CLEARLLAB 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 ISCH/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 a Navios flow cytometer. 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 along 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 System, including Software Instructions for Use, PN B47905

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, anotated, 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

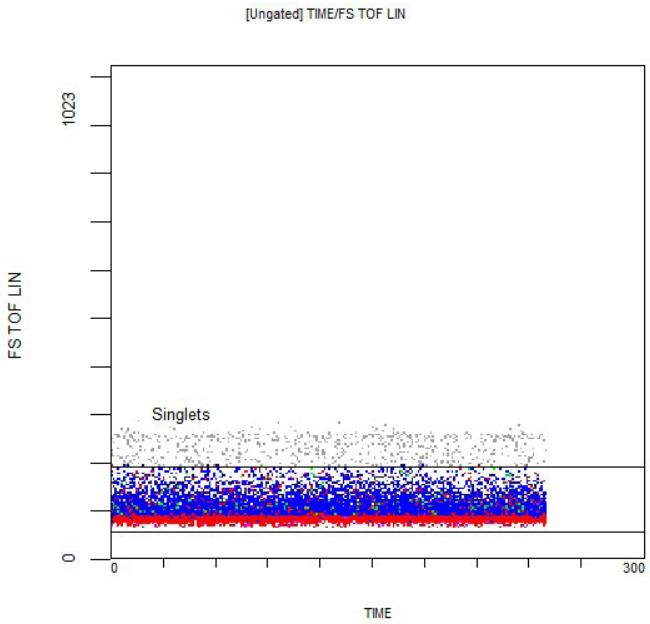
Case #1: Normal Whole Blood

Clinical Vignette

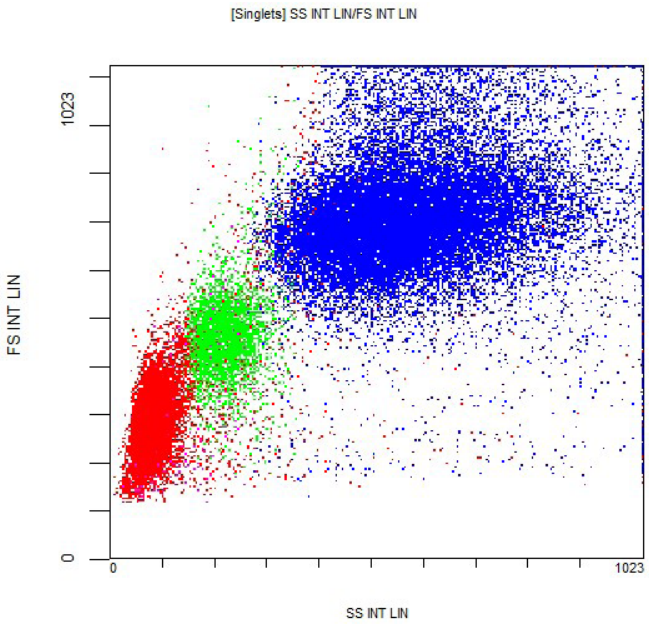
This 64-year-old male presents with mild lymphocytosis. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

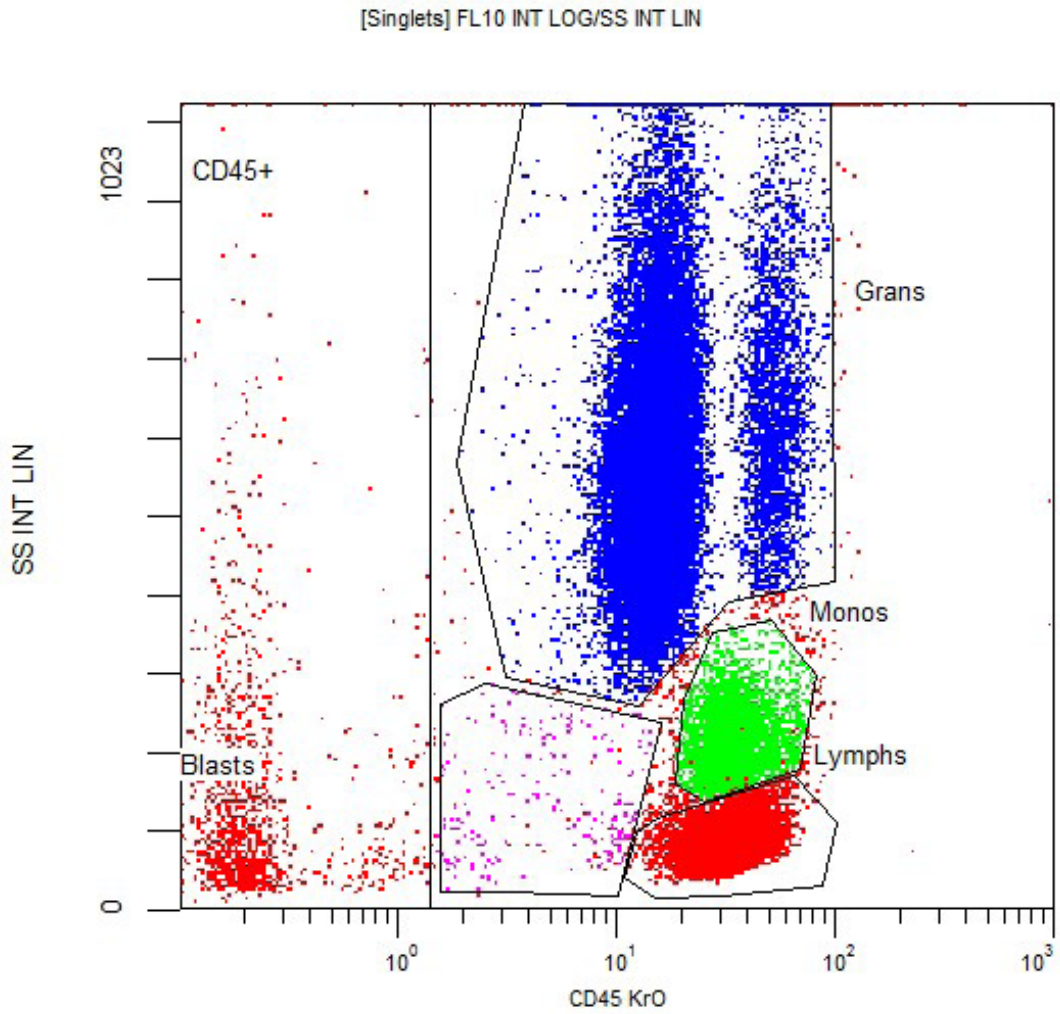
Access Case #1 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

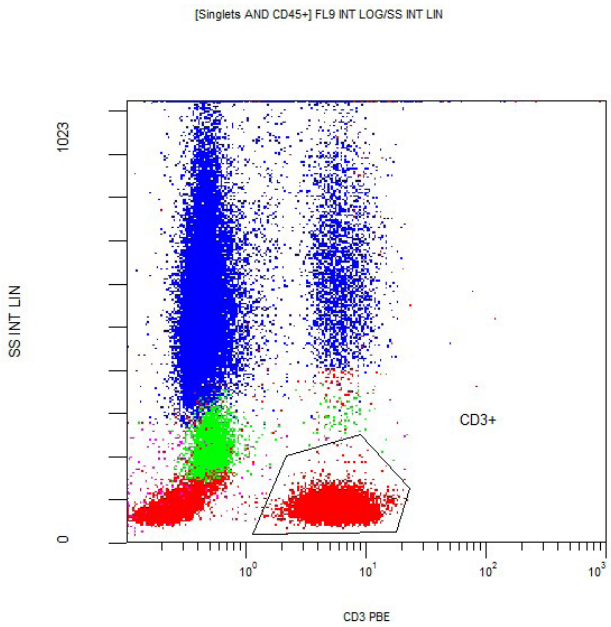


This Side Scatter/Forward Scatter dot plot demonstrates lymphocytes (red), monocytes (green), and granulocytes (blue).

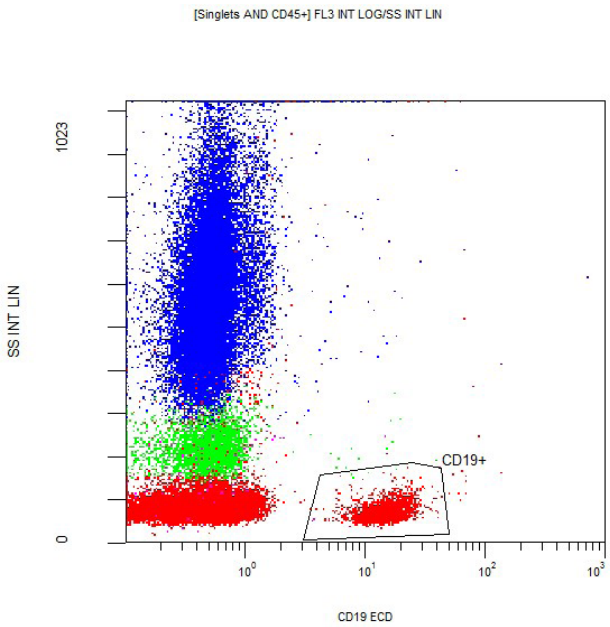


This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). The "Blast" gate contains only a few events. Note that the 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.

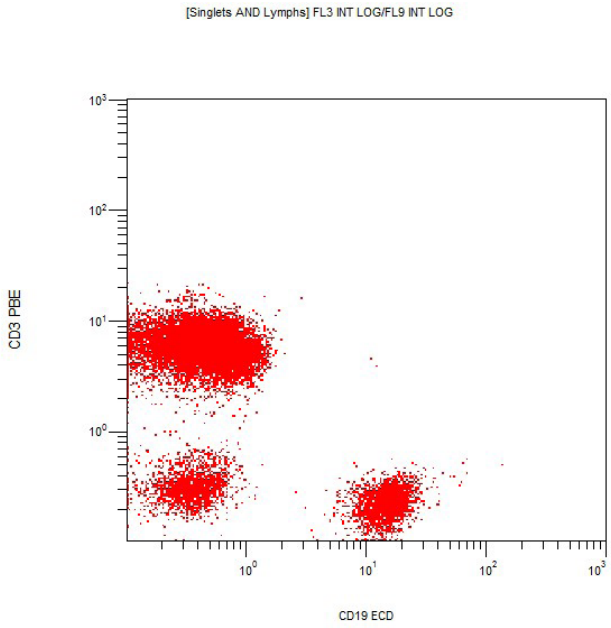
Note the mild degree of overlap in this case between apparent monocytes and lymphocytes. The gates should be adjusted such that they conform to the naturally occurring separations among the populations, but where no separation is observed an estimate based on experience may be used.



