CLEARLLAB 10C PANELS

IVD ANTIBODY COMBINATIONS FOR LEUKEMIA / LYMPHOMA* ANALYSIS

CASEBOOK

EVERY
event matters.

* Non-Hodgkin Lymphoma only

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 10C Panels IVD marked reagent for Leukemia and Lymphoma analysis on the Beckman Coulter Navios and Navios EX flow cytometers.

Sample 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 whole 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 10C 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 10C PANELS INTENDED USE

The ClearLLab 10C Panels are intended for in vitro diagnostic use for qualitative identification of cell populations by multiparameter immunophenotyping on the Navios and Navios EX flow cytometers. 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 K_2EDTA, Acid Citrate Dextrose (ACD) or Heparin), bone marrow (collected in K_2EDTA, Acid Citrate Dextrose (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 surface antigens listed below:

ClearLLab 10C Panels		Blue Laser					Red Laser			Violet Laser	
PN	**Tube**	**FITC**	**PE**	**ECD**	**PC5.5**	**PC7**	**APC**	**APC-A700**	**APC-A750**	**PB**	**KRO**
B96805	B Cell Tube	Kappa	Lambda	CD10	CD5	CD200	CD34	CD38	CD20	CD19	CD45
B96806	T Cell Tube	TCRγδ	CD4	CD2	CD56	CD5	CD34	CD7	CD8	CD3	CD45
B96807	M1 Cell Tube	CD16	CD7	CD10	CD13	CD64	CD34	CD14	HLA-DR	CD11b	CD45
B96808	M2 Cell Tube	CD15	CD123	CD117	CD13	CD33	CD34	CD38	HLA-DR	CD19	CD45

ClearLLab COMPENSATION KIT

	Blue Laser					Red Laser			Violet Laser	
PN	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700	APC-A750	PB	KRO
B74074	CD4	CD4	CD3	CD4	CD4	CD4	CD4	CD4	CD4	CD8

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 10C Panels meet recommendations for standardization as outlined by the Bethesda guidelines[2].

Additional information regarding ClearLLab 10C Panels is available at **beckman.com/ClearLLab**.

CASE SELECTION AND INTERPRETATION

The data presented in this case book was generated following the procedure detailed within the ClearLLab 10C Panel Instructions For Use (IFU) available at **beckman.com**.

Representative cases were selected from clinical trial data and were reviewed, annotated, and interpreted by:

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We wish to thank our Principal Investigators & clinical trial sites for their contribution to the clinical trial and to the development of this casebook:

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Analysis Protocols:
Download the ClearLLab 10C analysis protocol.

Analysis:
Download case specific Kaluza C analysis files.

RELATED DOCUMENTS

- ClearLLab 10C Application System Guide, PN C24688
- Kaluza C Flow Cytometry Software Instructions For Use, PN C10993
- Navios Flow Cytometer Instructions For Use, PN A96247
- Navios EX Flow Cytometer Instructions For Use, PN B73084AB
- ClearLLab 10C Panels Instructions For Use, PN C00197
- ClearLLab Compensation Beads Instructions For Use, PN C00201
- ClearLLab Compensation Kit Instructions For Use, PN B74074
- ClearLLab Control Cells Instructions For use, PN B99884
- ClearLLab Control Cells QC Analysis Protocols Download Addendum, PN C31984

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. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Swerdlow SH, et al. Blood. 2016;127:2375-90.
4. The 2016 revision of the World Health Organization classification of myeloid neoplasms and acute leukemia. Arber DA, et al. Blood 2016 127:2391-2405.
5. 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
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7. 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
8. 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
9. 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
10. 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.2110

CASES

The following Color Precedence Gating is applied to the cases:

- Lymphocytes (Gate Ly)/NK cells: red
- CD19+ B cells: orange
- CD3+ T cells: aqua
- Monocytes (Gate Mo): green
- Granulocytes (Gate Gr): blue
- CD45dim: purple
- Additional Aberrant populations: teal
- CD45 negative population: gray

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 familiarity with normal cell populations present in whole blood, bone marrow and lymph node tissue samples. The following are examples of normal samples stained with ClearLLab 10C panels.

PERIPHERAL WHOLE BLOOD

Case #1: Normal Whole Blood

Clinical Vignette

This 65 year old male presents with thrombocytopenia. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

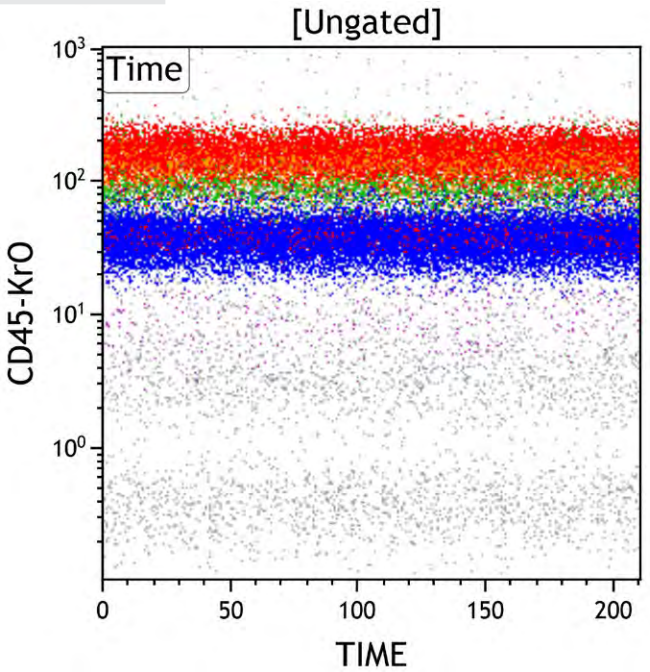

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

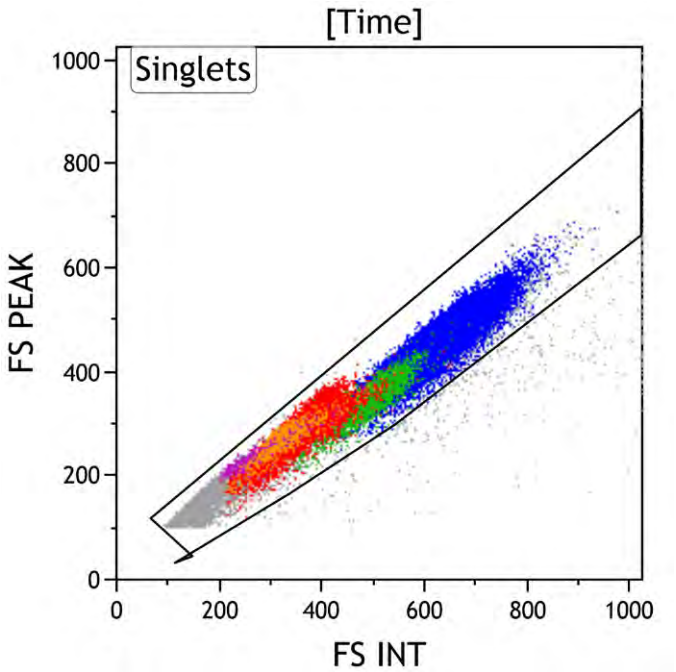

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

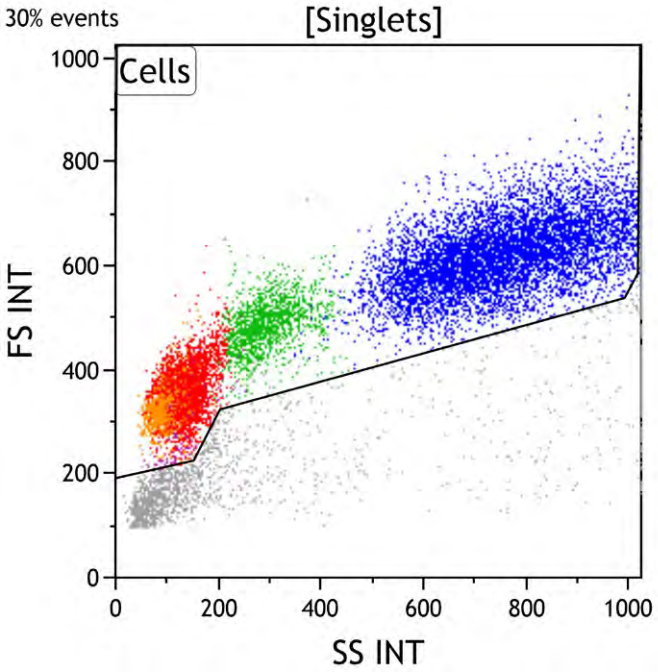

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

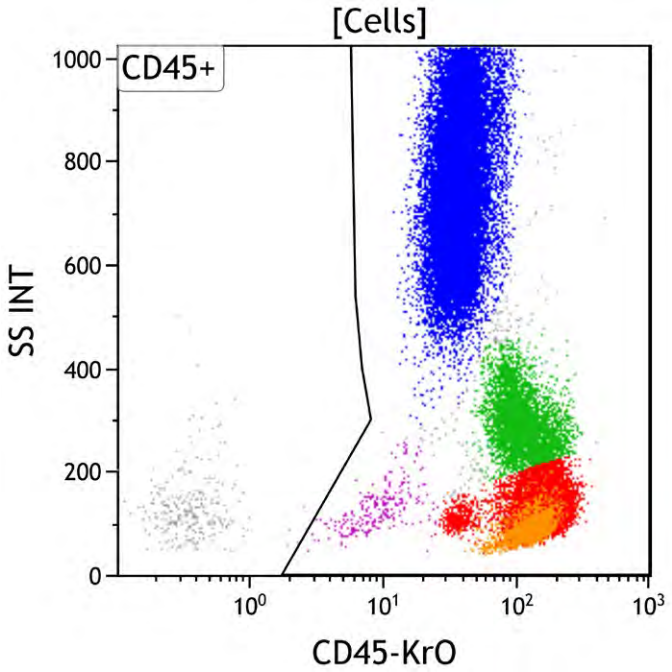

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

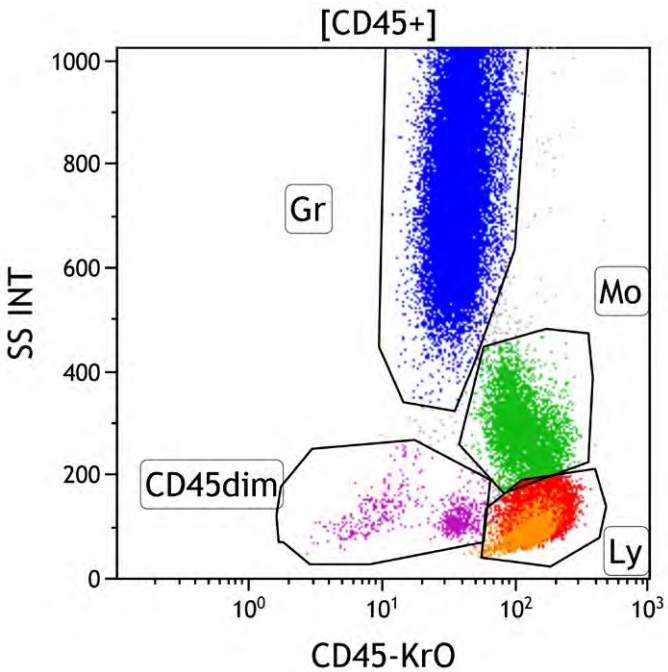

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

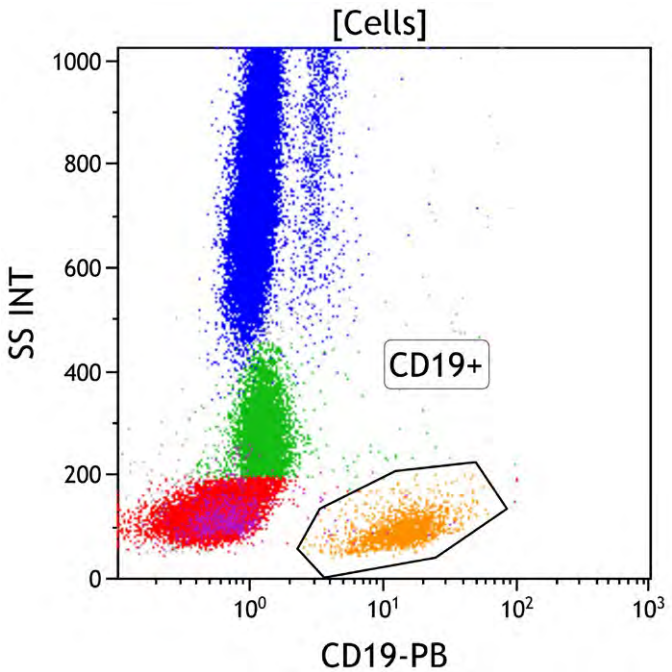

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B Cell Tube

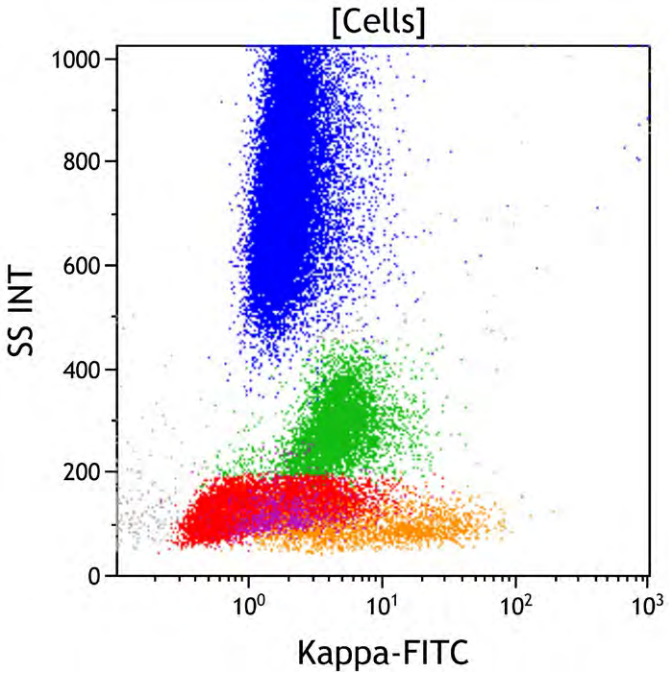

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot.

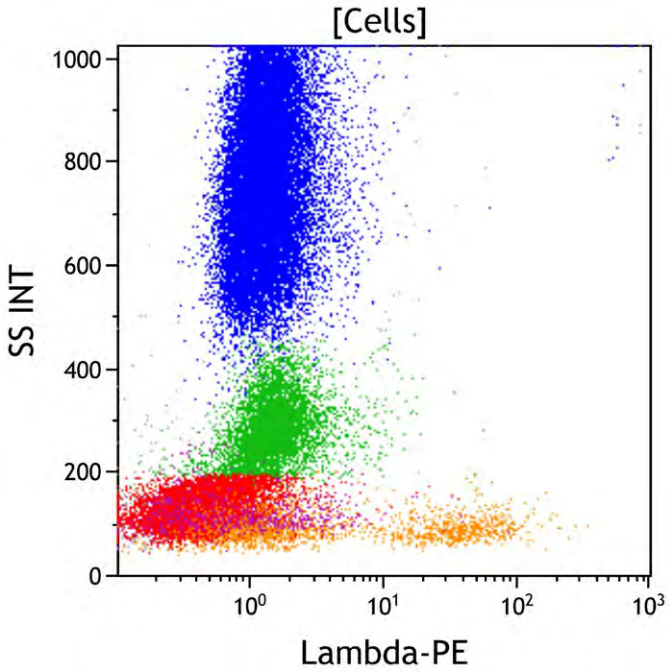

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot.

