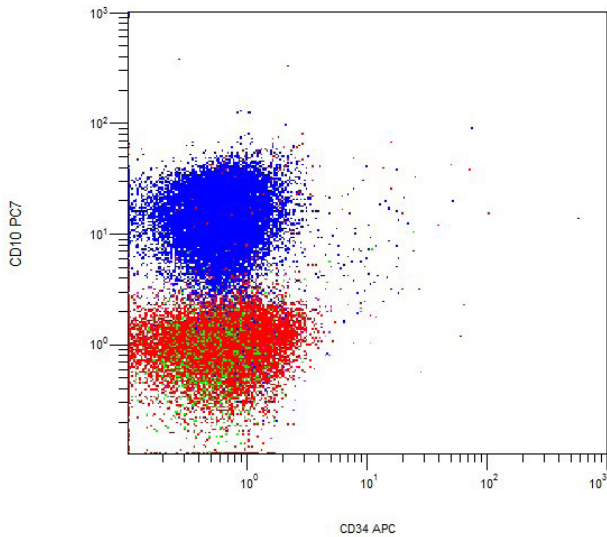
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). As noted elsewhere in this case, B lymphocytes are present but represent a minority of lymphocytes.

This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes and NK cells are present. The blue events noted on the diagonal are consistent with high background fluorescence.
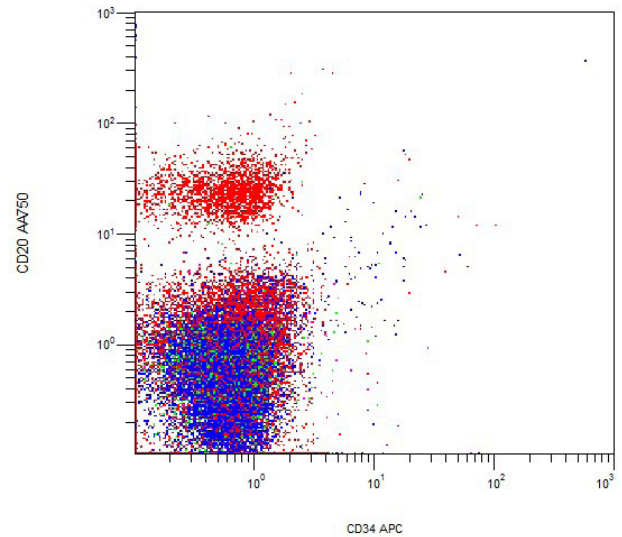
This CD34/CD10 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). As noted elsewhere in this analysis, granulocytes (blue) express CD10.
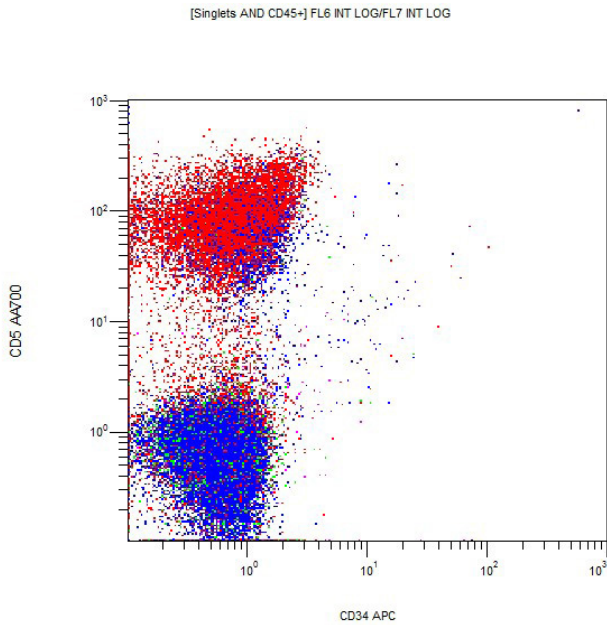
This CD34/CD20 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No co-expression of CD20 and CD34 is noted.

No Immunophenotypic Abnormality > Peripheral blood > Case #1: Normal Whole Blood

[Singlets AND CD45+] FL6 INT LOG/FL7 INT LOG

This CD34/CD5 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No co-expression of CD34 and CD5 is noted.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies no immunophenotypically aberrant populations in this case. Note that correlation with clinical and laboratory data is recommended, and that a malignant process cannot be ruled solely on the basis of this assay.

This is the end of the preview. If you want to download the entire casebook, please visit becls.co/3n8GUsD.