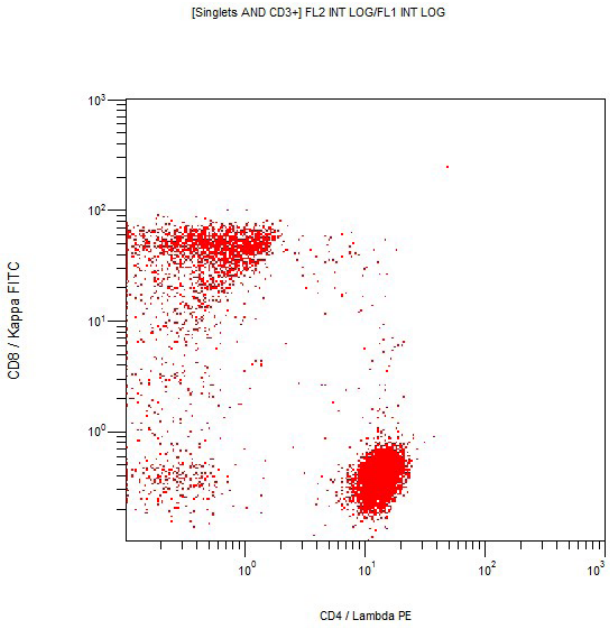
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.



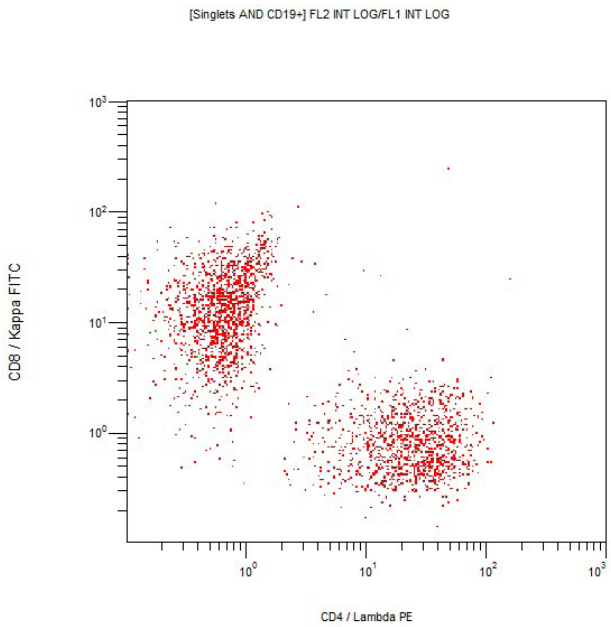
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only.



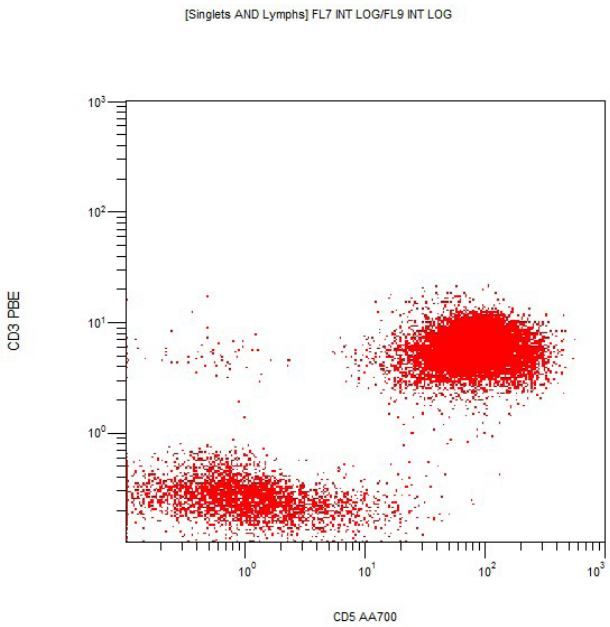
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present.



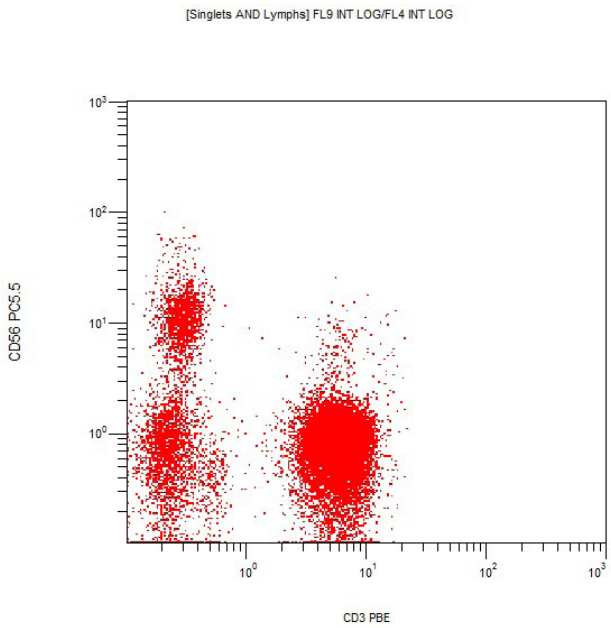
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes. A small population of CD4 and CD8 dual negative T lymphocytes is noted.



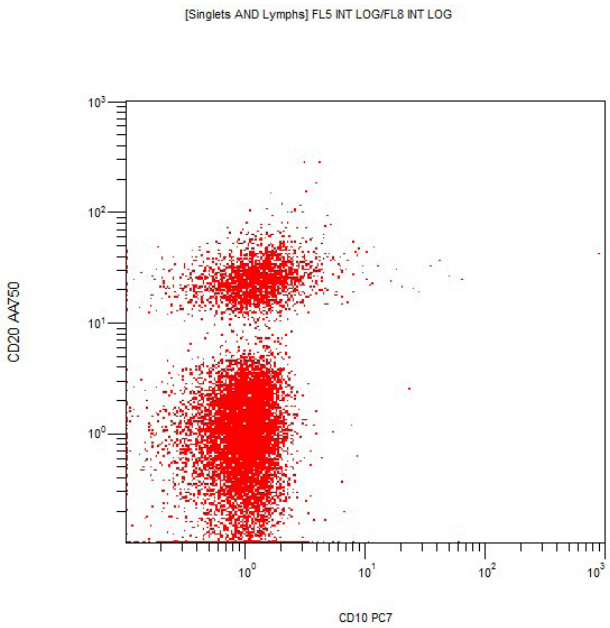
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations.



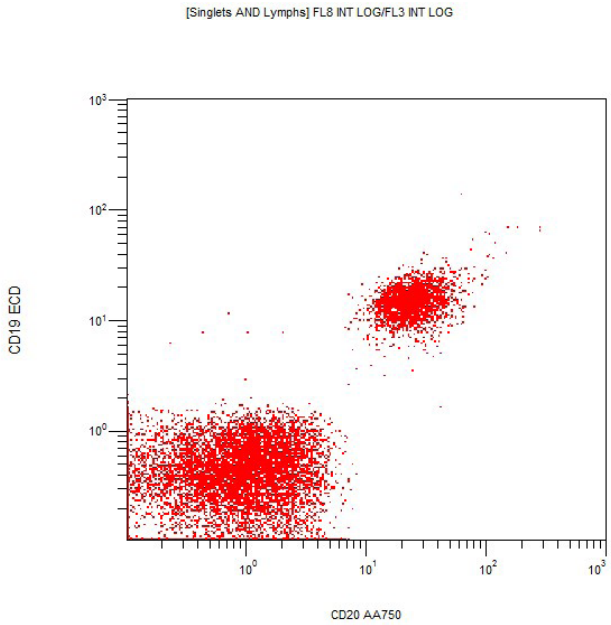
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable CD3 positive, CD5 positive T lymphocytes comprise the majority of cells. The remaining cells are a mixture of B lymphocytes and NK cells.



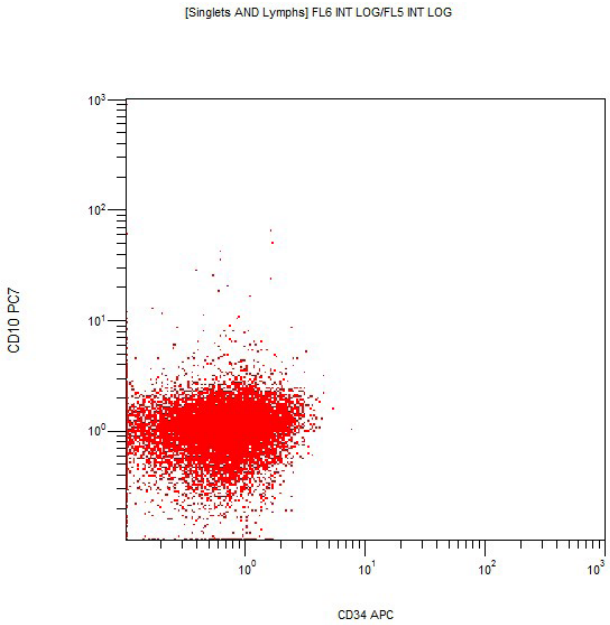
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. A small population of CD56 positive T lymphocytes is noted. The CD3 and CD56 dual negative cells are B lymphocytes.



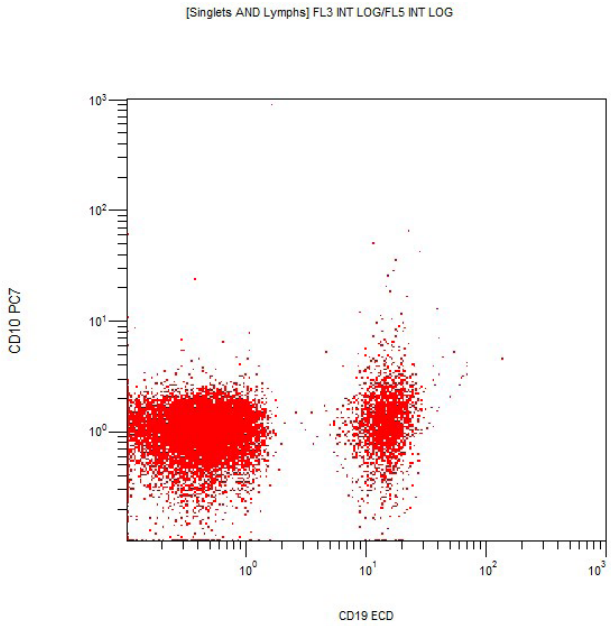
This CD10/CD20 dot plot is gated on Lymphocytes. No distinct population of B lymphocytes that co-express CD10 is noted.