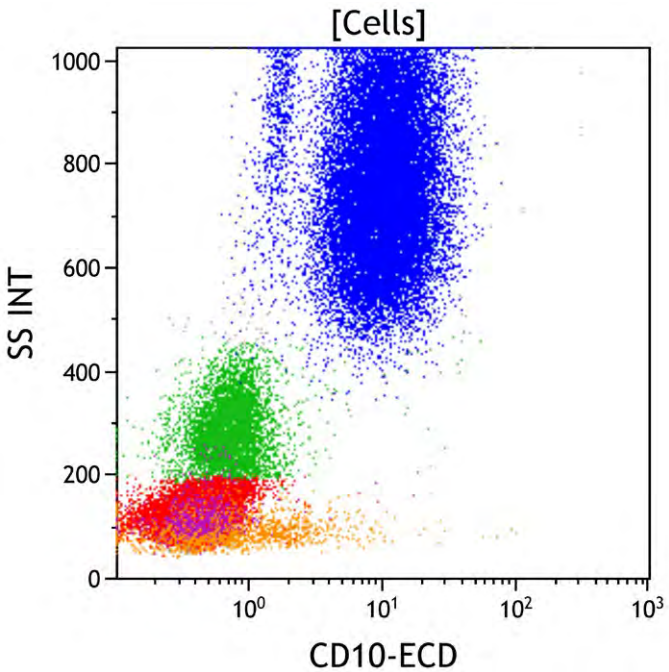

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

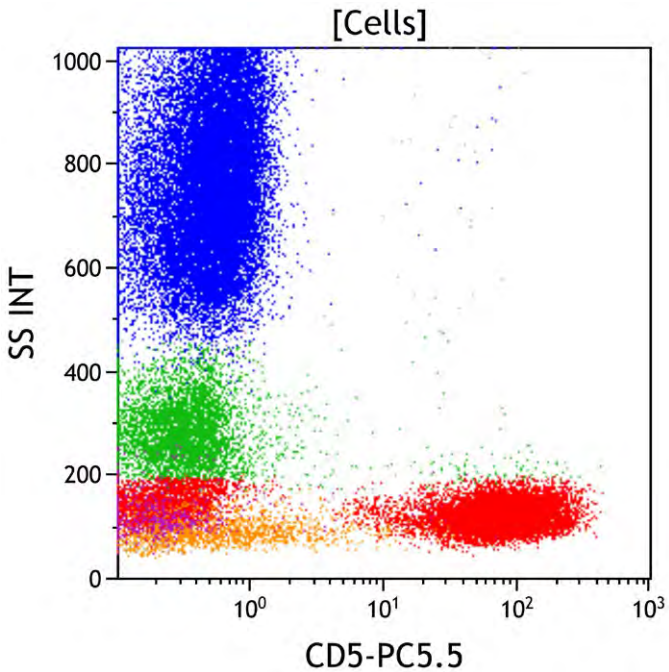

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red, lower right), as well as dimly in a subset of mature B cells (orange). These lymphoid cells typically have low side scatter.

B Cell Tube

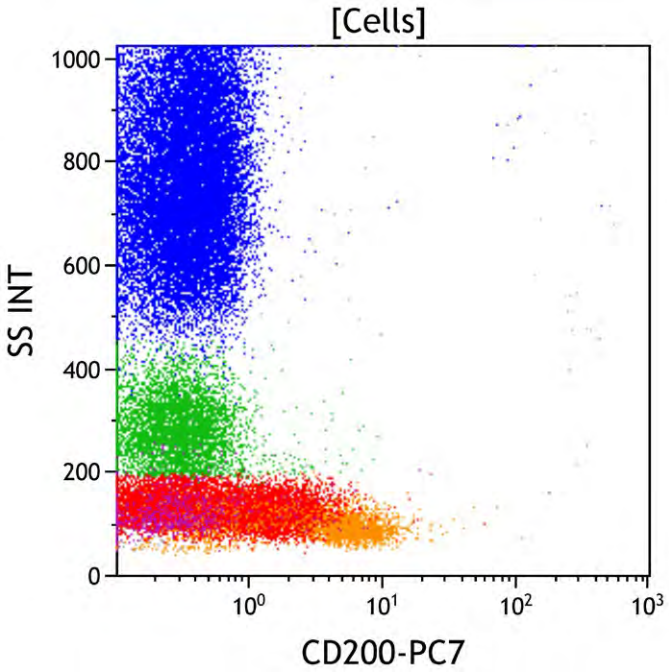

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive).

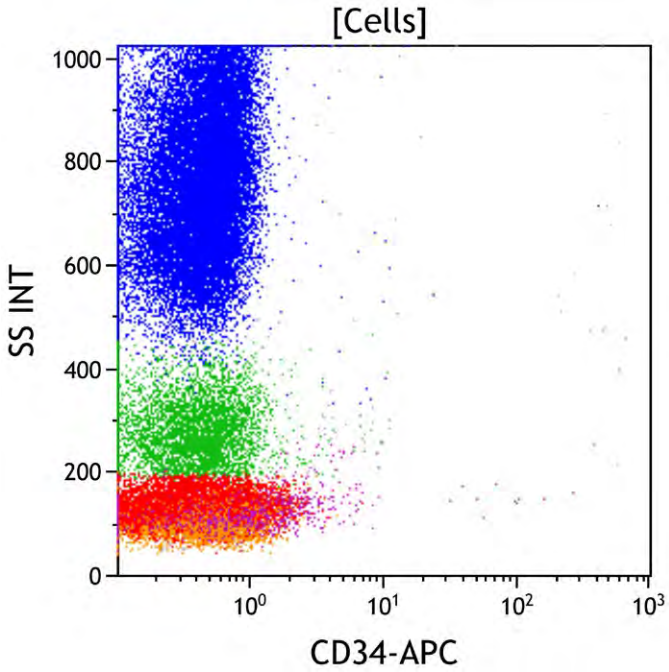

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

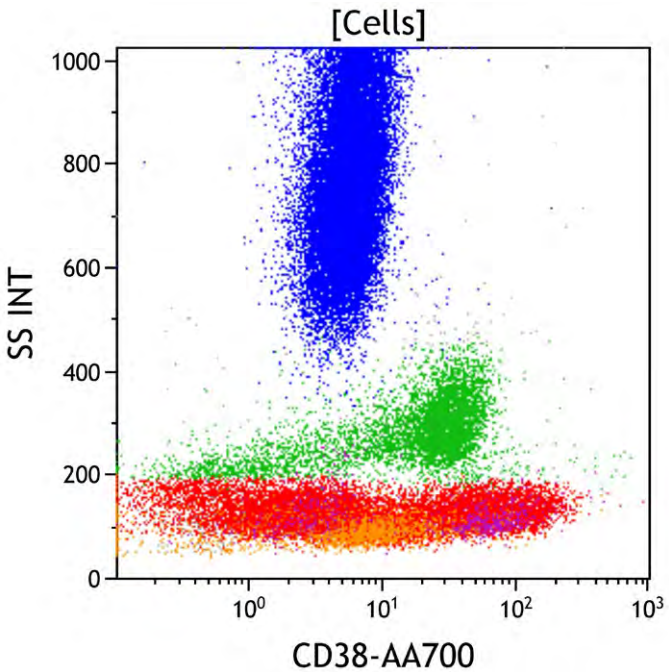

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes.

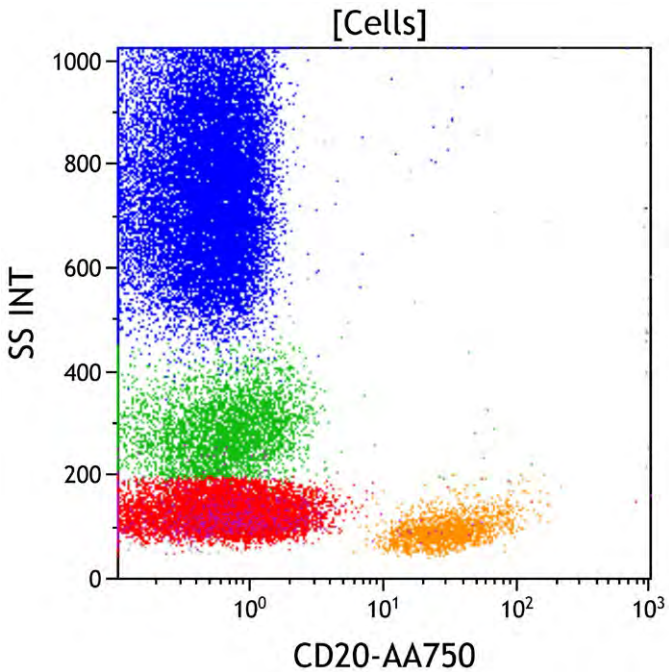

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.

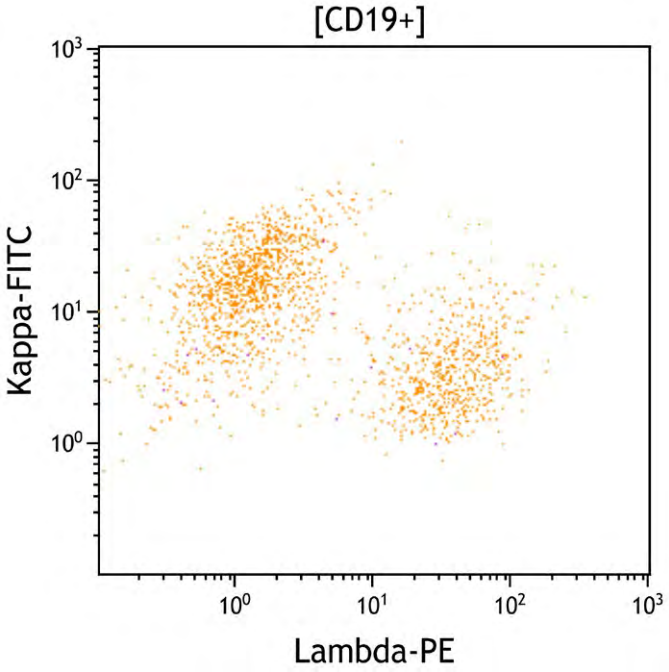

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

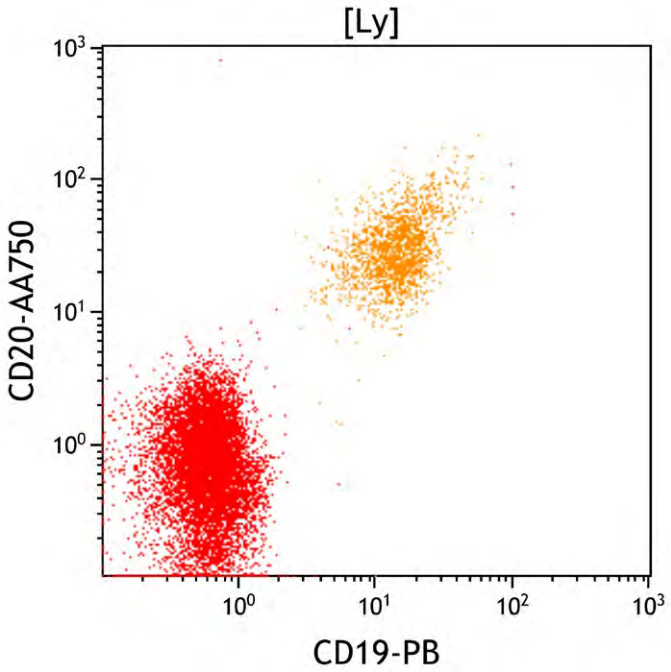

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression.

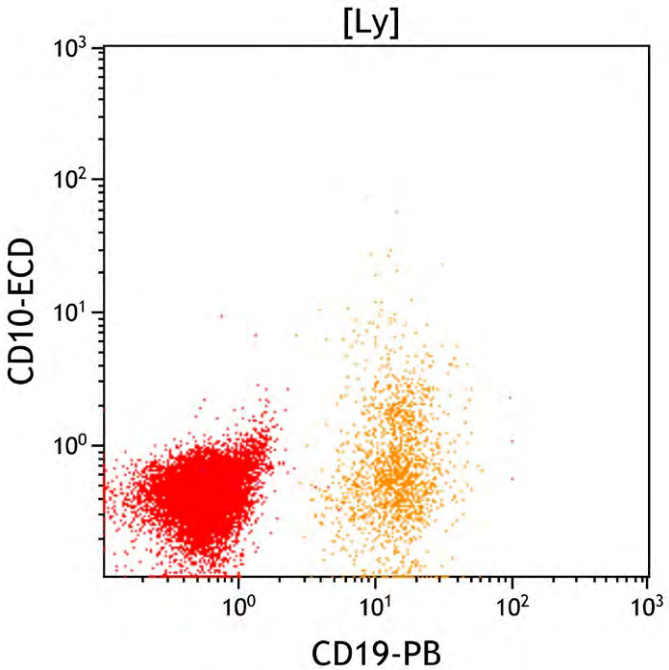

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates.

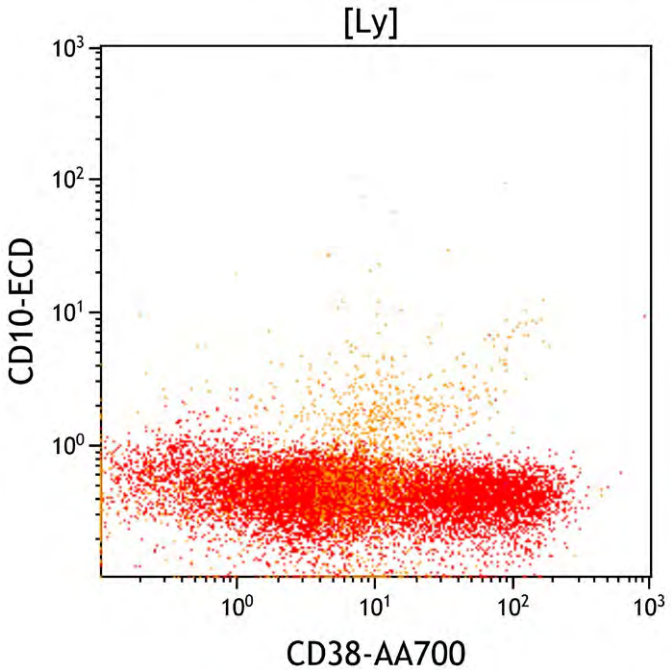

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state.

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B Cell Tube

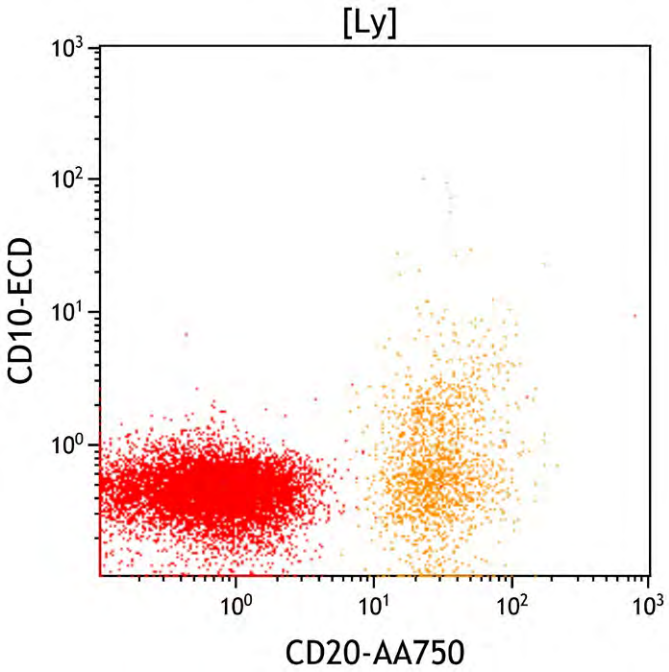

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20 without CD10.