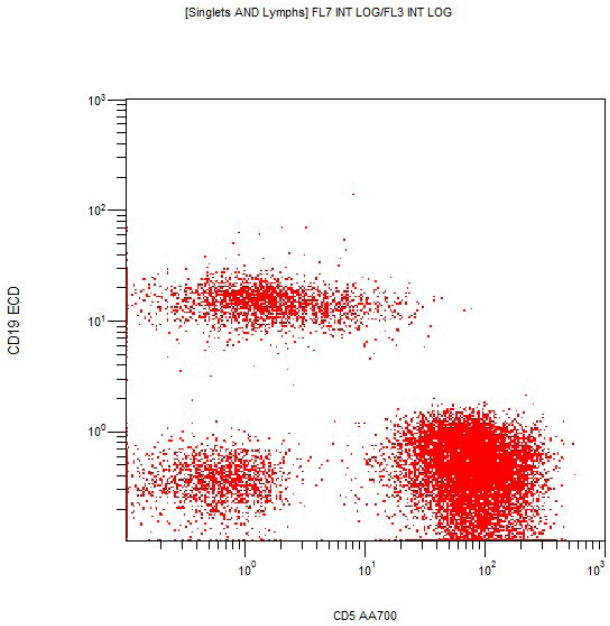
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display expected normal co-expression of CD19 and CD20.



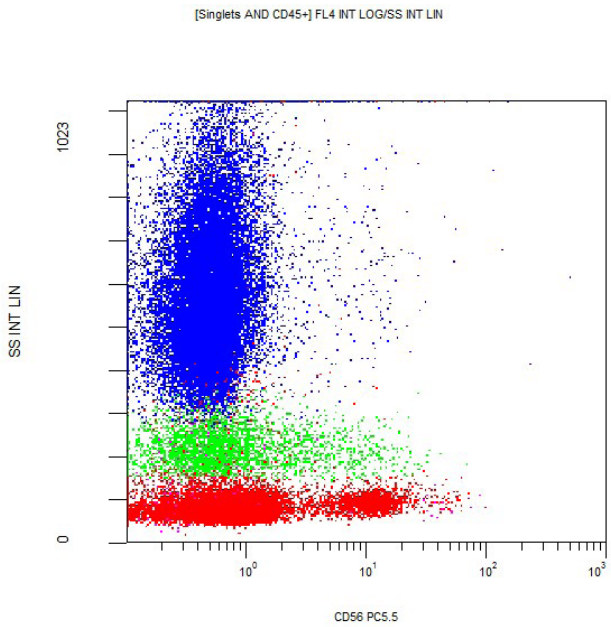
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, as expected for mature peripheral blood lymphocytes.



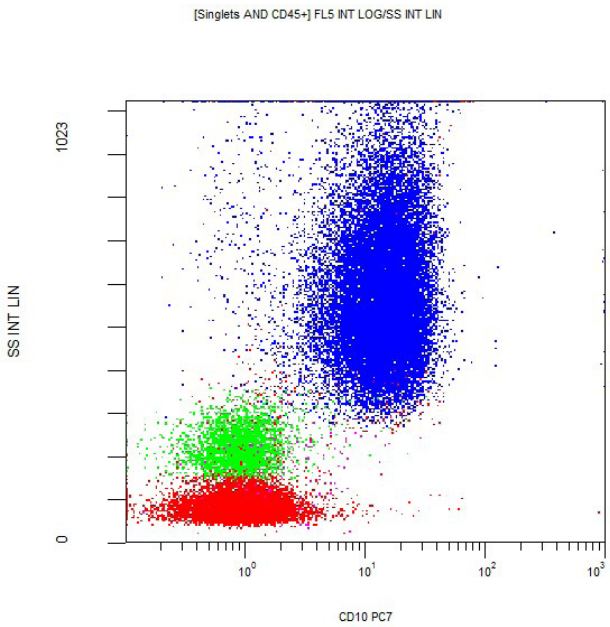
This CD19/CD10 dot plot is gated on Lymphocytes. B lymphocytes comprise the minority of lymphocytes in this peripheral blood sample, as expected.



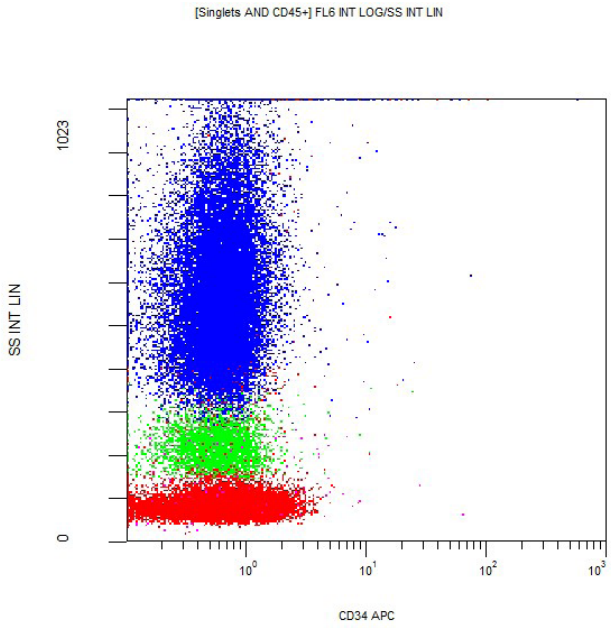
This CD5/CD19 dot plot is gated on Lymphocytes. Possible low density co-expression of CD5 and CD19 is identified on a small subset of B lymphocytes. Additional analysis of this population could be performed in order to establish or rule out immunoglobulin light chain restriction within this compartment.



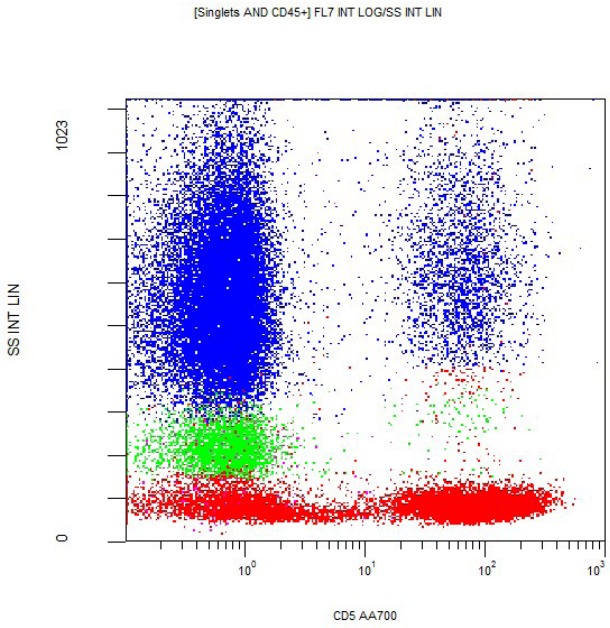
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population 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.



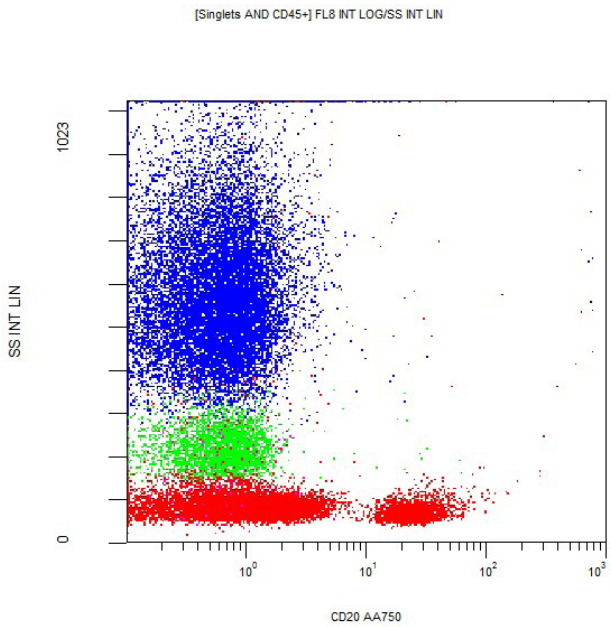
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are positive for CD10.



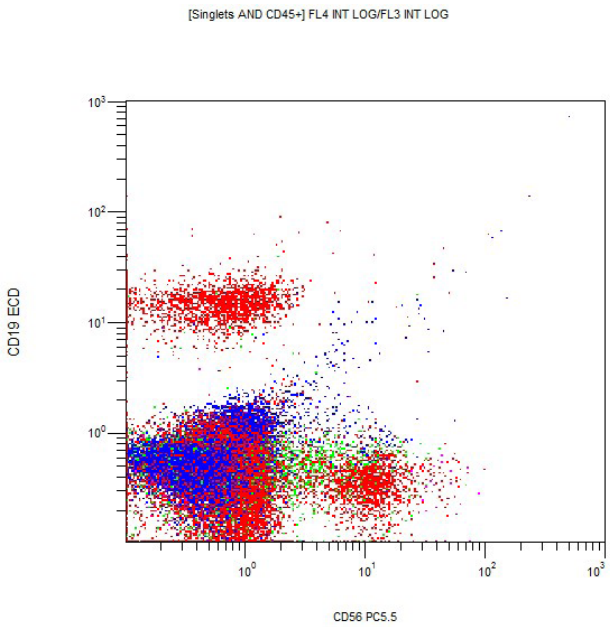
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No significant CD34 positive population is present.



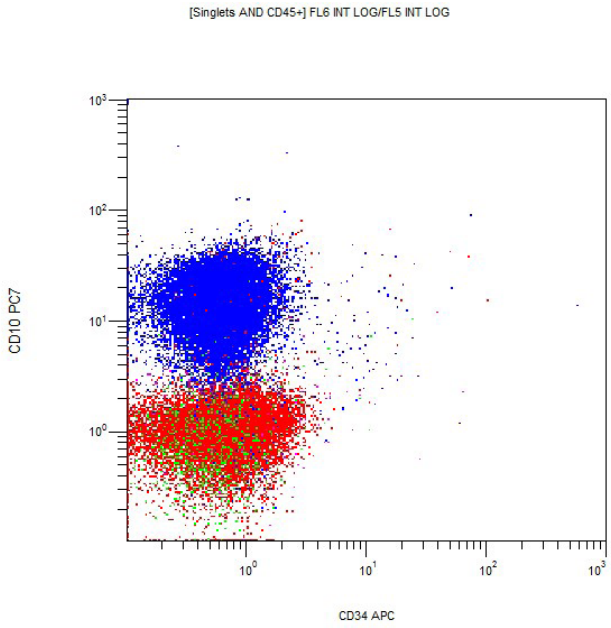
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The majority of the lymphocytes (red) express CD5, consistent with T lymphocytes.



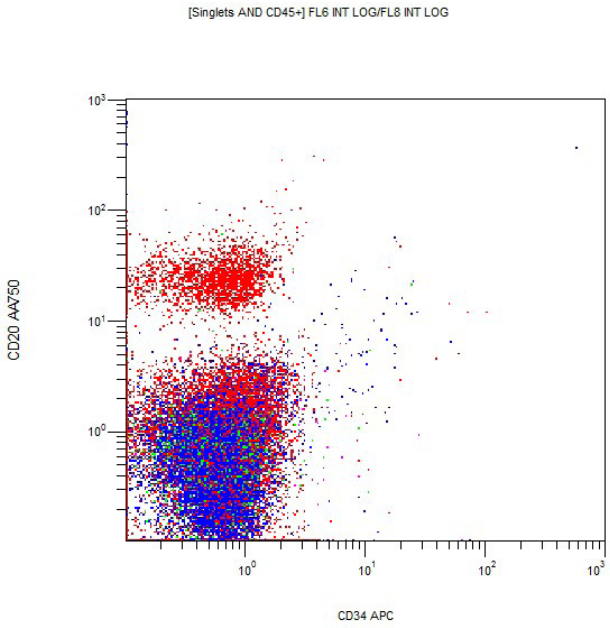
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). As noted elsewhere in this case, B lymphocytes are present but represent a minority of lymphocytes.



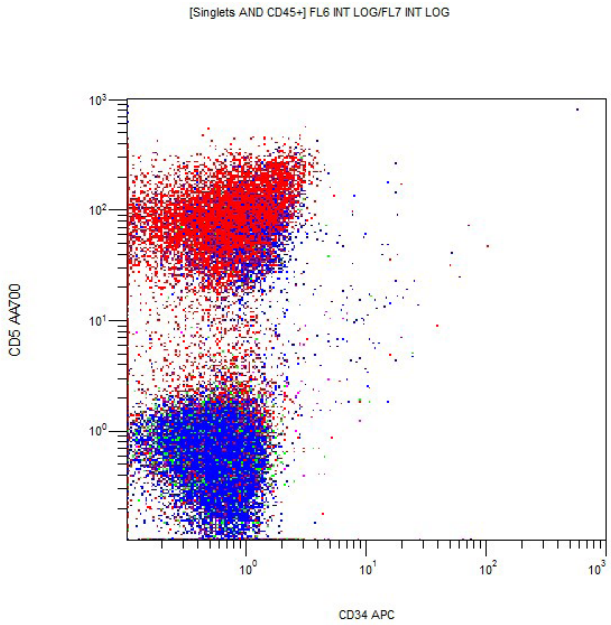
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes and NK cells are present. The blue 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.



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.

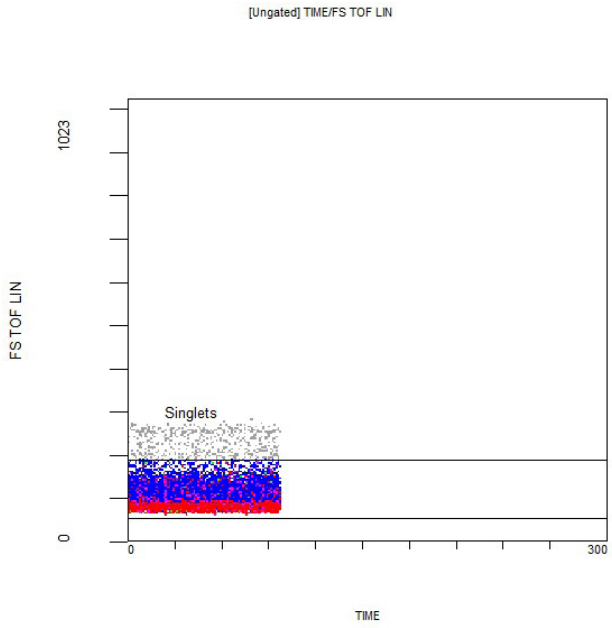
Case #2: Normal Bone Marrow

Clinical Vignette

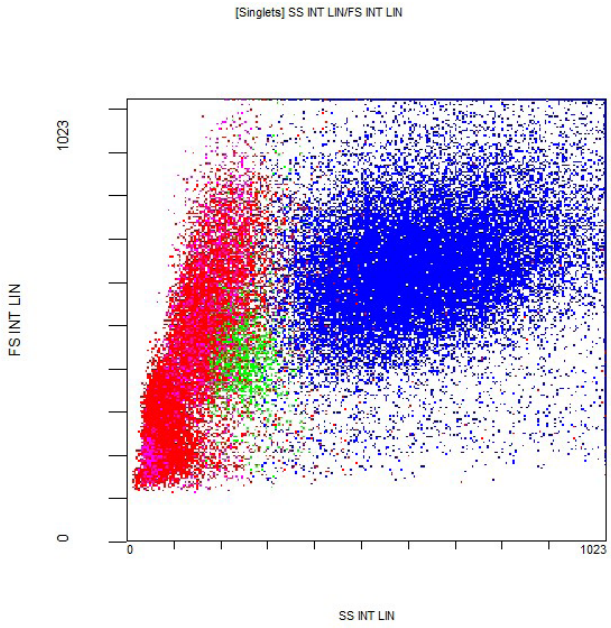
This 75-year-old female presents with thrombocytopenia. A bone marrow aspirate sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

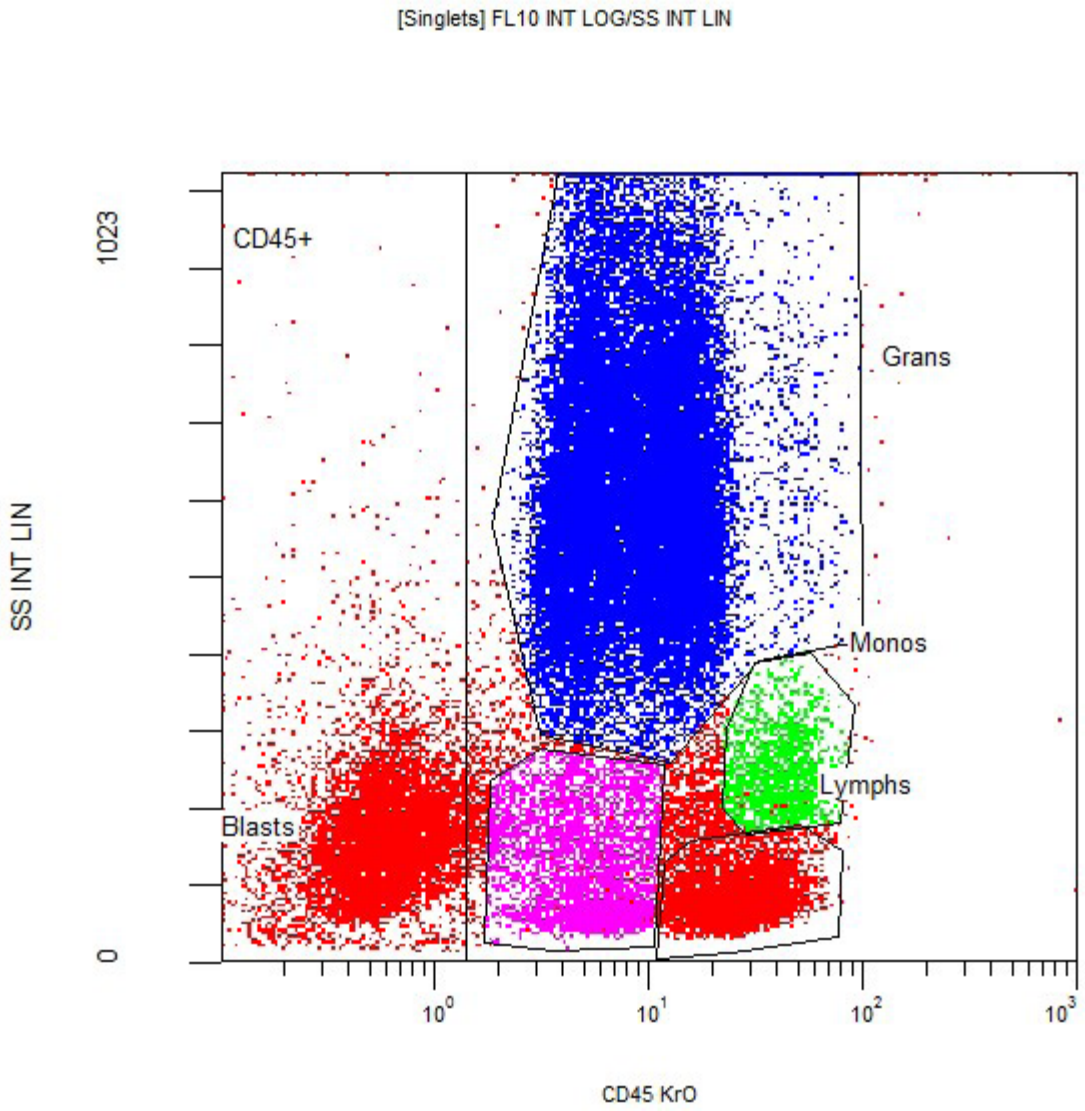
Access Case #2 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

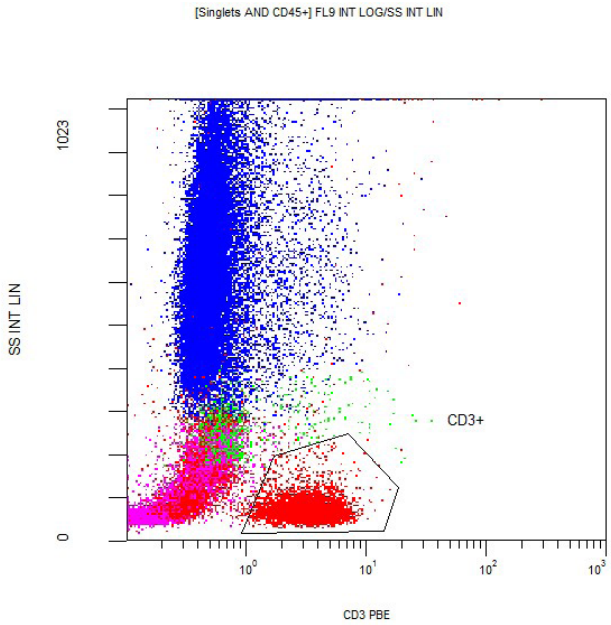


This Side Scatter/Forward Scatter dot plot demonstrates lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink).