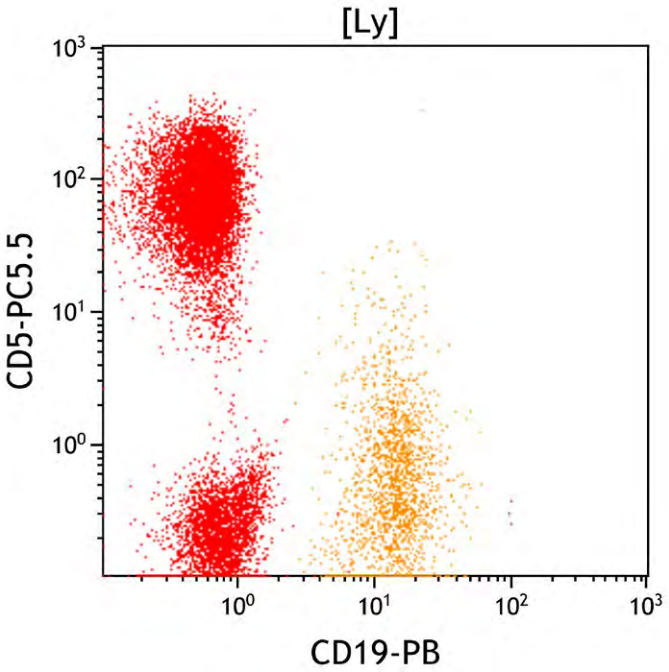

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red, upper left), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

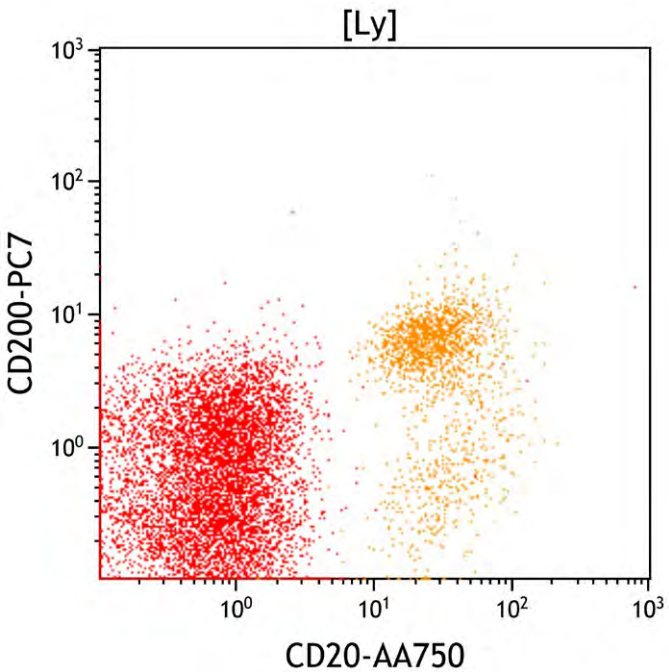

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

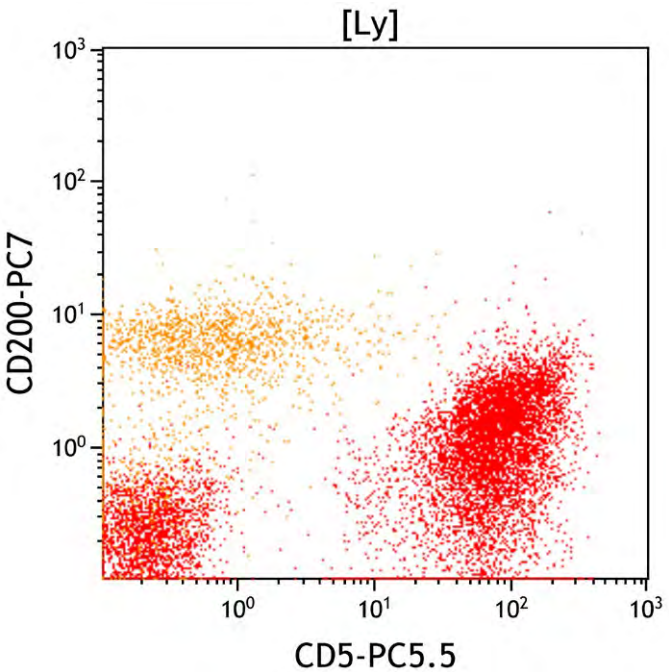

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

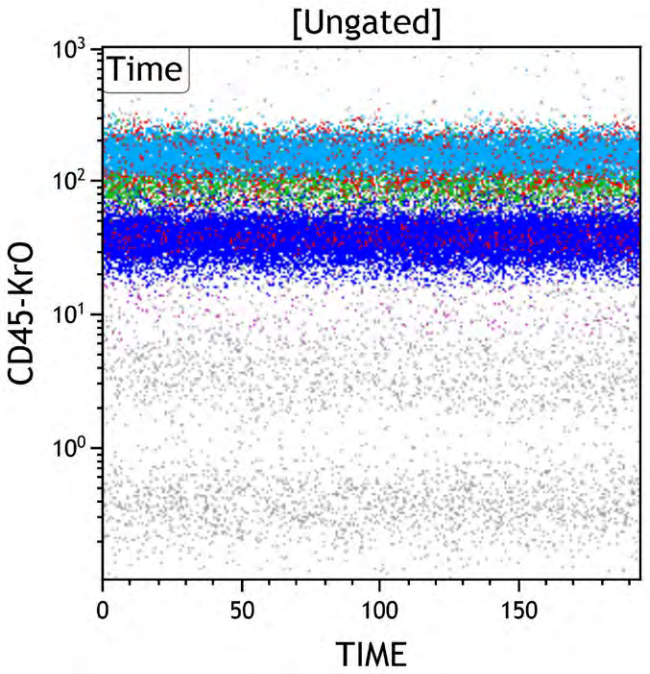

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

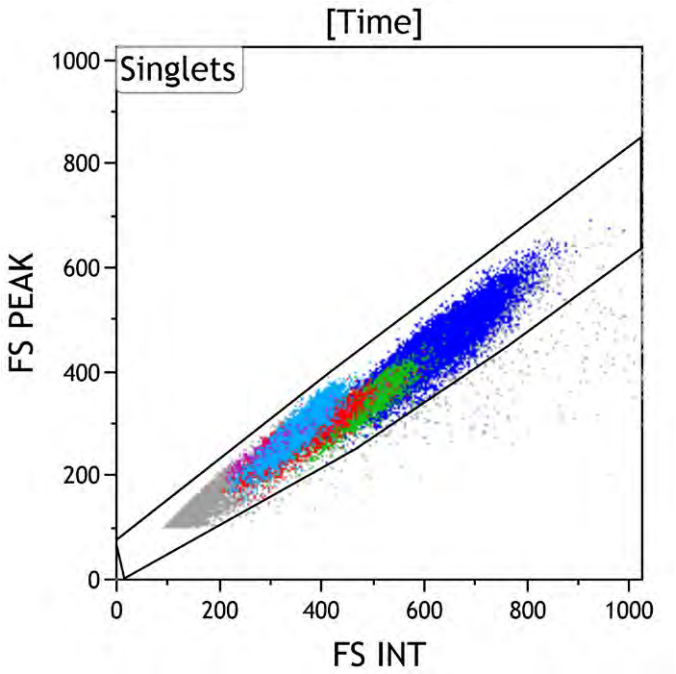

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

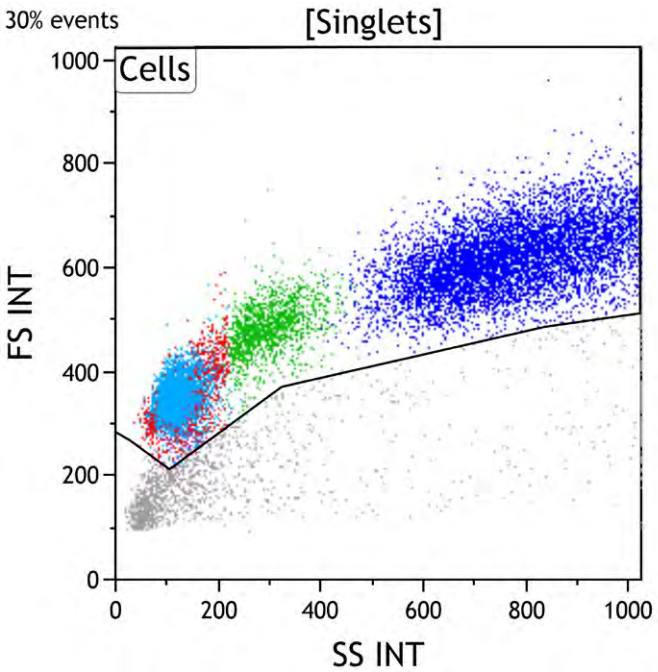

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

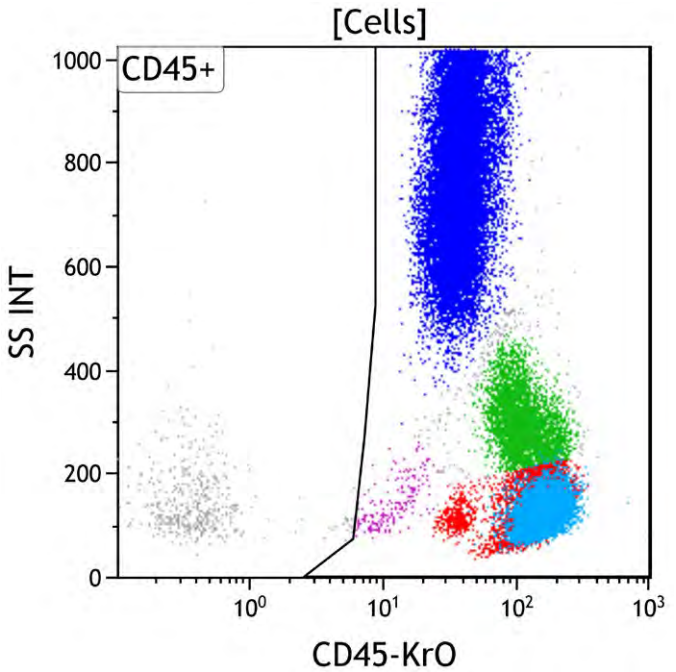

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

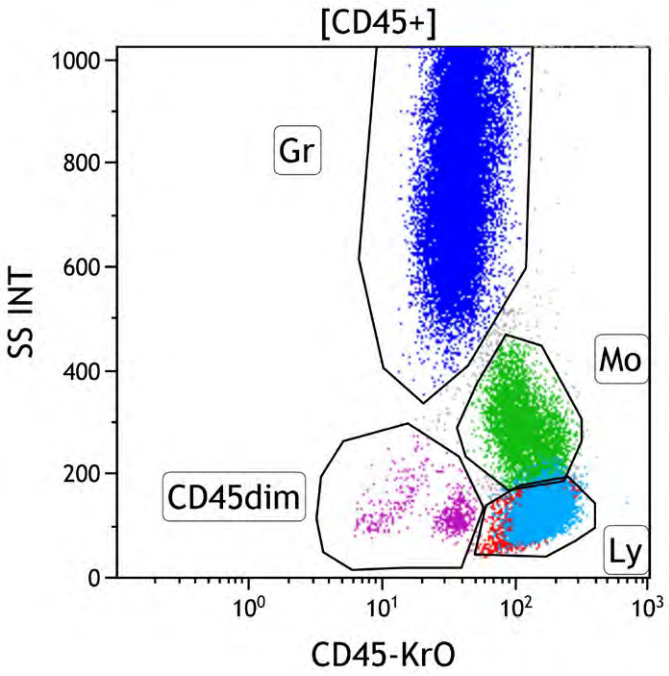

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

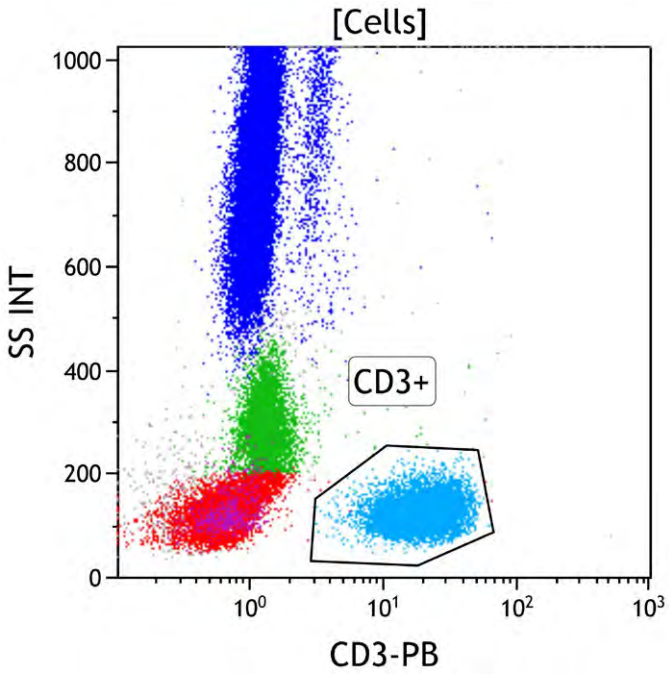

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

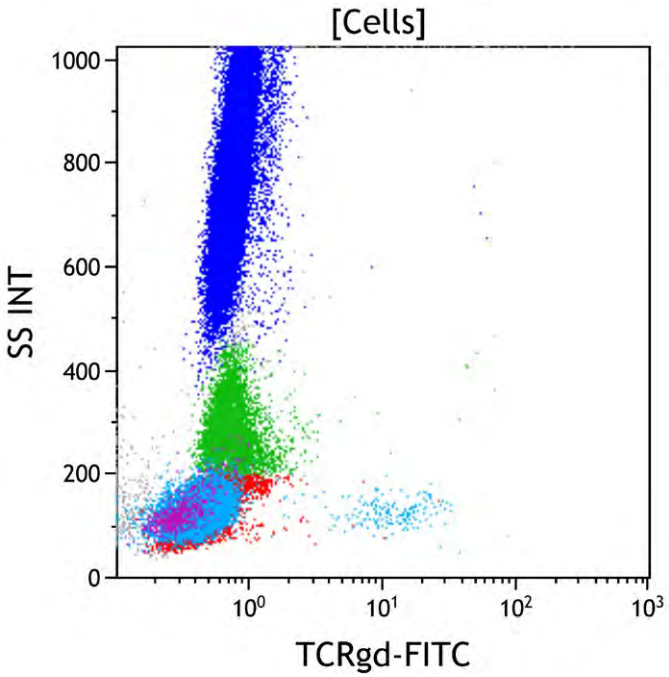

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

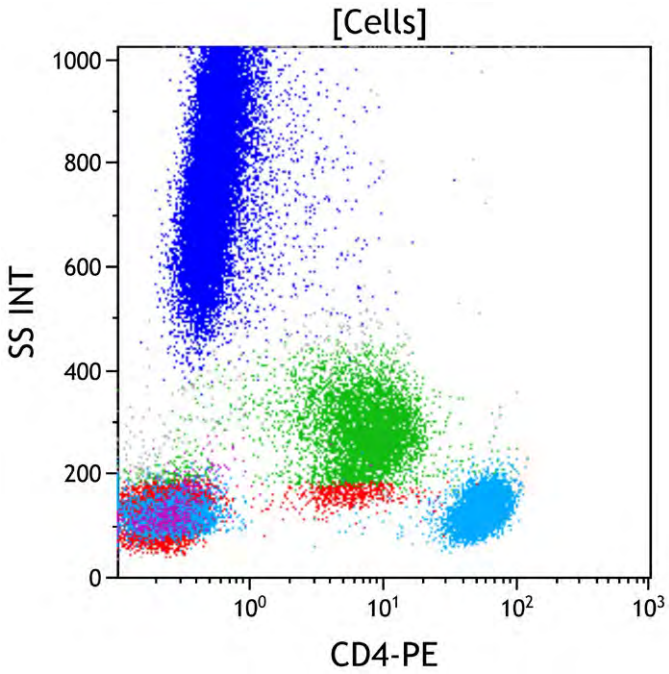

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells.

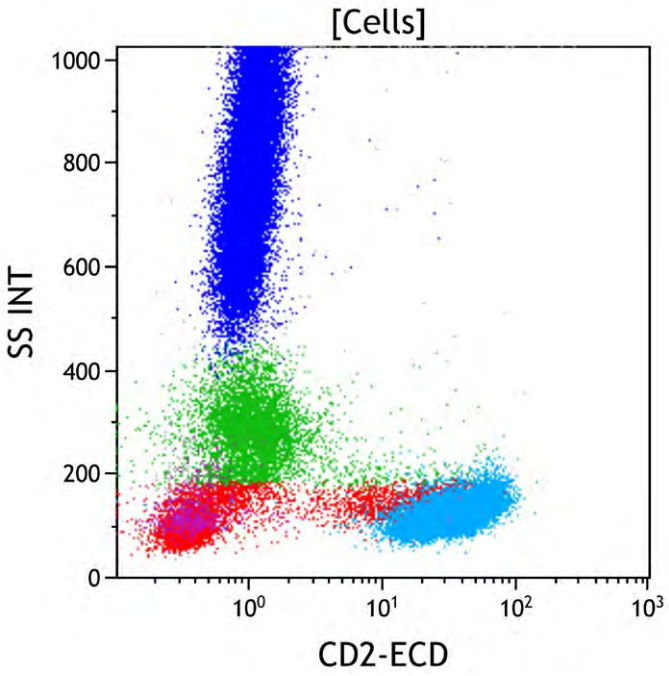

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

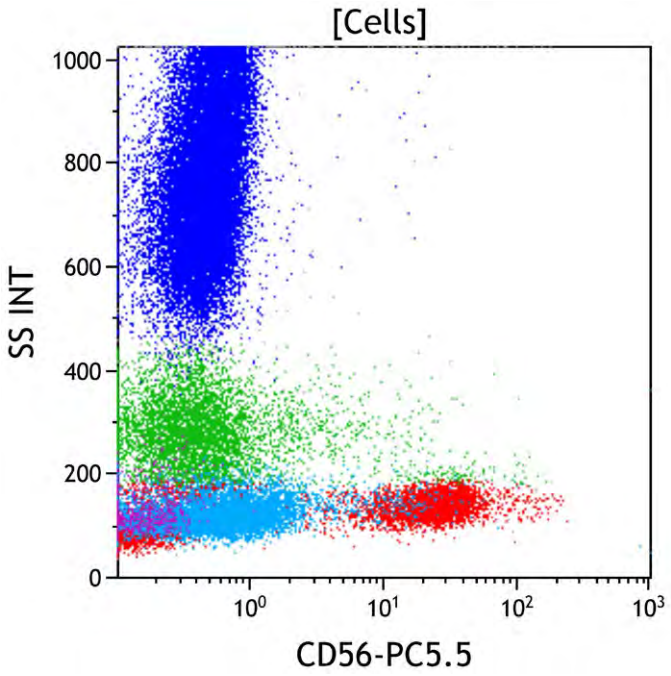

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

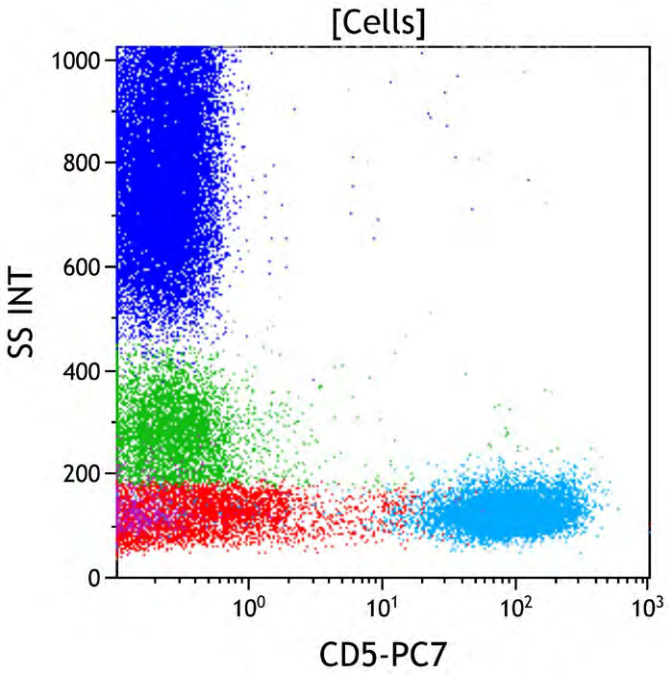

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

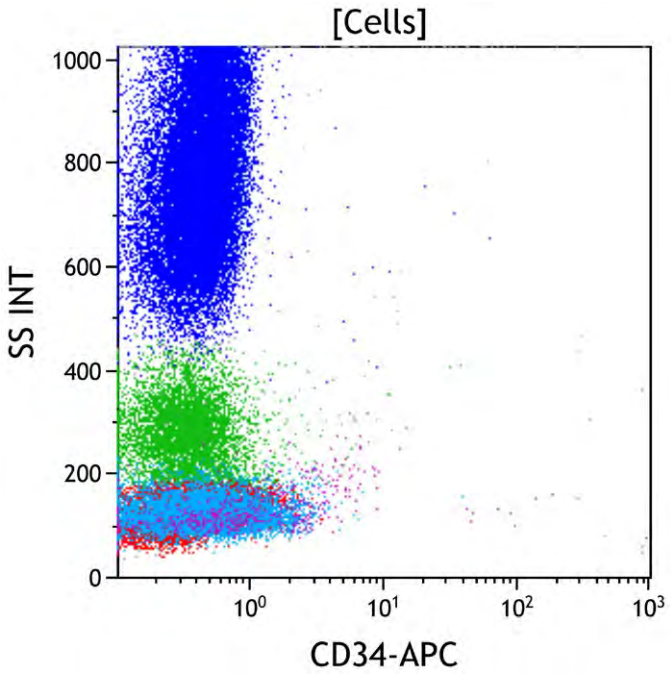

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

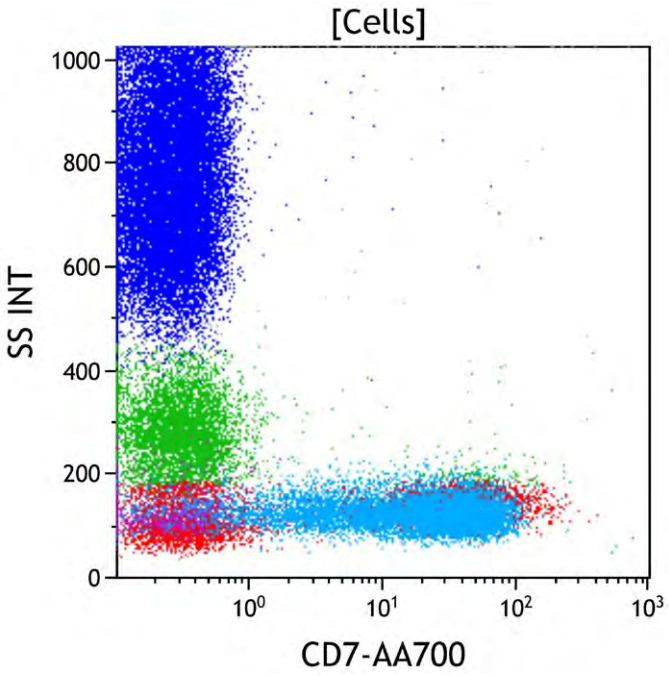

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

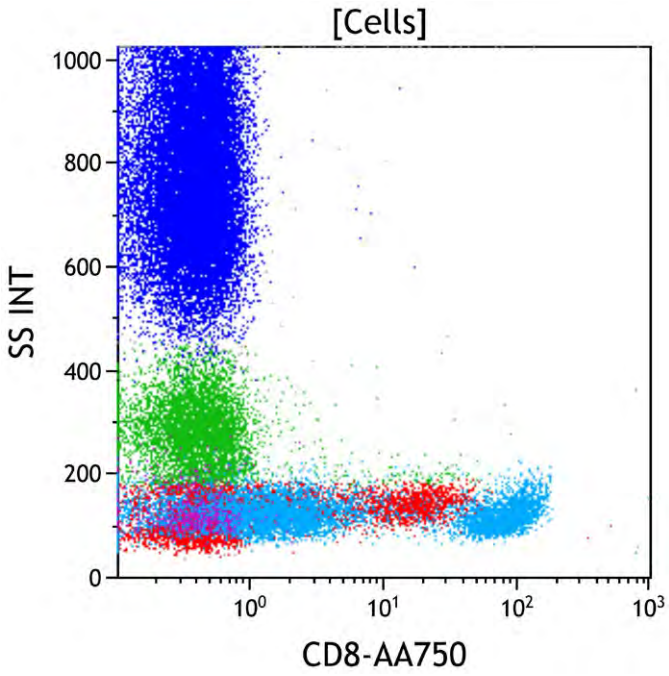

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

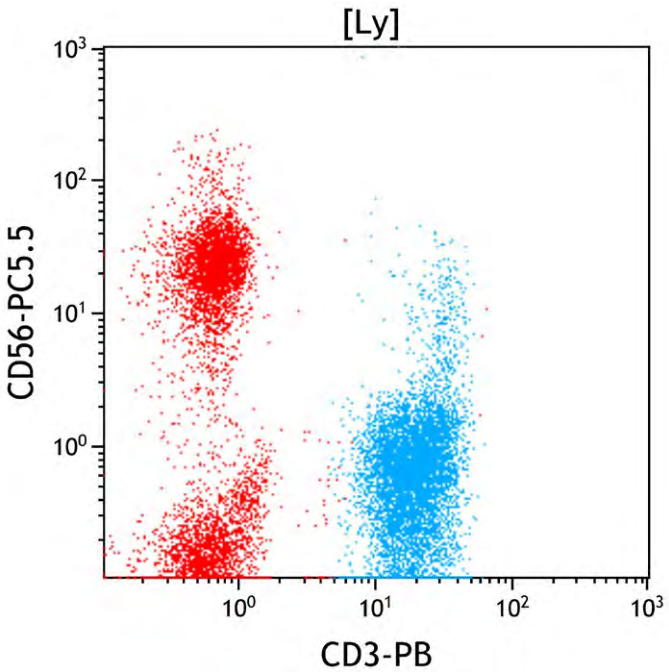

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

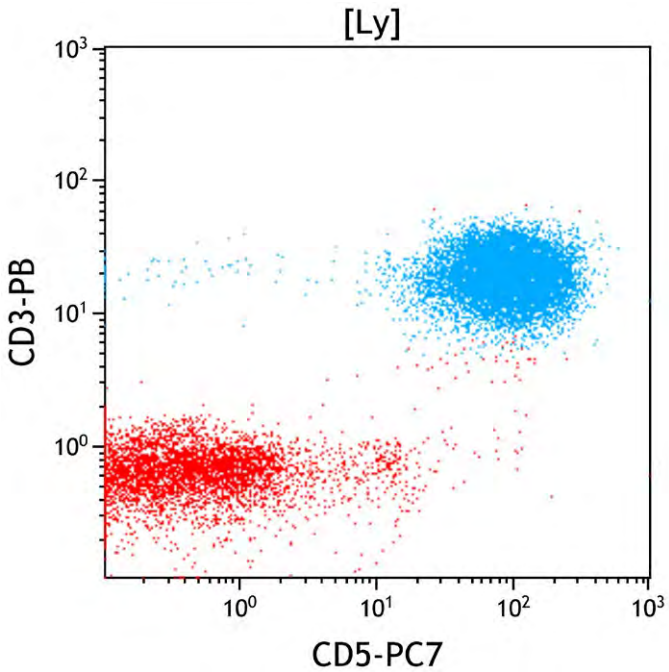

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

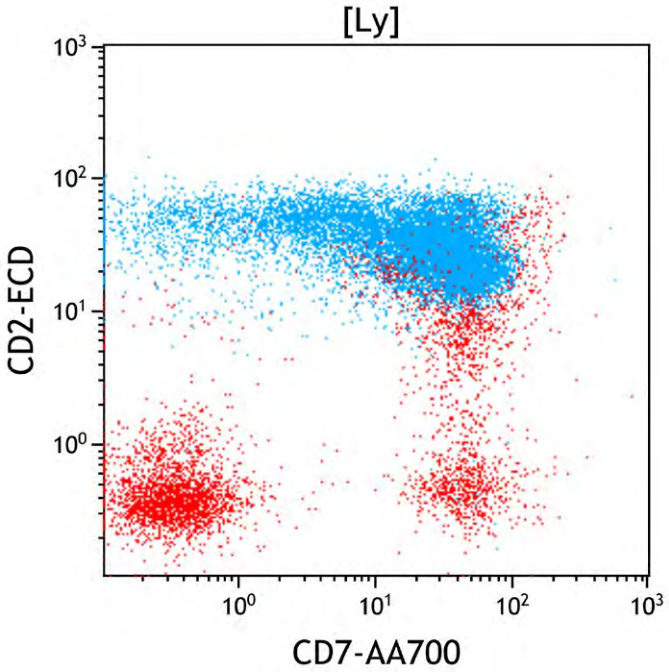

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

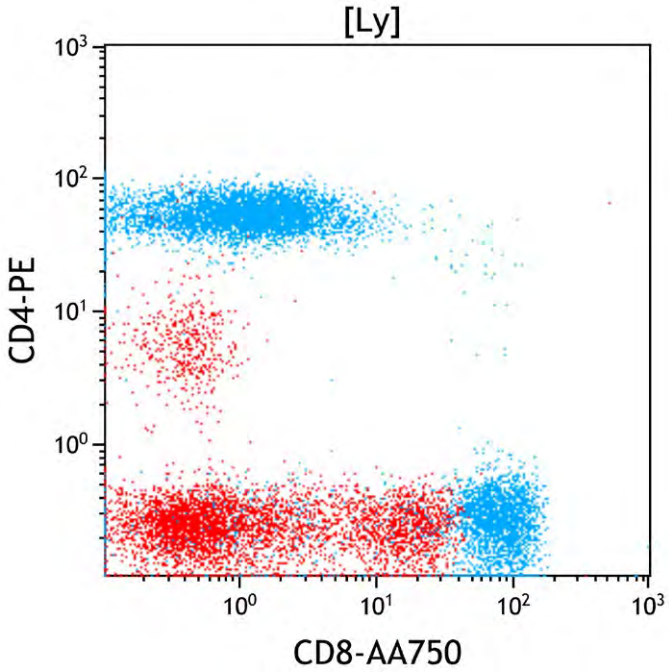

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, middle left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