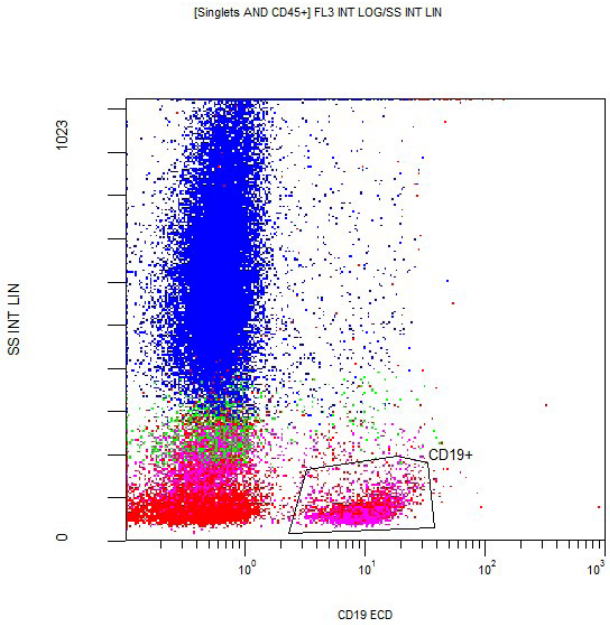


This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). Additionally, the "Blast" gate (pink) has been used to define cell that do not fall into any of the other gates and express low density CD45. Normal bone marrow contain several low density CD45 populations, and the size and distribution of these populations vary with patient age. The Blast gate may also contain debris. Note that the label for the Blast gate is not immediately adjacent to it---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 that the overlap among the various populations in this particular case renders it somewhat difficult to distinguish among them. 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.

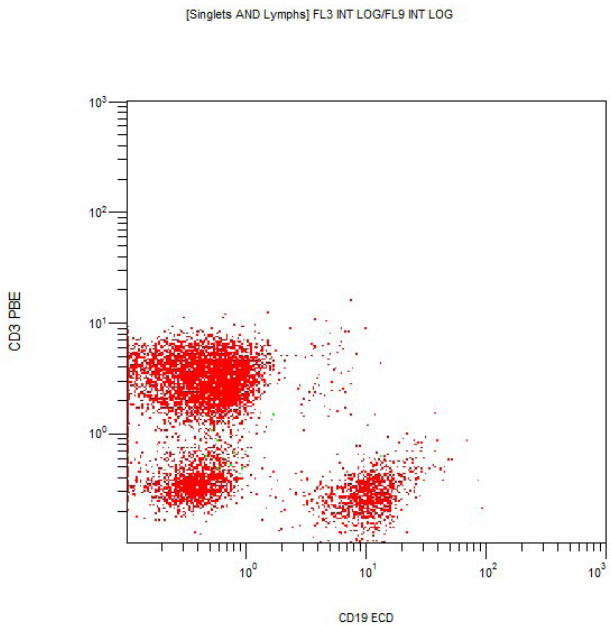


This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.

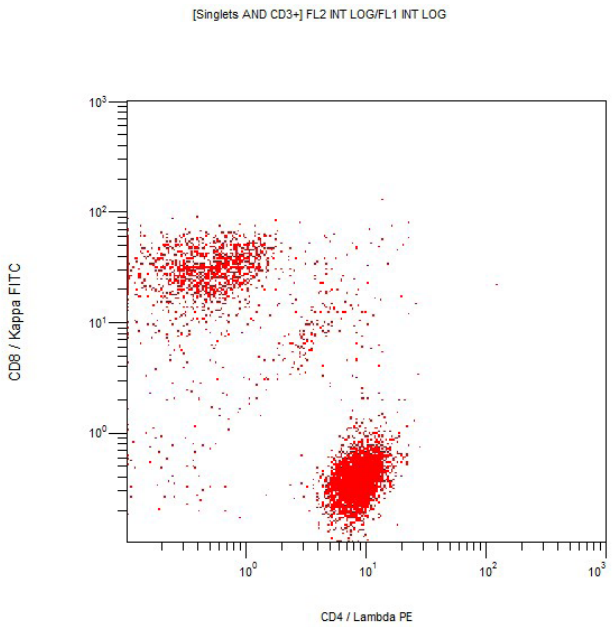


This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only. Depending on the number of B cell progenitors ("hematogones") in the sample and how you've drawn your lymphocyte (red) and blast (pink) gates, you may or may not see a mixture of pink and red cells in the CD19+ gate. In this instance at least some of the hematogones are included in the blast gate and are thereby depicted as pink.

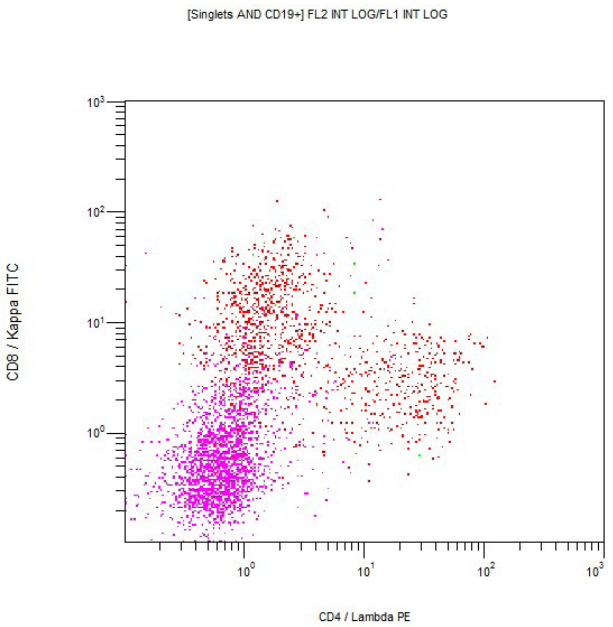
Note that this display is gated on CD45 positive events and consequently does not display events that are very low density or negative for CD45. As B lymphoblastic leukemia (B ALL) frequently displays very low density CD45, care should be taken to include CD45 negative events when B ALL is suspected.



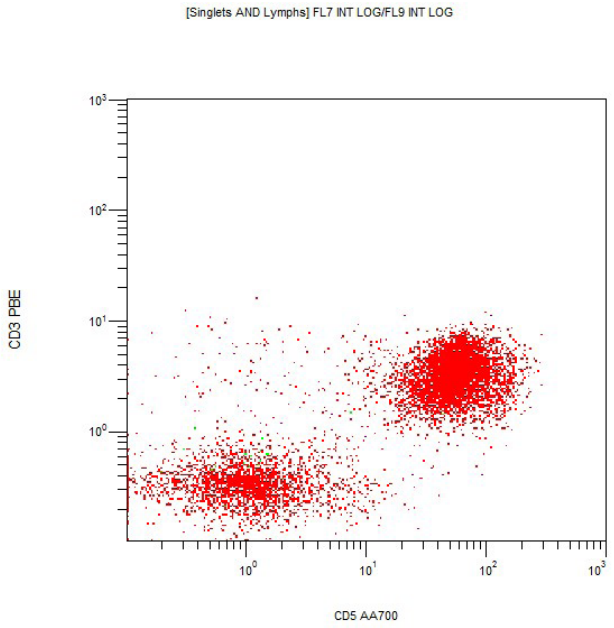
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present.



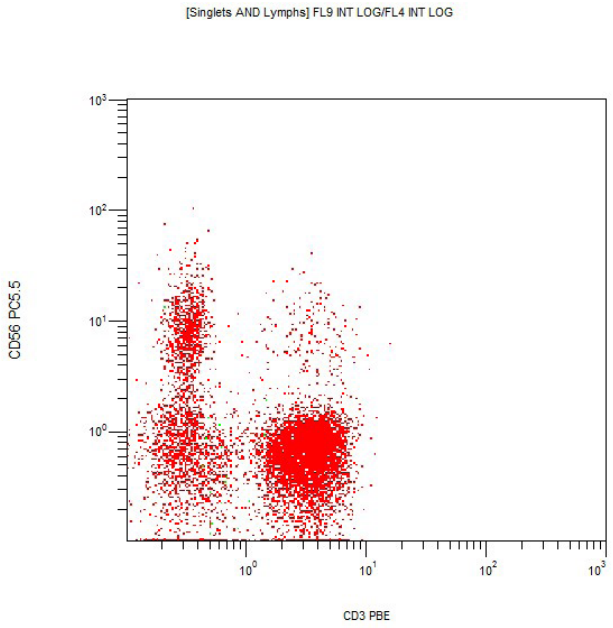
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes. A small population of CD4 and CD8 dual negative T lymphocytes is noted. The CD4 and CD8 dual positive events on the diagonal here are consistent with nonspecific staining of debris.



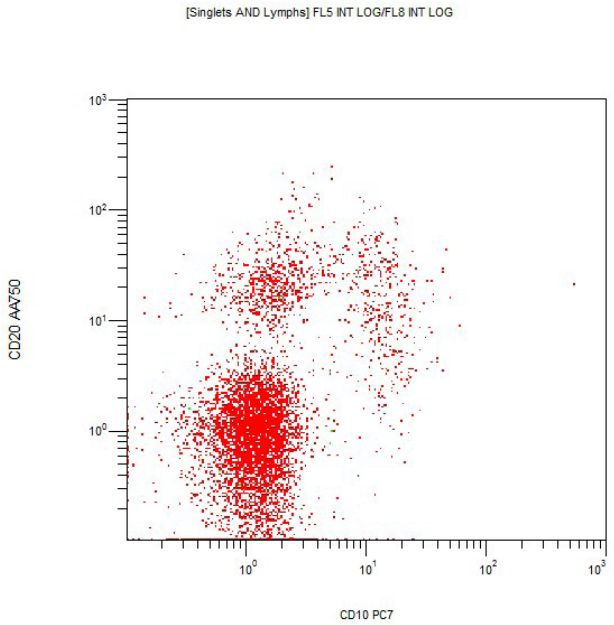
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. Note that the pink events are, as expected, negative for both Kappa and Lambda surface immunoglobulin light chains as they represent hematogones i.e. B cell progenitors.



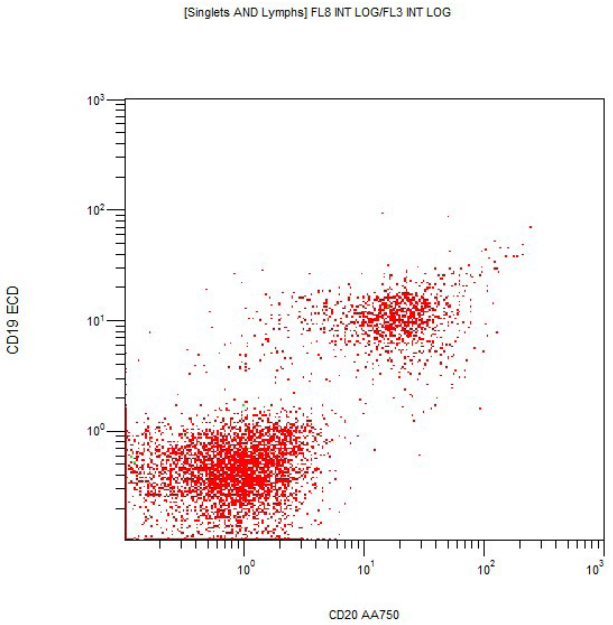
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable CD3 positive/CD5 positive T lymphocytes comprise the majority of cells. The remaining cells are a mixture of B lymphocytes and NK cells.