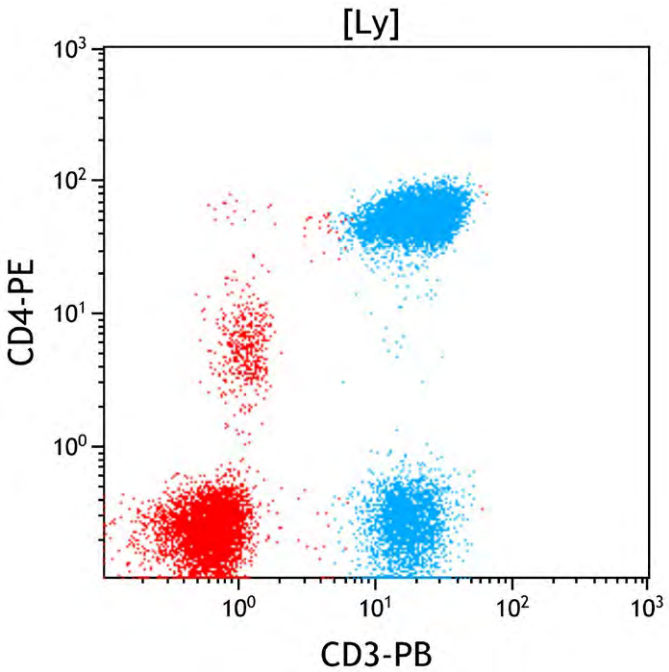

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

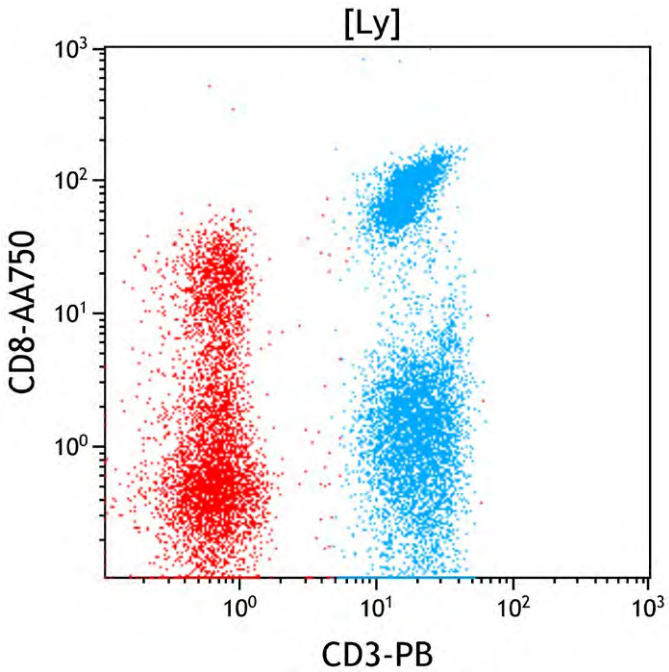

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

T Cell Tube

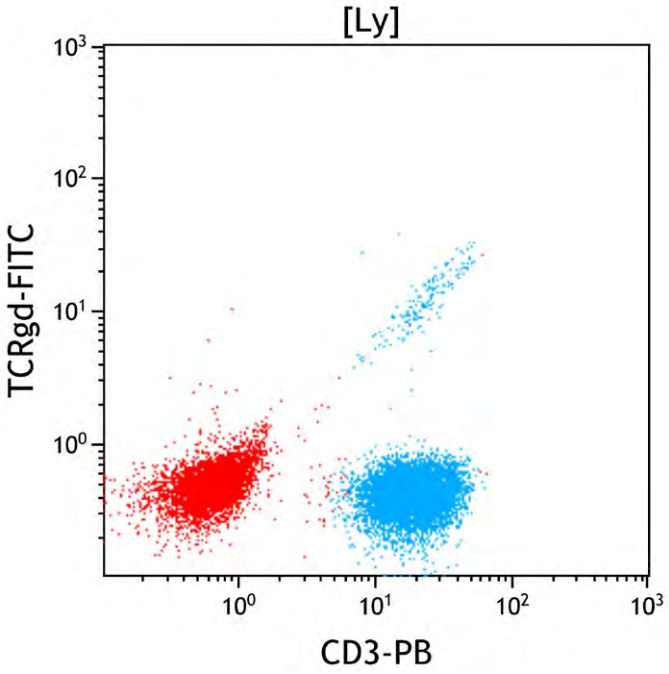

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

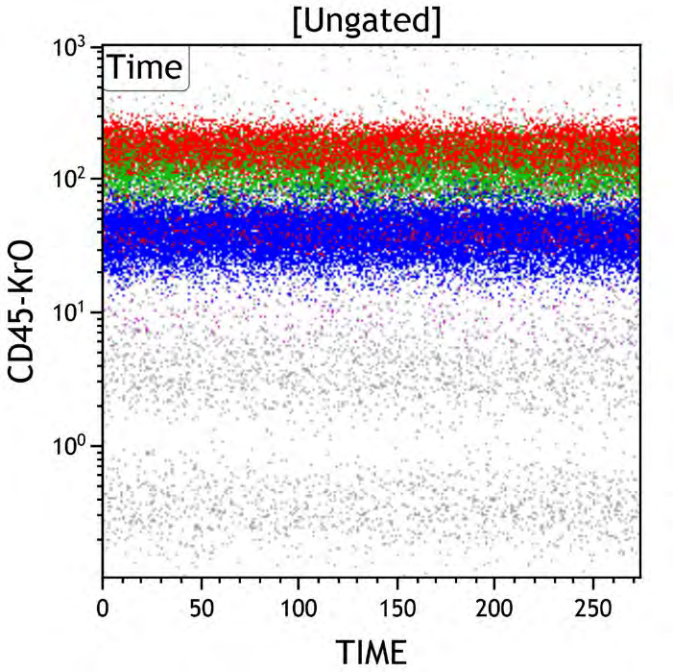

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

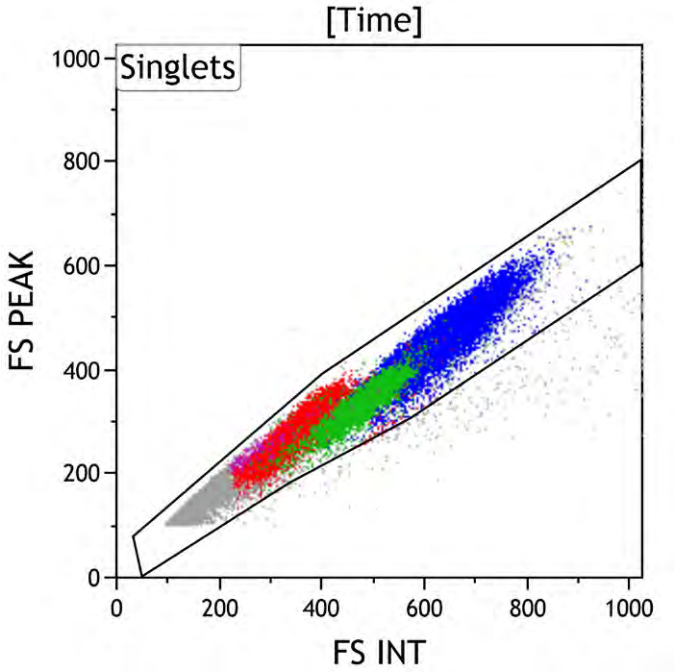

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

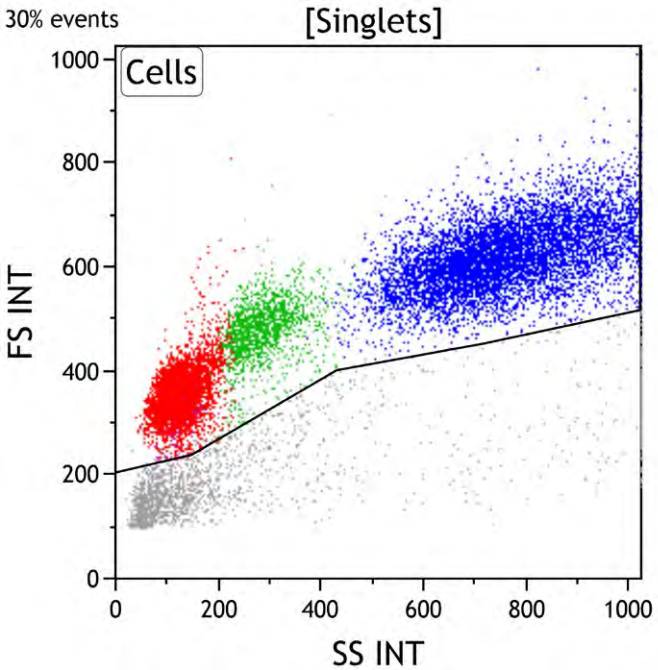

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

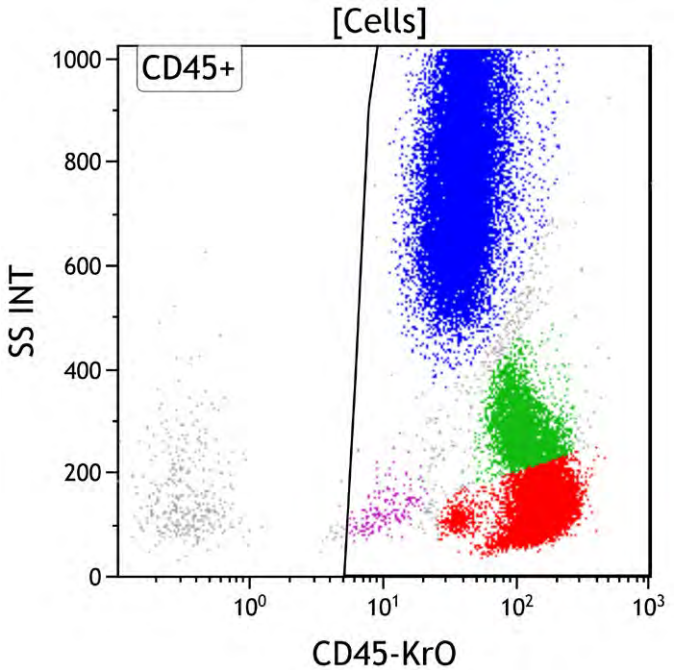

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

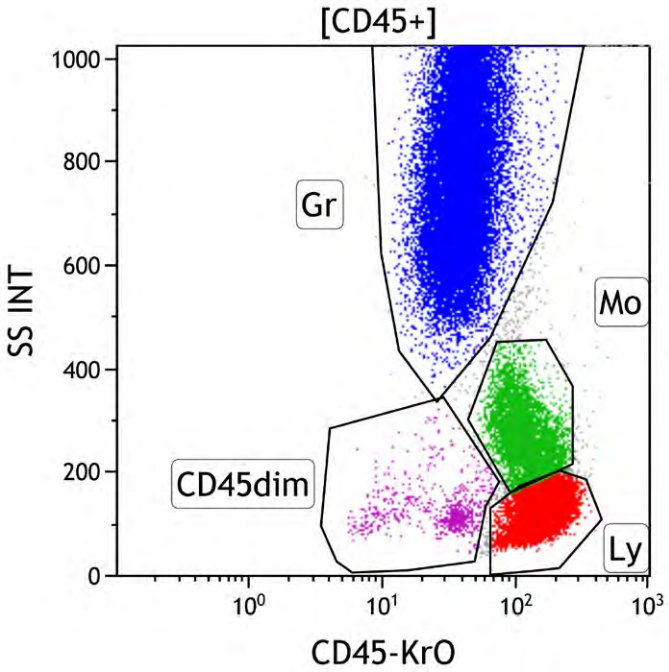

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

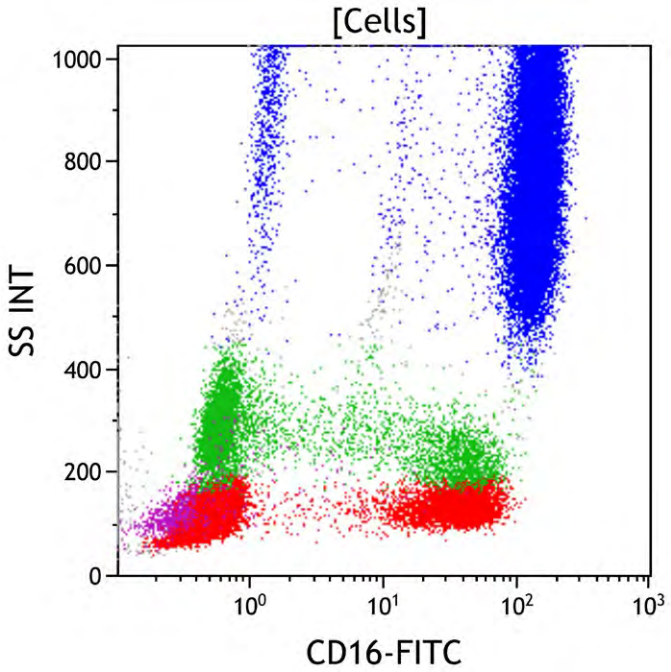

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green).

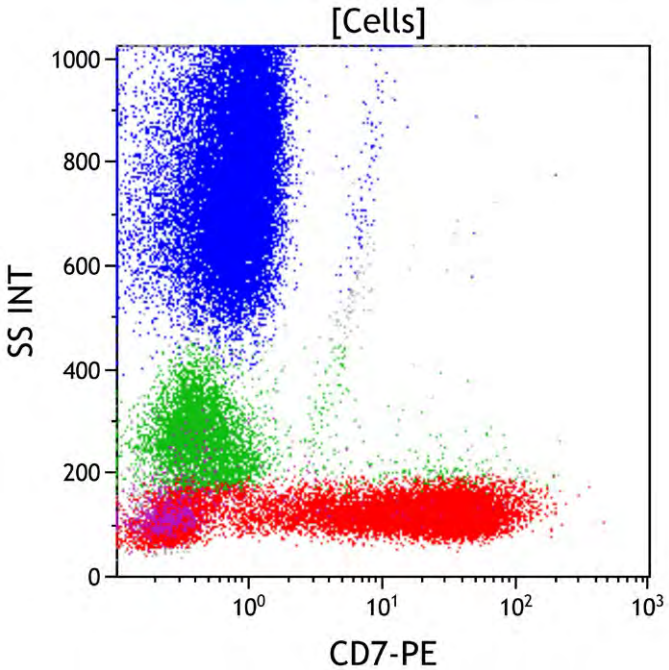

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red, lower right). It is also expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

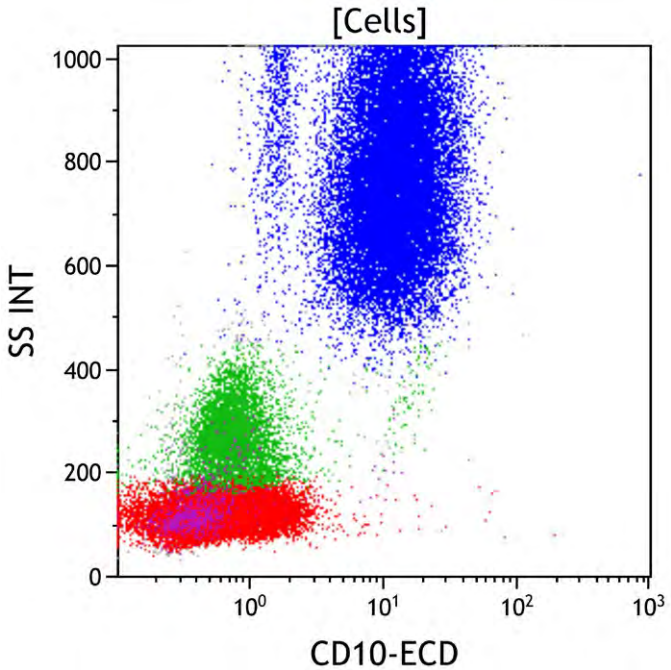

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

M1 Cell Tube

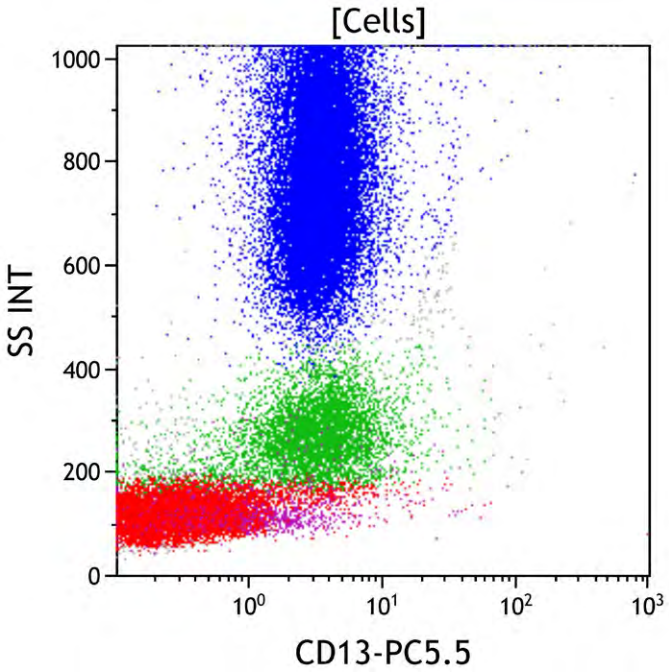

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), mature monocytes (green), and myeloid progenitors (purple).

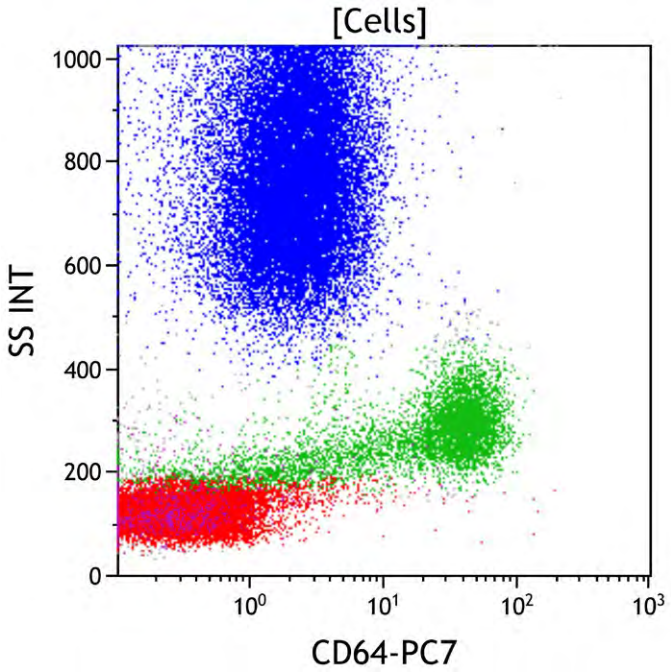

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature monocytes (green). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphocytes (red) or most CD34 positive progenitors. Activated mature monocytes express CD64 at lower level and have lower side scatter.

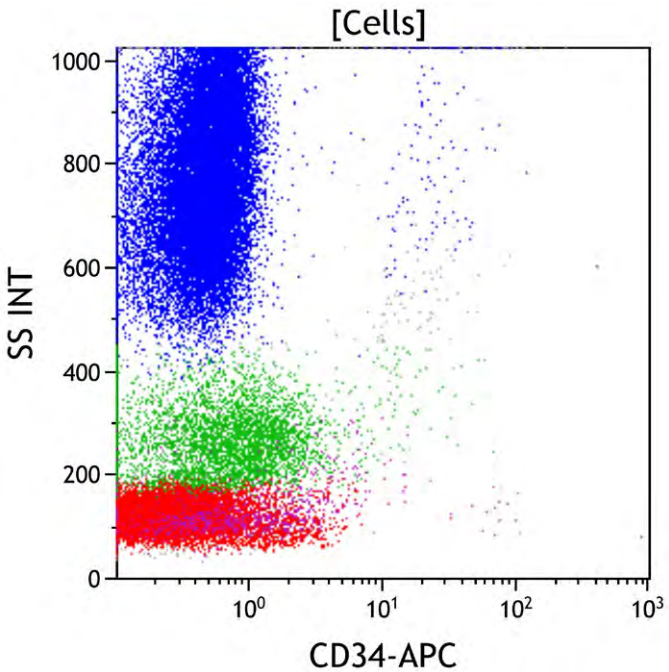

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

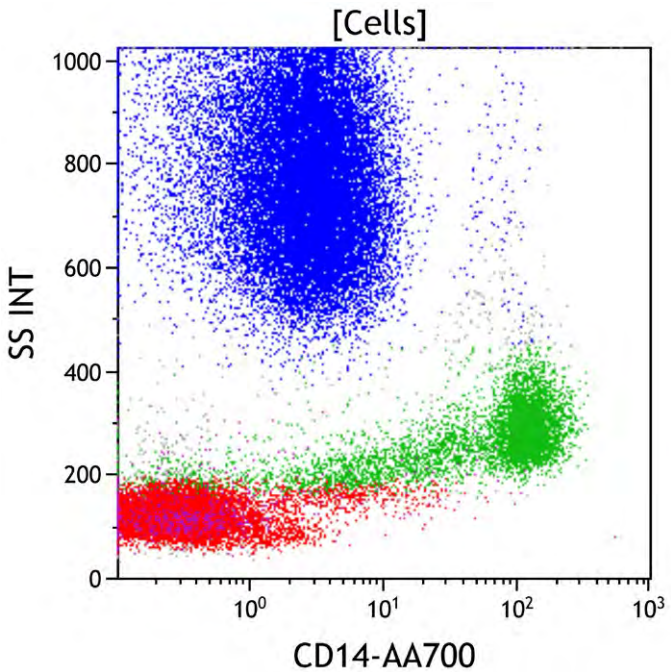

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a low level on mature granulocytes (blue). Activated mature monocytes express CD14 at a lower level and have lower side scatter.

M1 Cell Tube

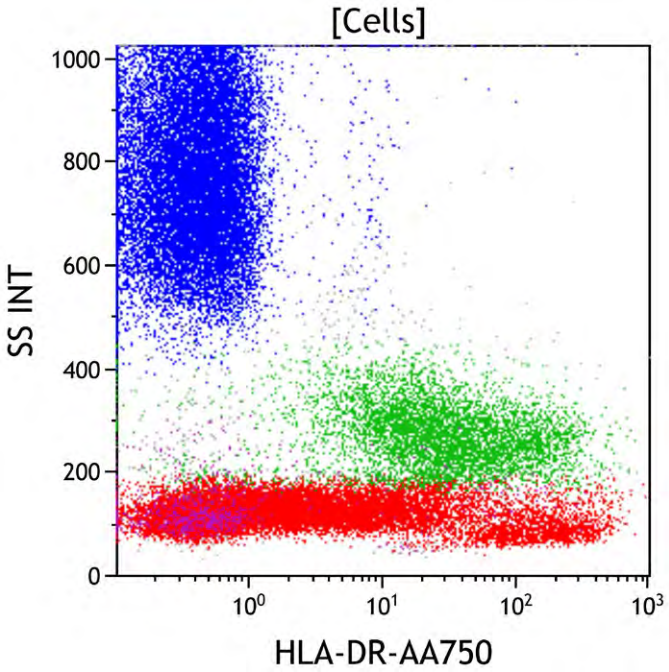

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells, and activated T cells.

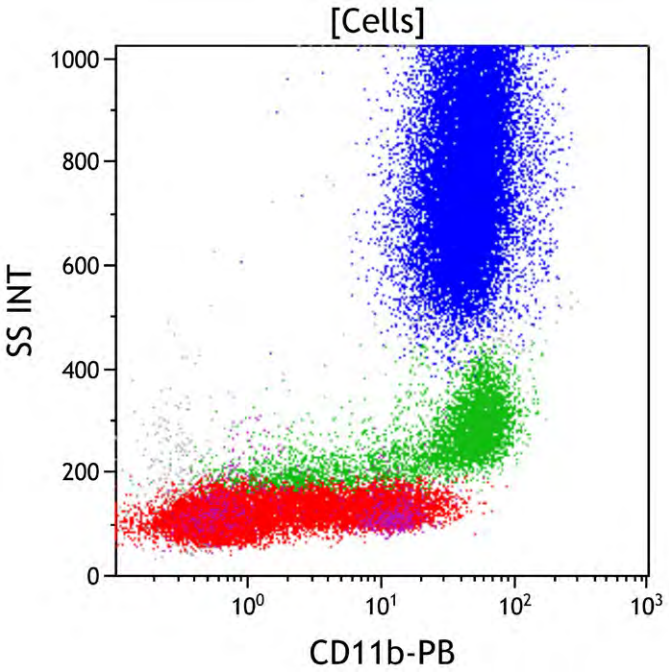

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (blue) and on monocytes (green). CD11b is also expressed on NK cells and basophils.

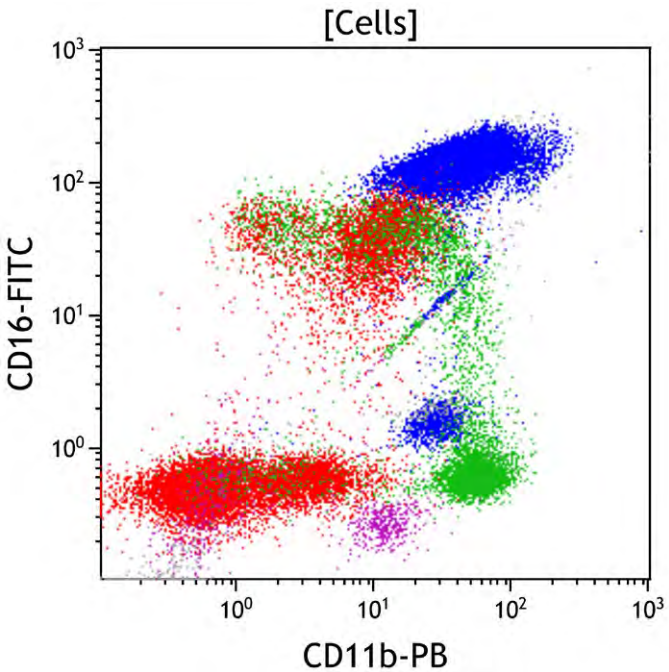

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), immature and mature granulocytes (blue) and NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red, upper right). Activated mature monocytes express CD16 at a variable level and are CD11b positive.

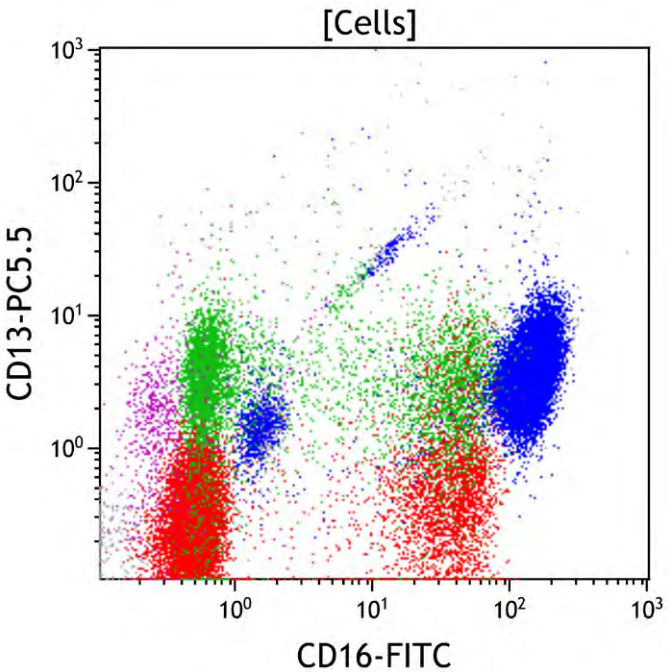

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red, lower right).

M1 Cell Tube

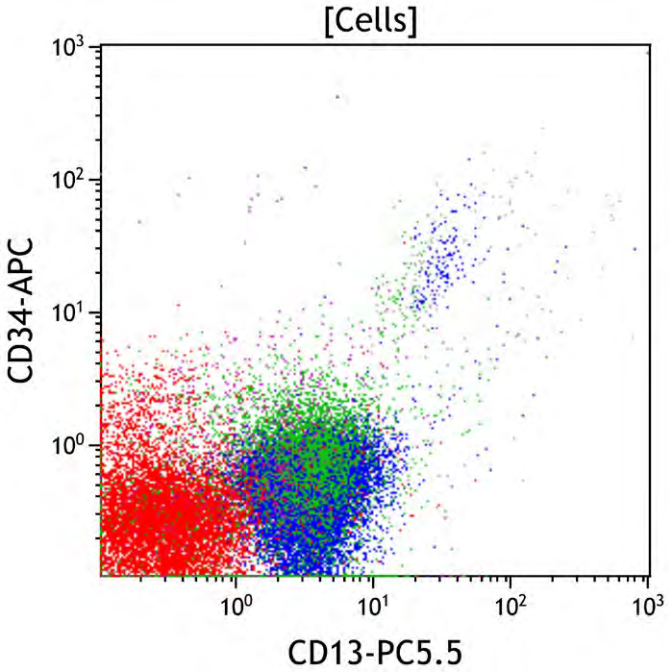

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34.

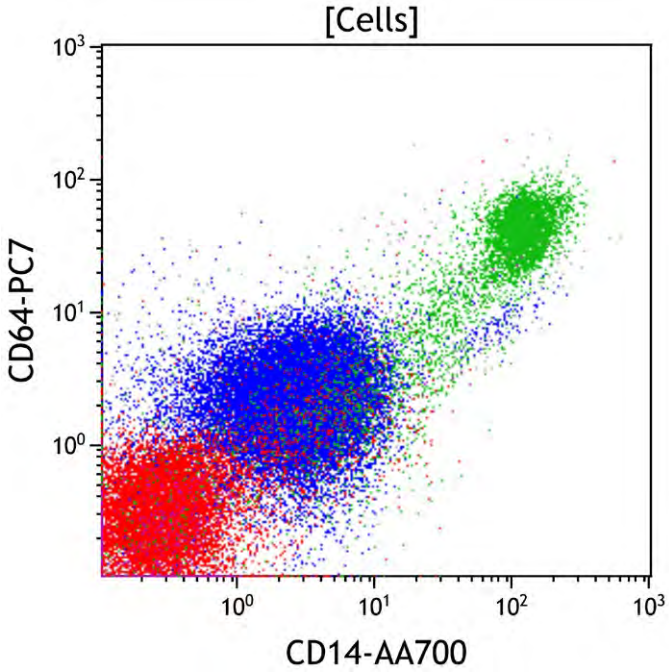

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes (blue). Activated mature monocytes express CD14 and CD64 at a variably lower level.

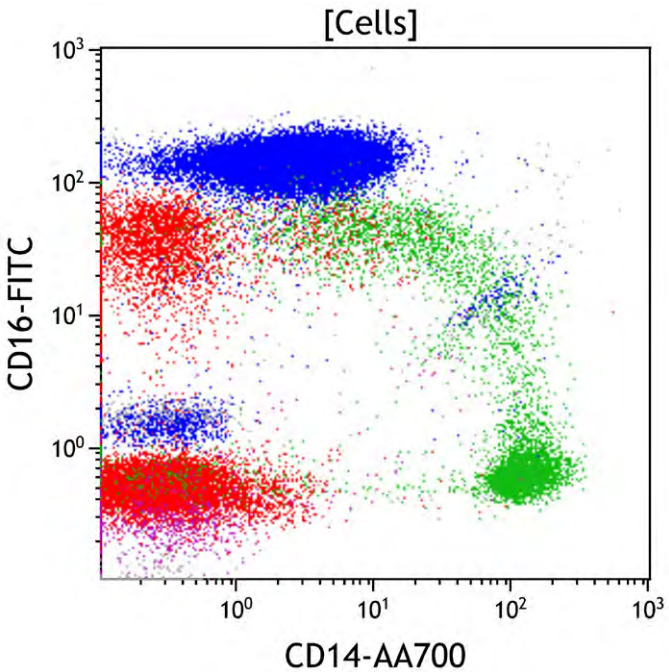

Figure 19. This CD14 vs CD16 dot plot shows all viable cells. CD14 is expressed at a high level on monocytes (green) and a lower level on granulocytes (blue, upper left). CD16 is expressed on granulocytes and a subset of NK cells (red, middle left). Activated mature monocytes express CD14 and CD16 at a variably lower level.

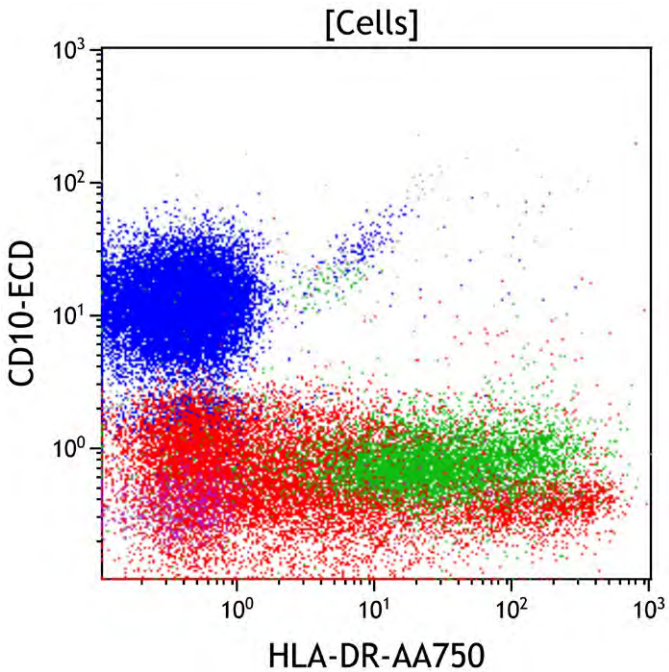

Figure 20. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (Green), B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue).

M1 Cell Tube

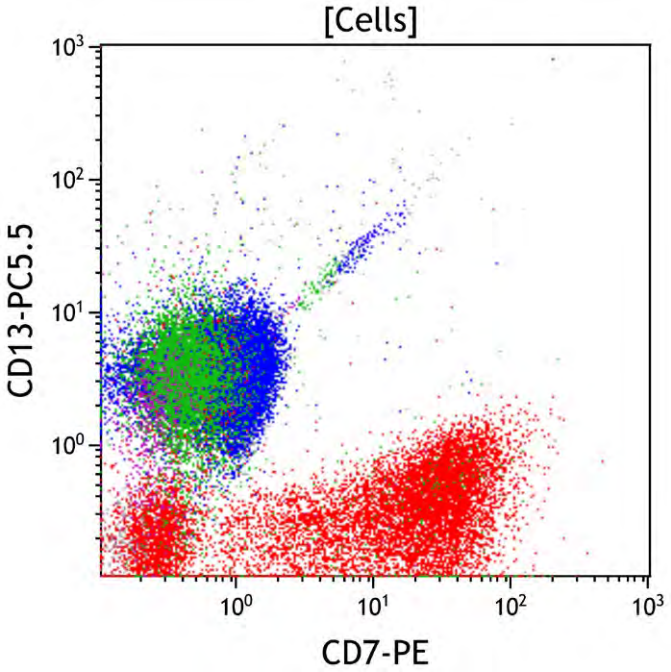

Figure 21. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red, lower right). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