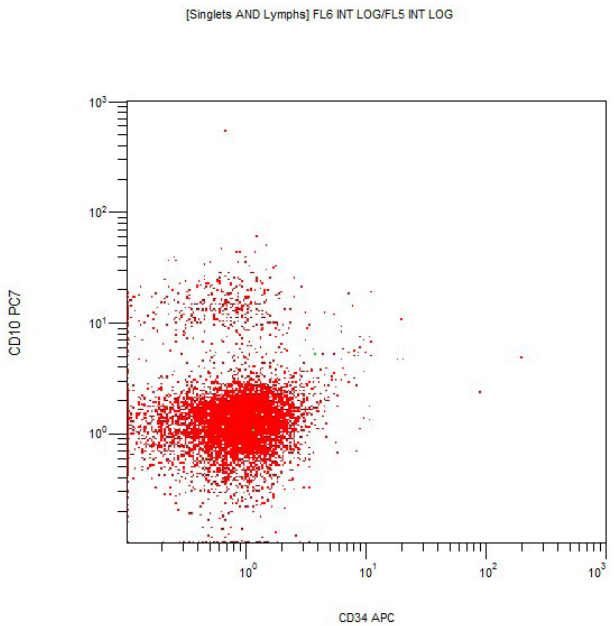
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. A small population of CD56 positive T lymphocytes is noted. The CD3 and CD56 dual negative cells are B lymphocytes.



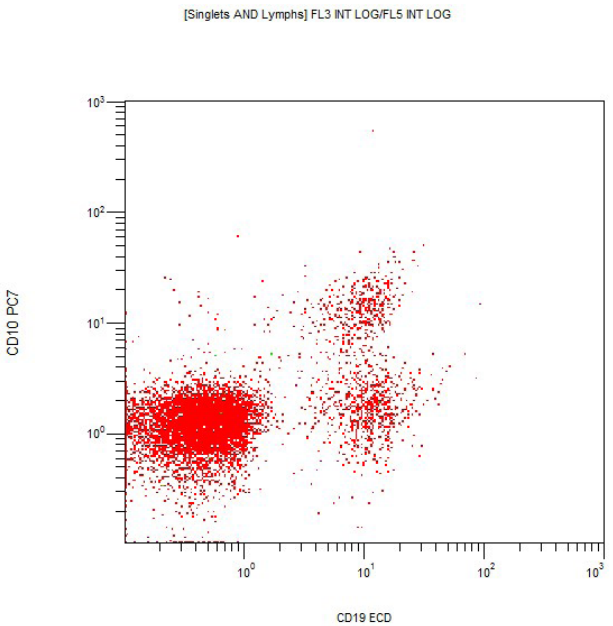
This CD10/CD20 dot plot is gated on Lymphocytes. A distinct population of B lymphocytes that co-express CD10 is noted. These cells are hematogones that were captured in the lymphocyte gate rather than the blast gate.



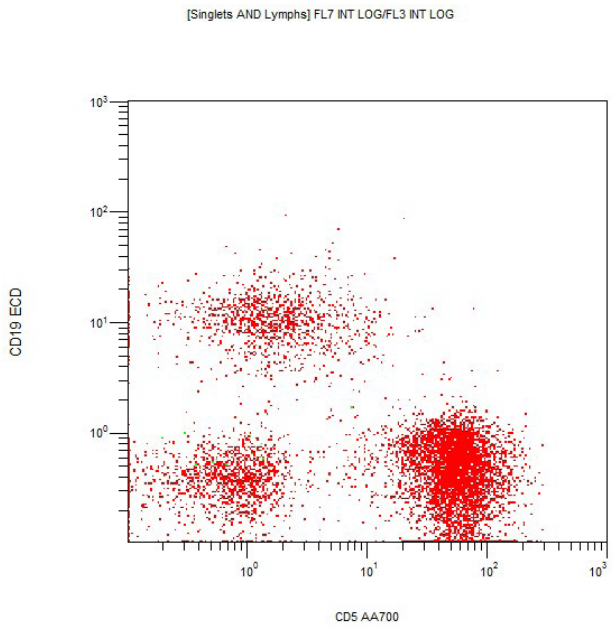
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display expected normal co-expression of CD19 and CD20. As there are also hematogones in this gate, increased heterogeneity for CD19 and CD20 expression is noted relative to mature B lymphocytes.



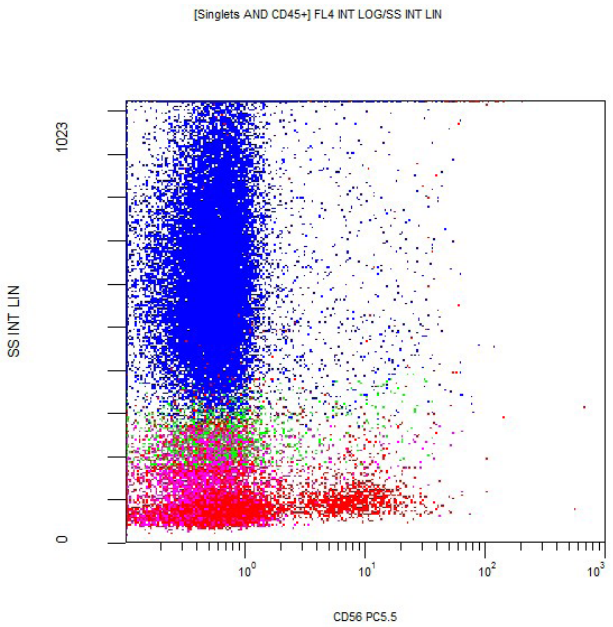
This CD34/CD10 dot plot is gated on Lymphocytes. The small subset of cells that express CD10 is consistent with hematogones. Note that there is very little, if any, co-expression of CD34. This finding typical of more mature hematogones---more mature hematogones also express higher density CD34 relative to less mature hematogones, and are therefore more likely to have been included in the lymphocyte gate.



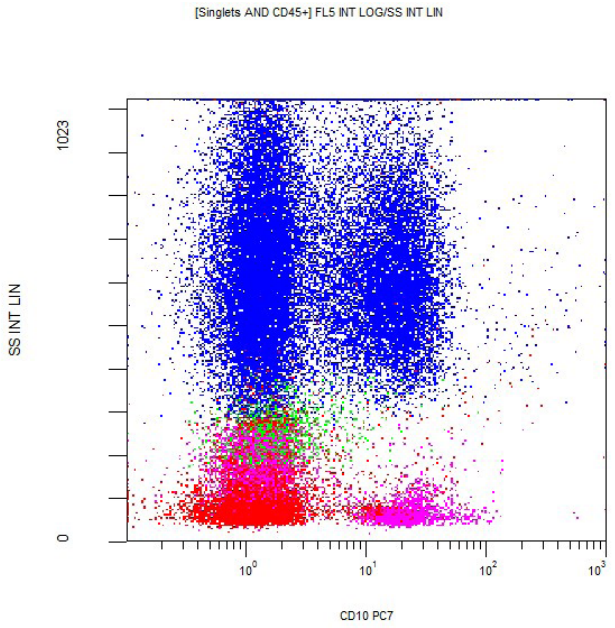
This CD19/CD10 dot plot is gated on Lymphocytes. The CD19 positive, CD10 negative population is comprised of normal mature B lymphocytes whereas the CD19 and CD10 dual positive population is comprised of hematogones.



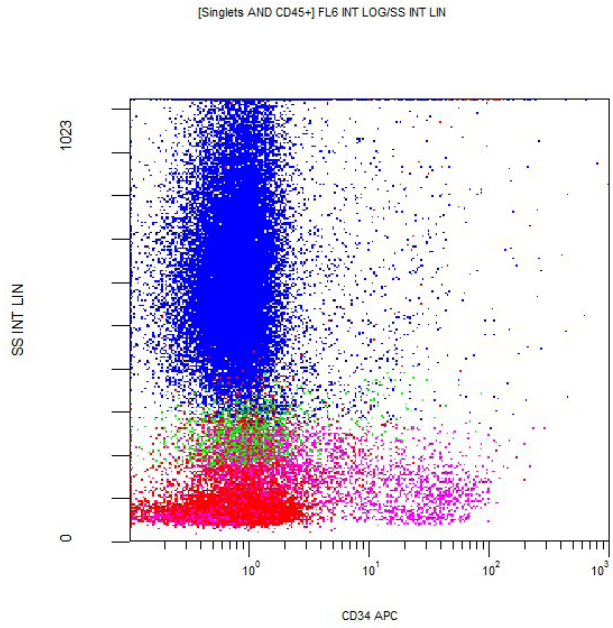
This CD5/CD19 dot plot is gated on Lymphocytes and shows the expected distribution.



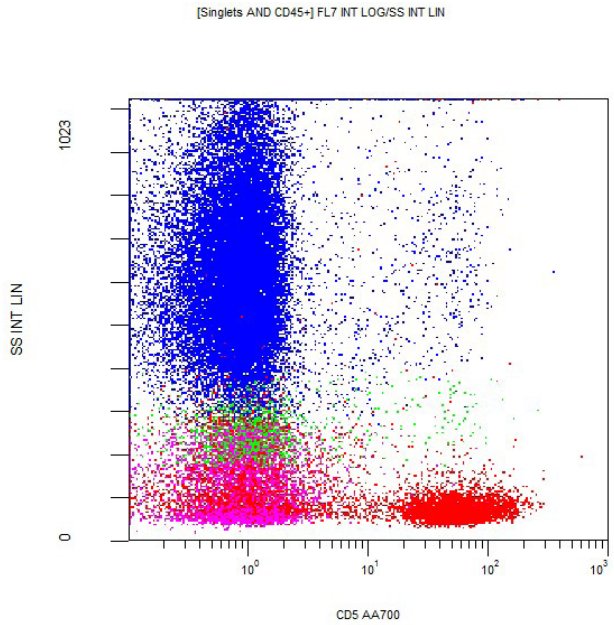
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells.