M2 Cell Tube

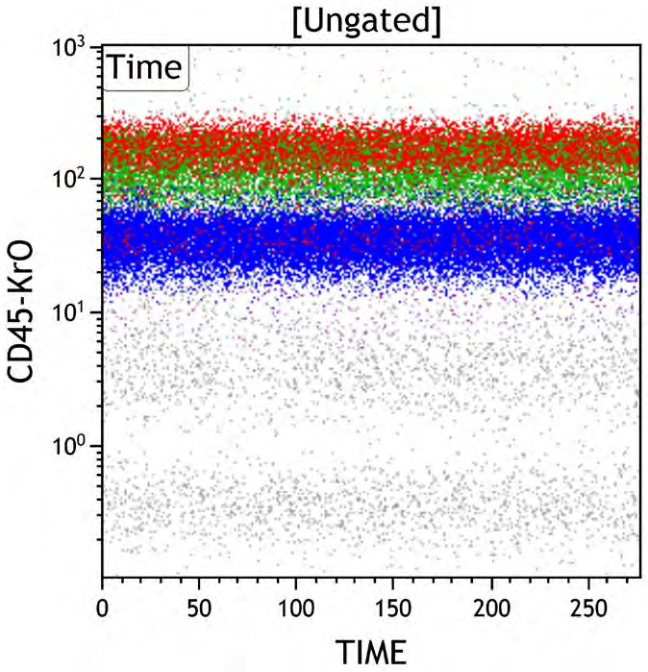

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

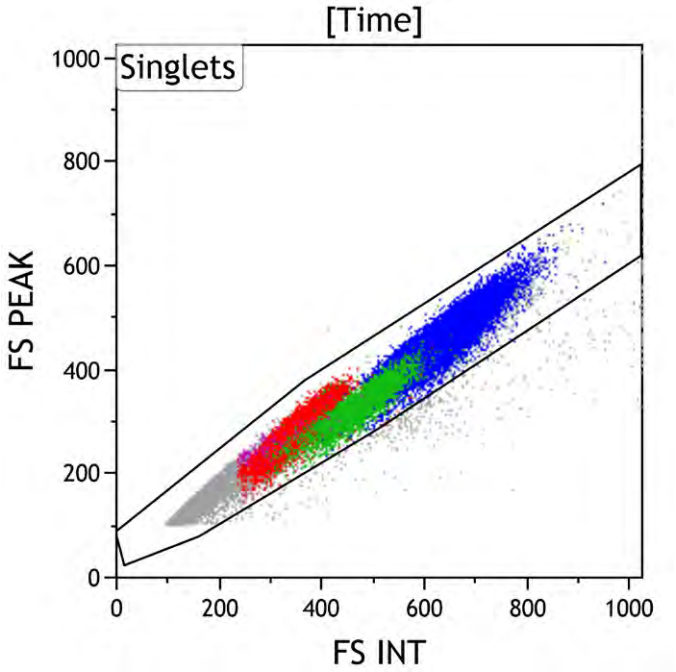

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

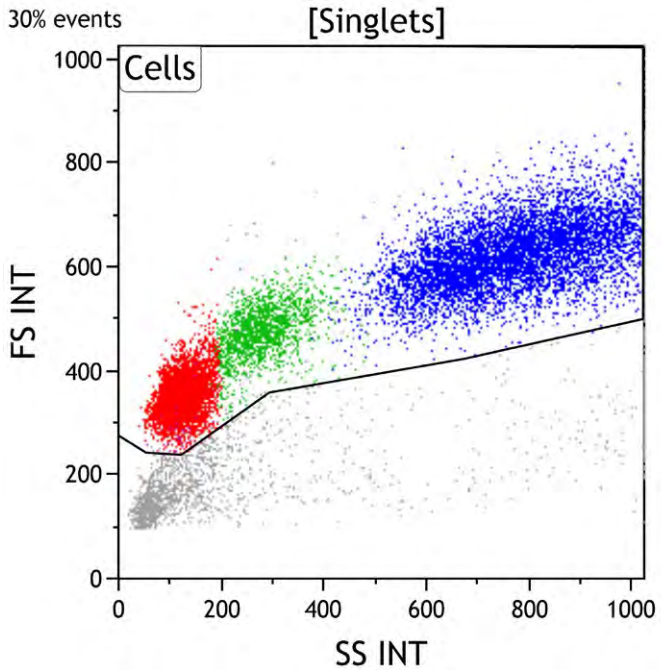

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

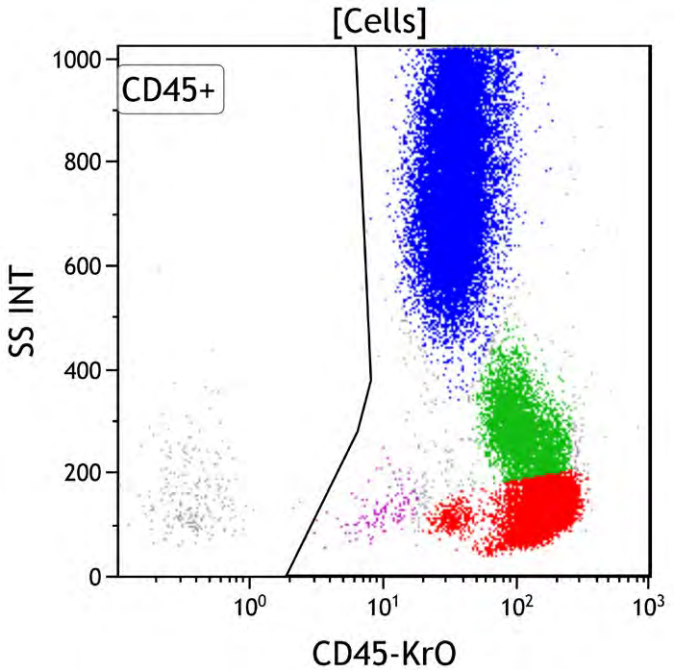

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

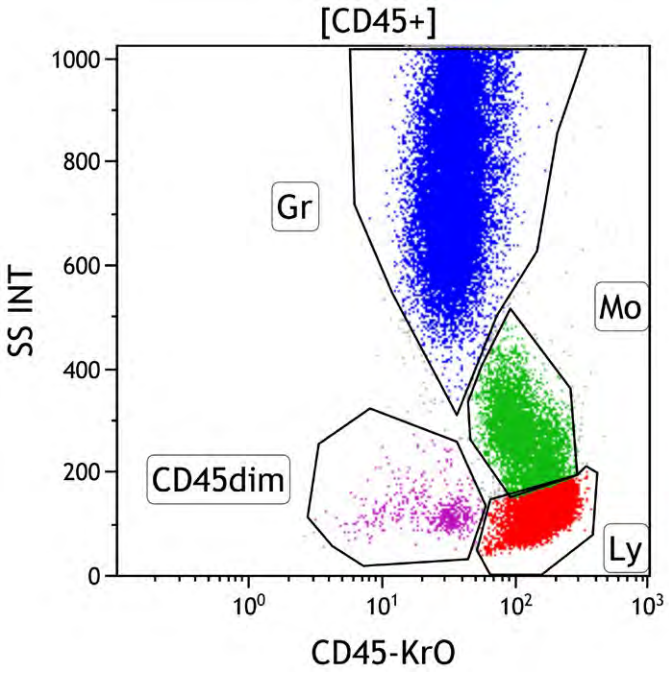

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

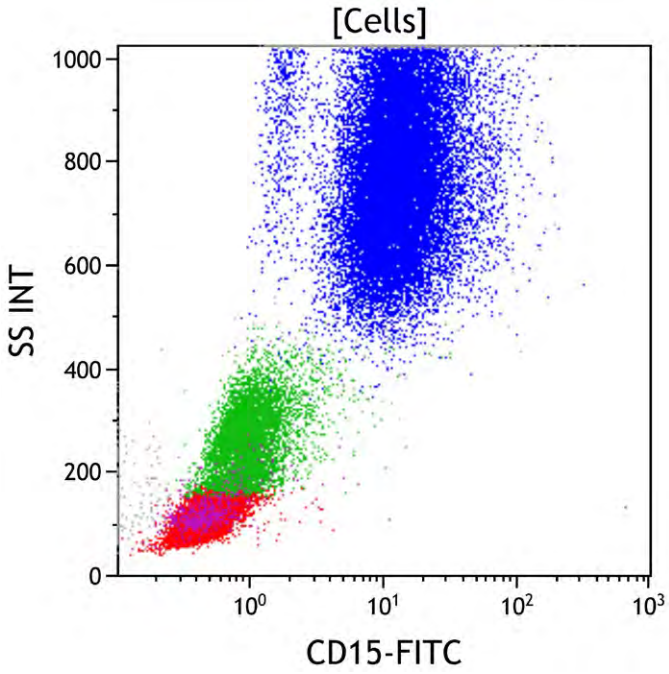

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes (green).

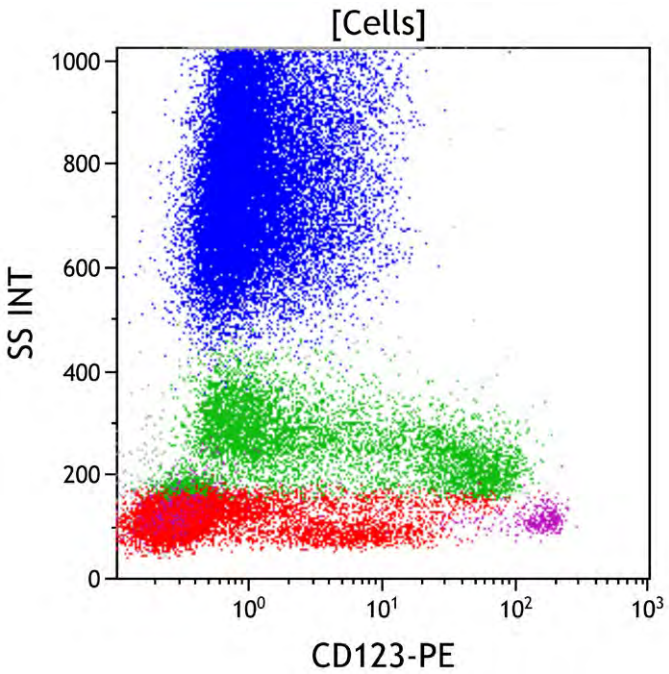

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green).

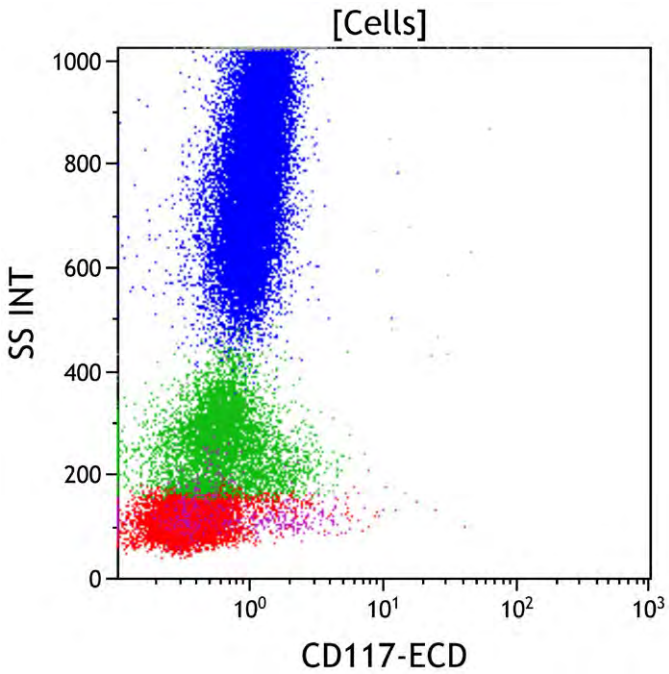

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Mature granulocytes, monocytes, and lymphocytes are negative for CD117.

M2 Cell Tube

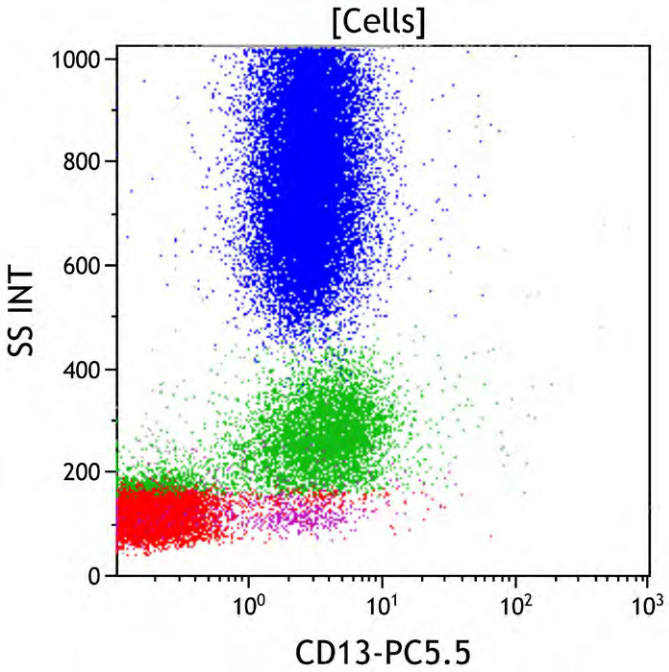

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and mature monocytes (green) and variably on myeloid progenitors.

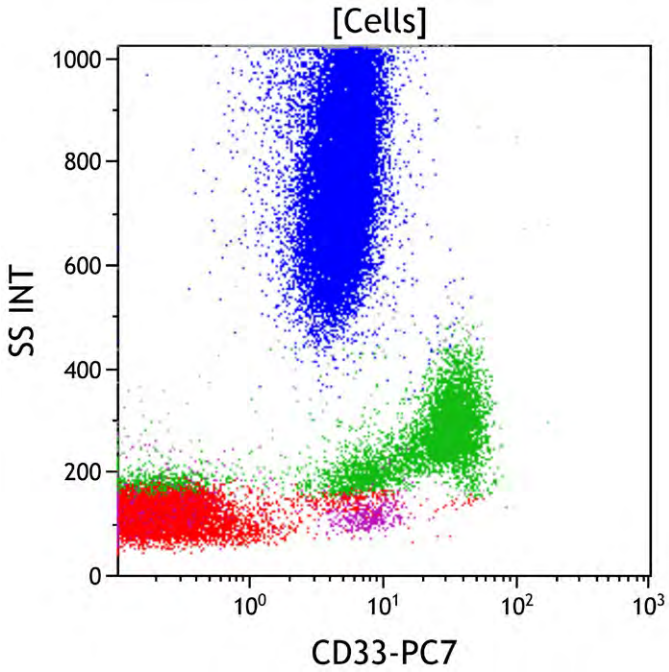

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors.

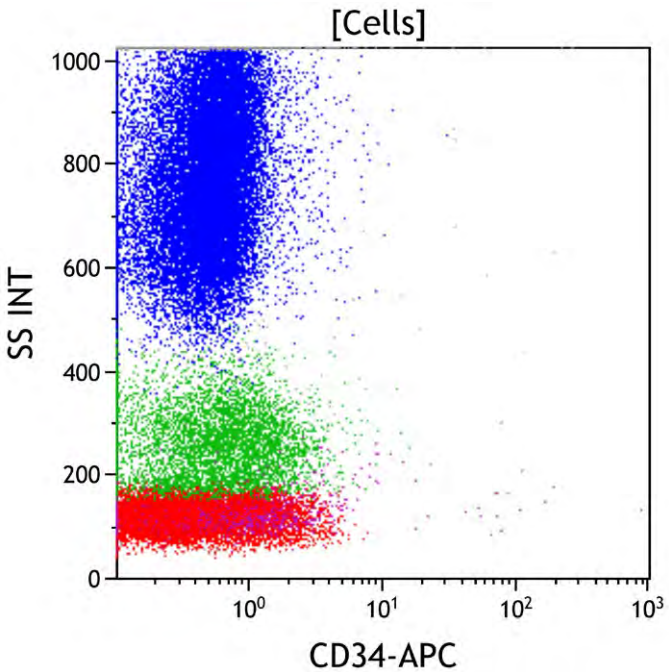

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

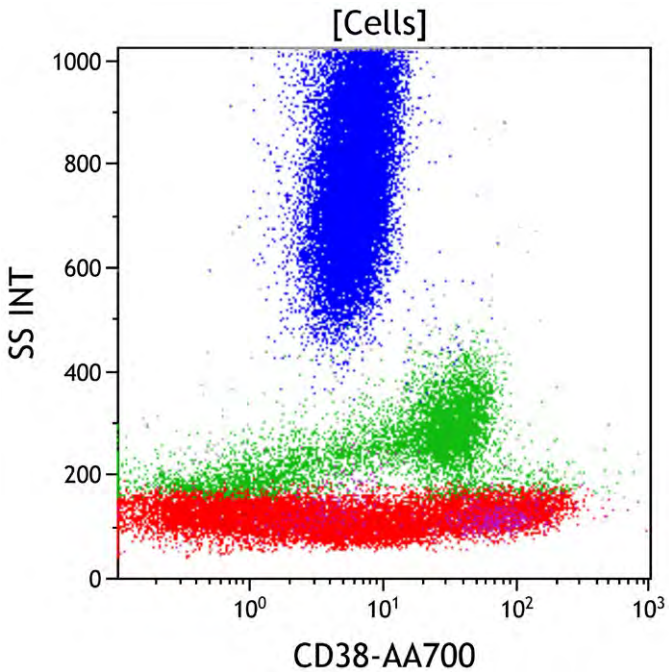

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red).

M2 Cell Tube

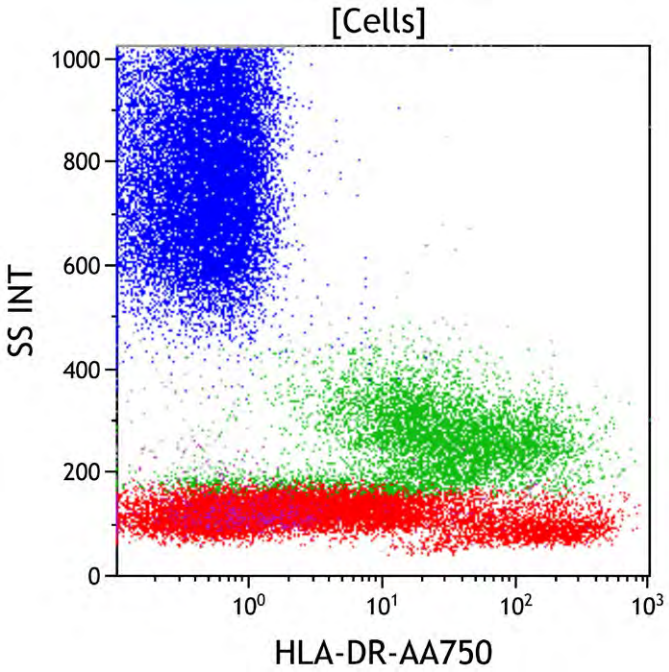

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells (red, lower right), and activated T cells.