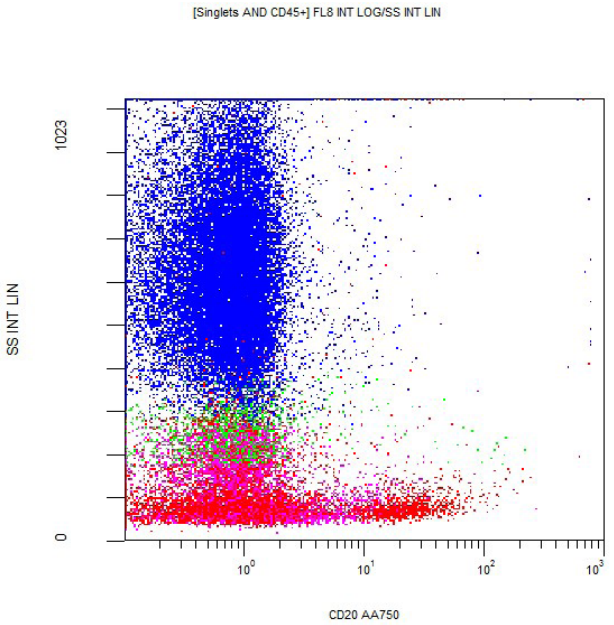
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are partially positive for CD10. The events within the blast gate (pink) with low side scatter that express CD10 are hematogones.



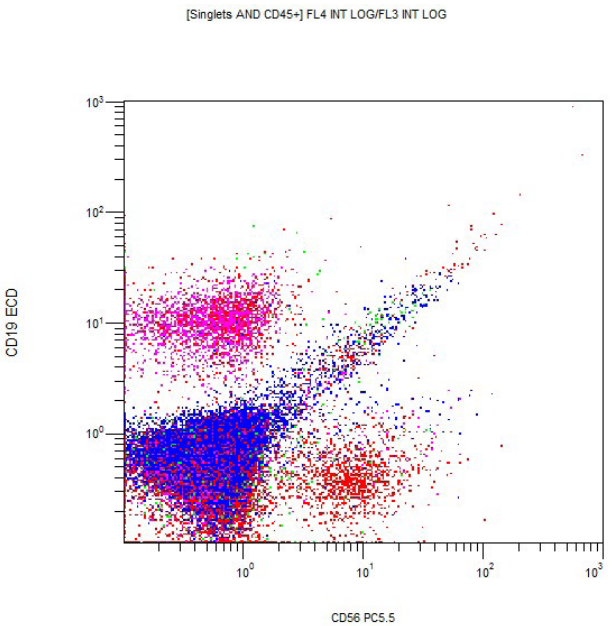
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). CD34 expression is noted, but the events do not form a tightly clustered pattern due to the mixture of different cell types that express CD34 in this sample.



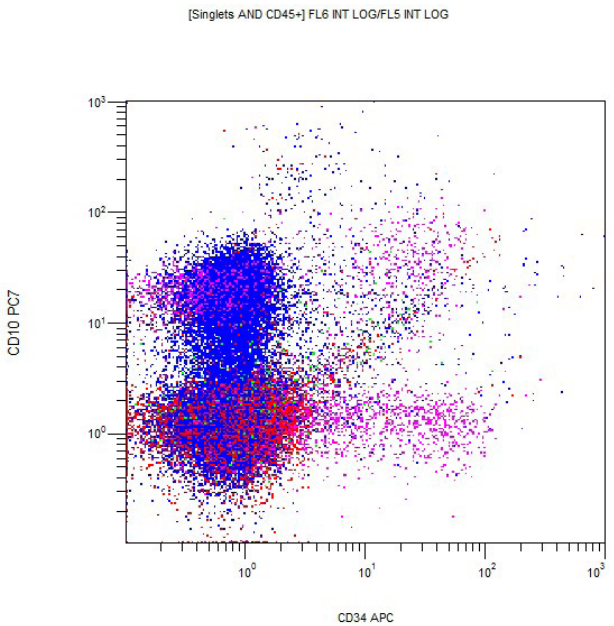
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Many of the lymphocytes (red) express CD5, consistent with T lymphocytes.



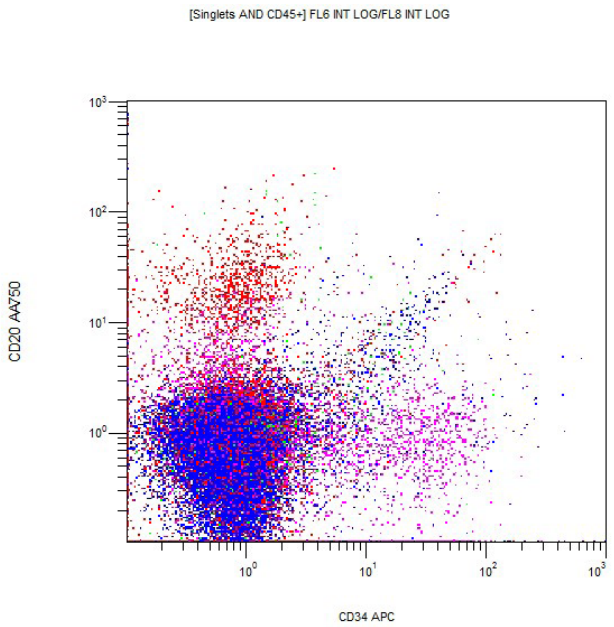
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The heterogeneity in CD20 expression noted here is due to the mixture of mature B lymphocytes and hematogones.



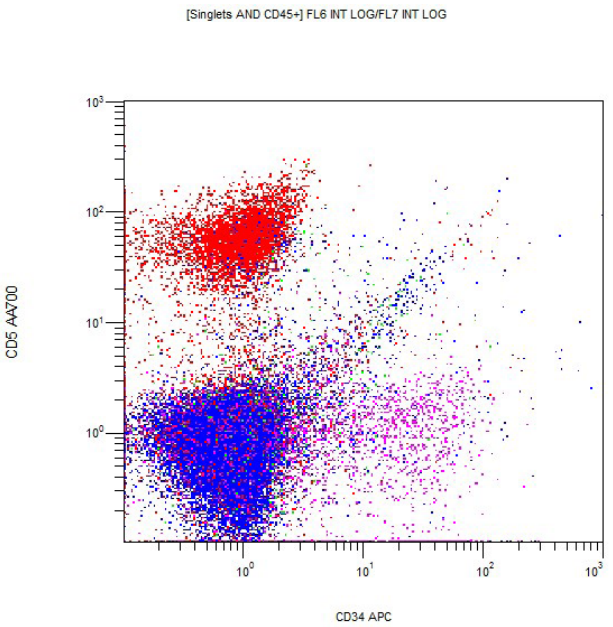
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes (red), NK cells (red), and hematogones (pink) are all 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. The pink events in the blast gate include more mature hematogones (CD10 positive, CD34 negative), myeloblasts (CD10 negative, CD34 positive), and less mature hematogones (CD10 and CD34 dual positive).



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). The CD20 negative, CD34 positive events are myeloblasts, show in pink because they were included in the original blast gate.



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. The CD5 negative, CD34 positive events are myeloblasts, show in pink because they were included in the original blast gate.

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.

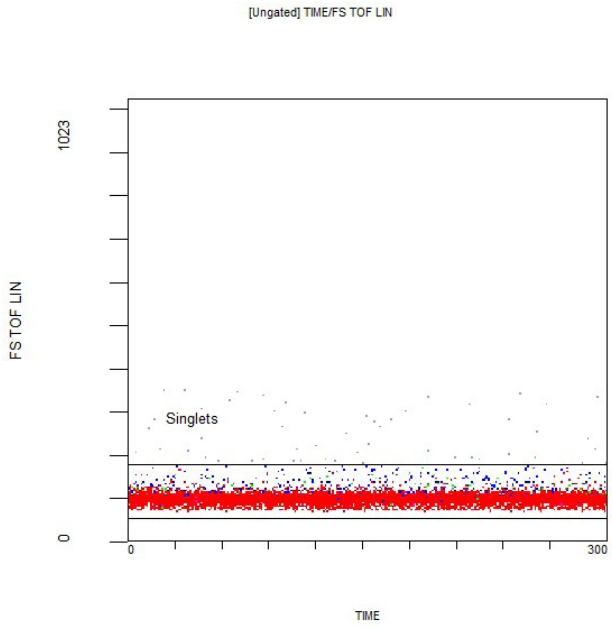
Case #3: Normal Lymph Node

Clinical Vignette

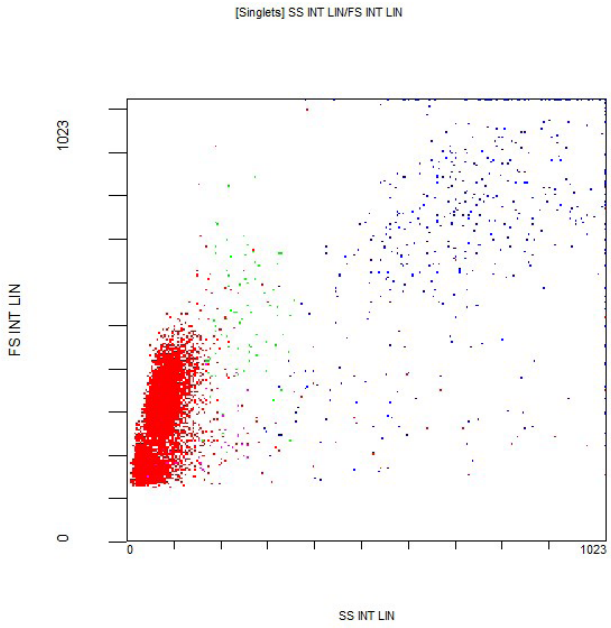
This 32-year-old female presents with lymphadenopathy. A lymph node biopsy sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