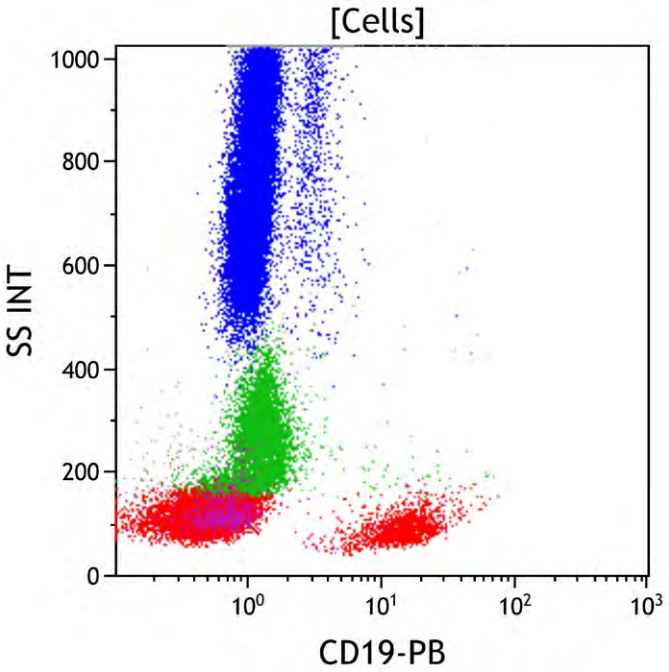

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on immature and mature B cells (red, lower right), as well as most plasma cells. These cells typically have low to moderate side scatter.

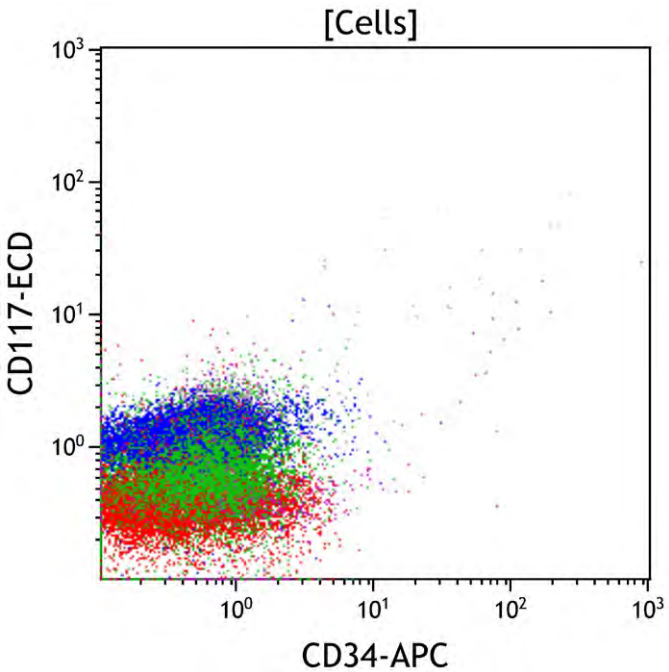

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34 and CD117. CD34 and CD117 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

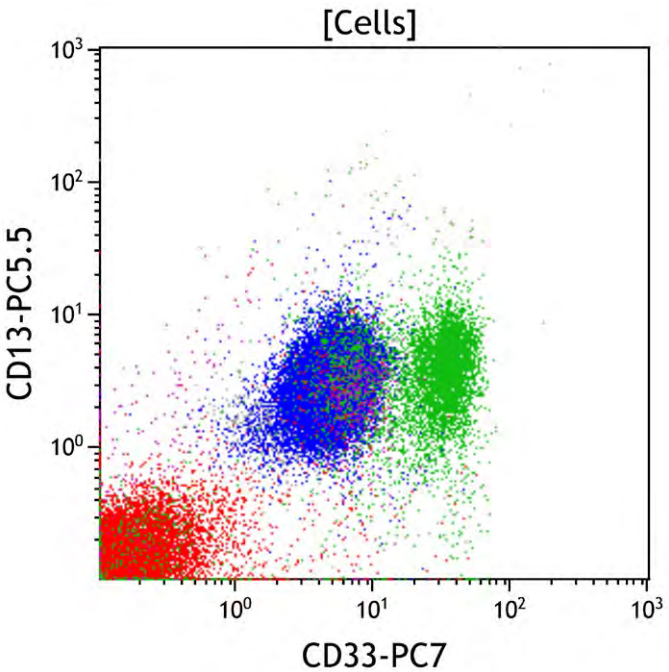

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Lymphocytes (red) do not express either CD13 or CD33.

M2 Cell Tube

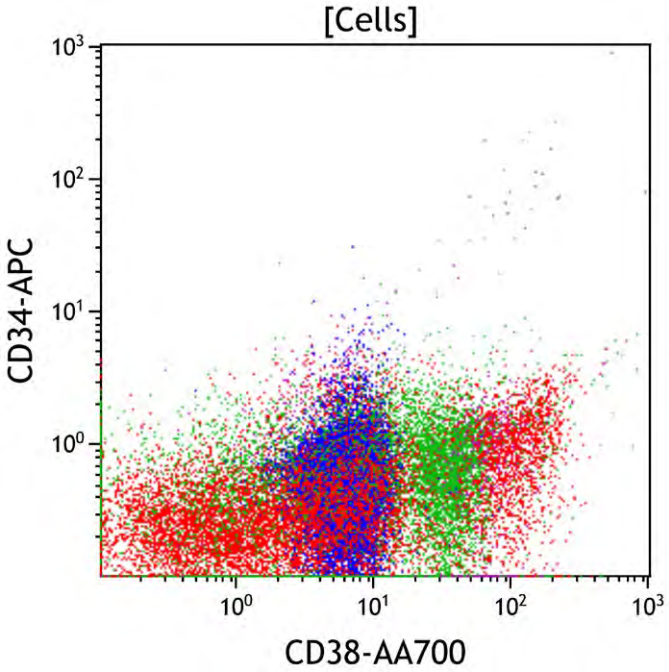

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is an activation marker. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes, monocytes, and lymphocytes are negative for CD34 with variable CD38. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

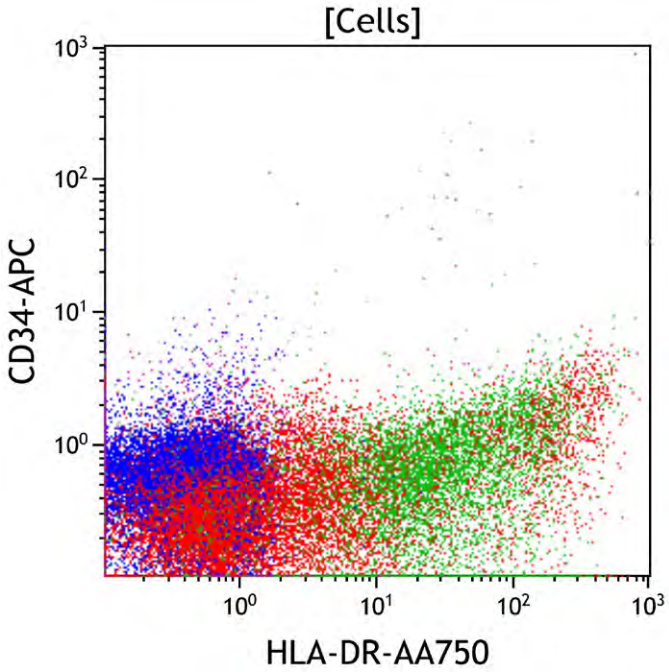

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

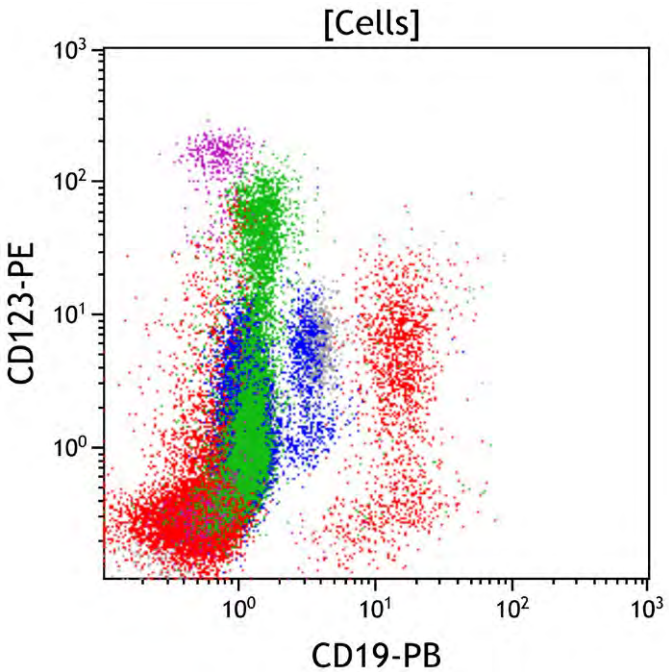

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red, right) normally do not express significant CD123. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

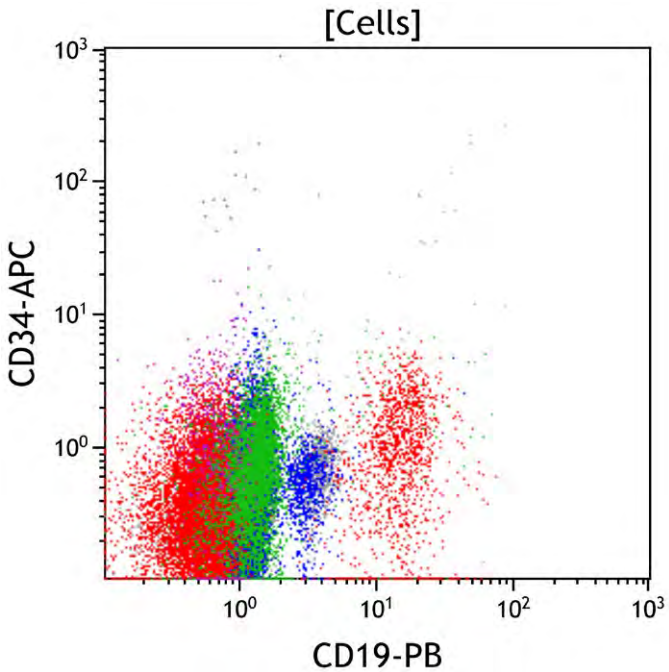

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

M2 Cell Tube

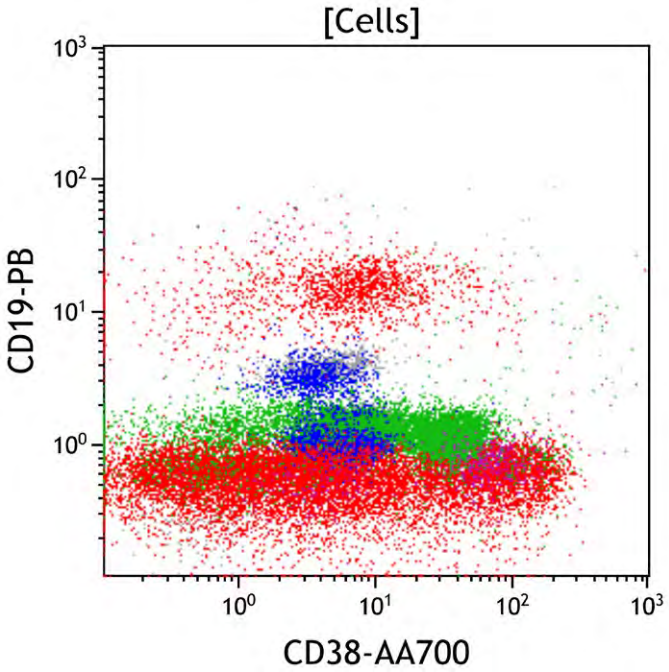

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells show intermediate expression of CD38 (red, upper). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

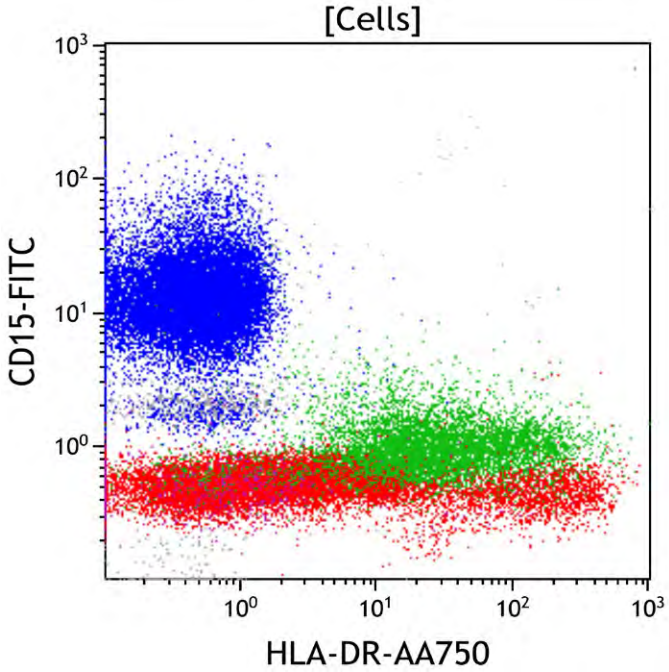

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Mature granulocytes do not express HLA-DR (blue).

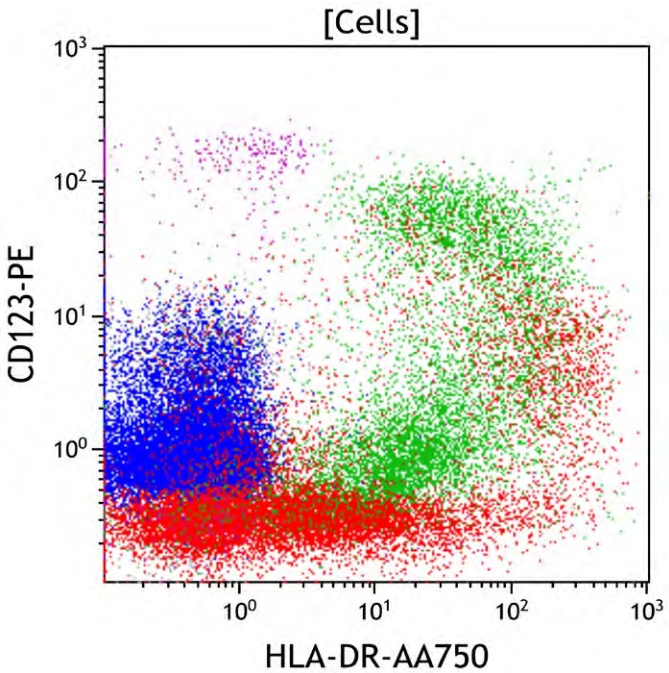

Figure 23. This HLA-DR vs CD123 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Mature granulocytes do not express HLA-DR (blue). CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors.

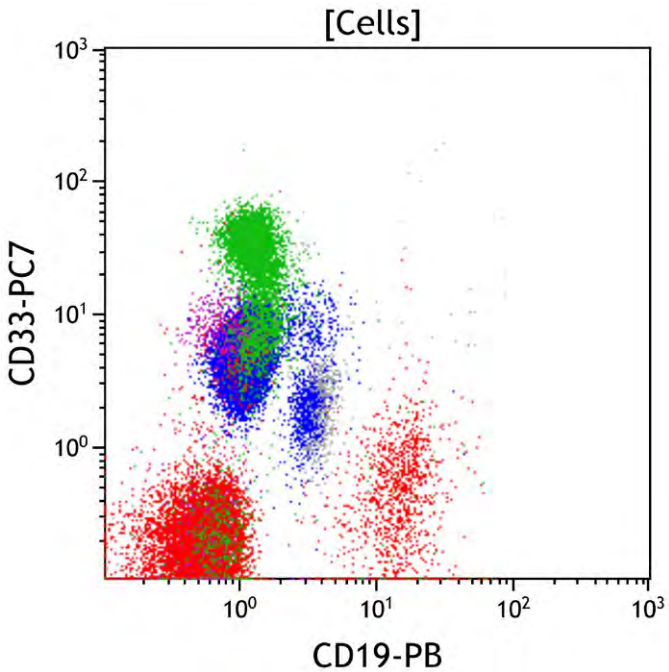

Figure 24. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red, lower right). CD33 is expressed by monocytes (green) and granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identified no immunophenotypically aberrant populations in this case. No white cell abnormality is identified by morphology. Note that correlation with clinical, morphologic, and laboratory data is recommended, and that a malignant process cannot be ruled solely on the basis of this assay.