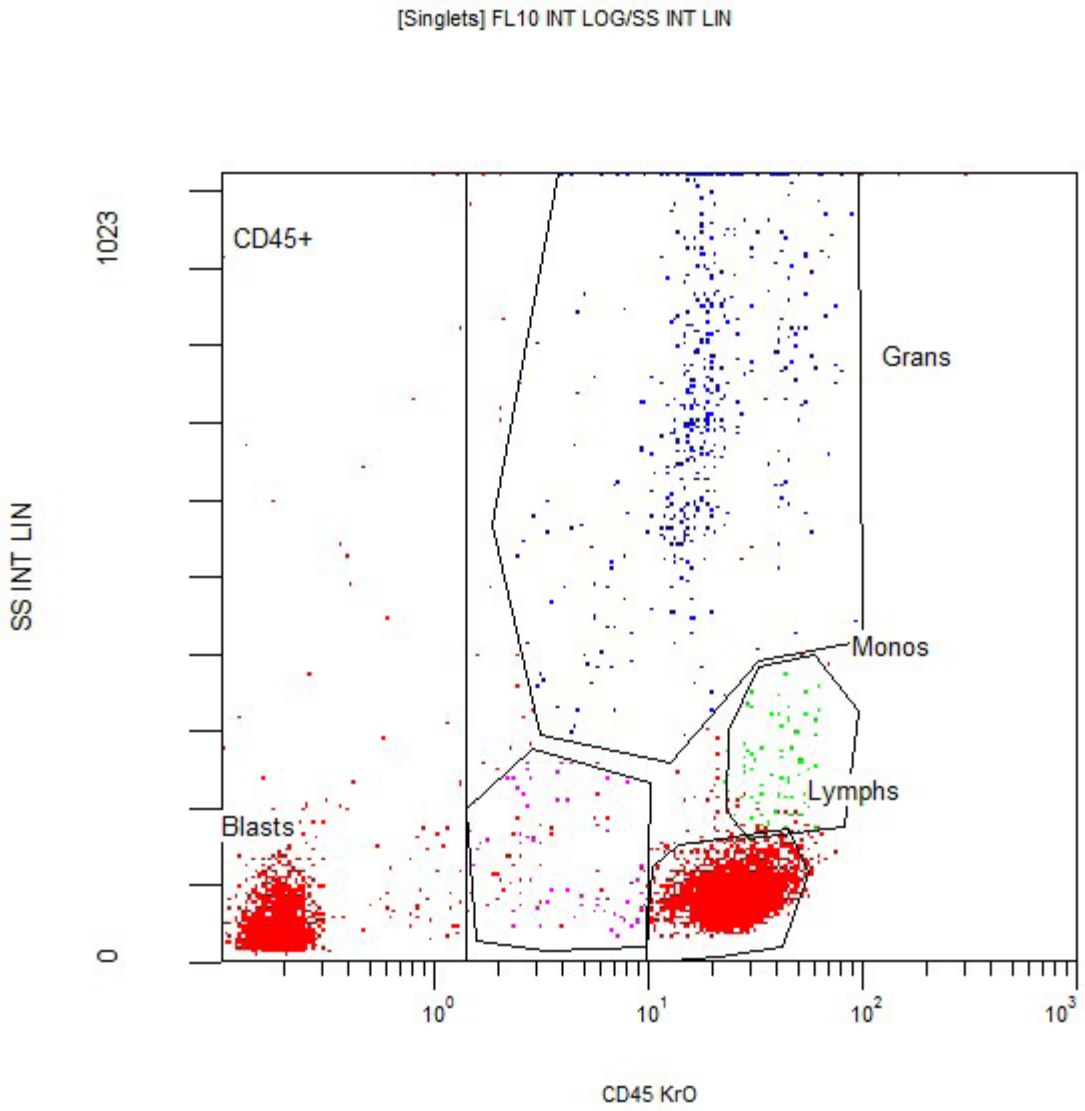
Access Case #3 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

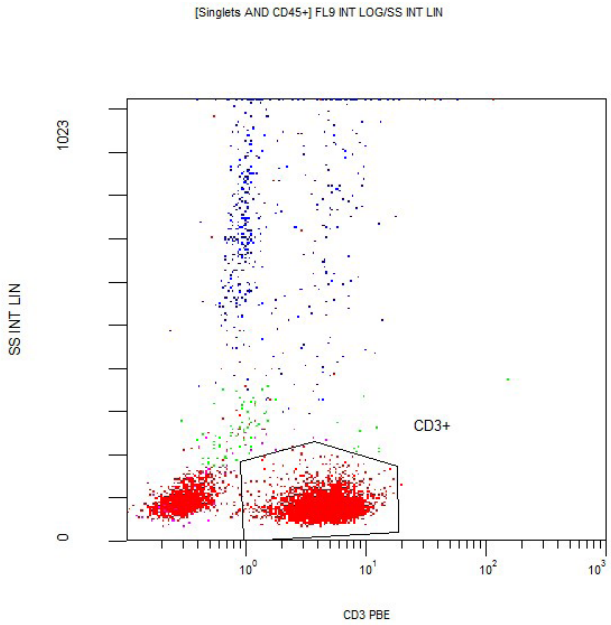


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of apparent lymphocytes (red) as well as a few monocytes (green) and granulocytes (blue). Note that the events with very low forward scatter seen here may represent debris.

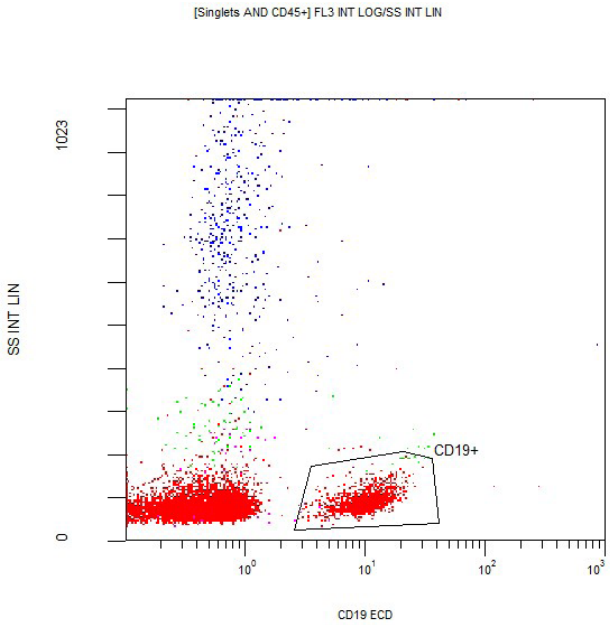


This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). The "Blast" gate contains only a few events. Note that the 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 or. In this case the CD45 negative events are consistent with debris, consistent with the findings in the Side Scatter/Forward Scatter dot plot.

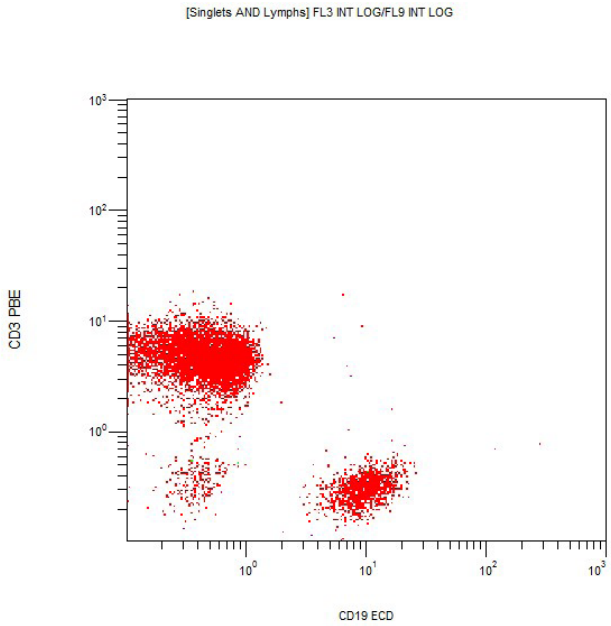
Note that the minimal overlap among the various populations in this particular case renders it easy to distinguish among them.



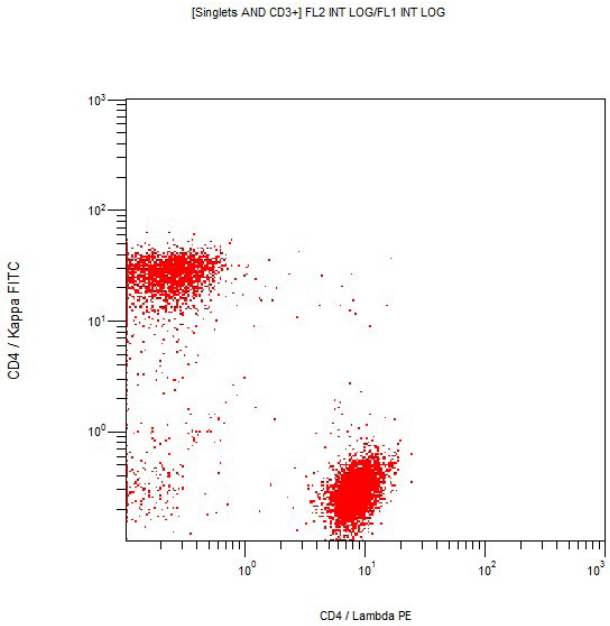
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.



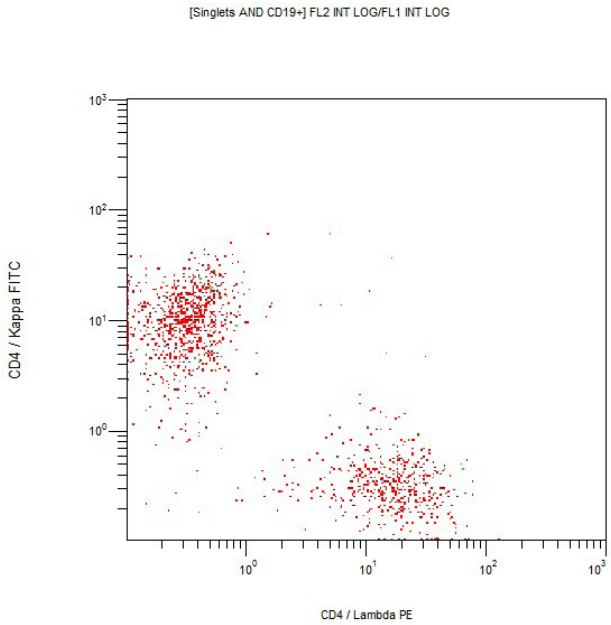
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only.



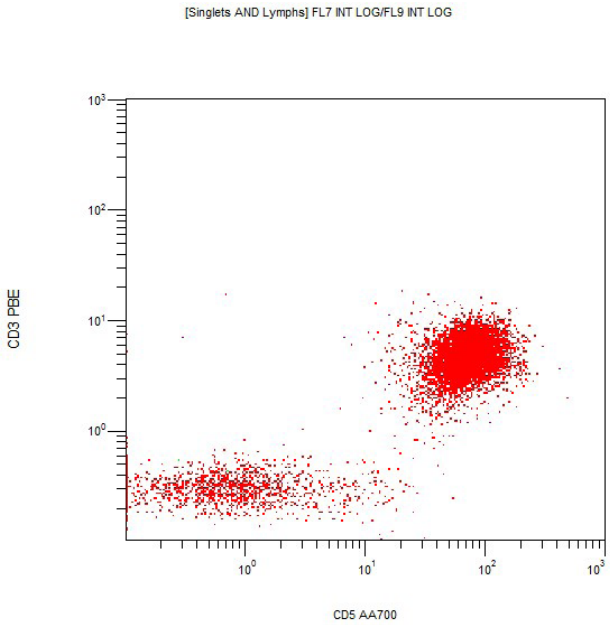
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis.



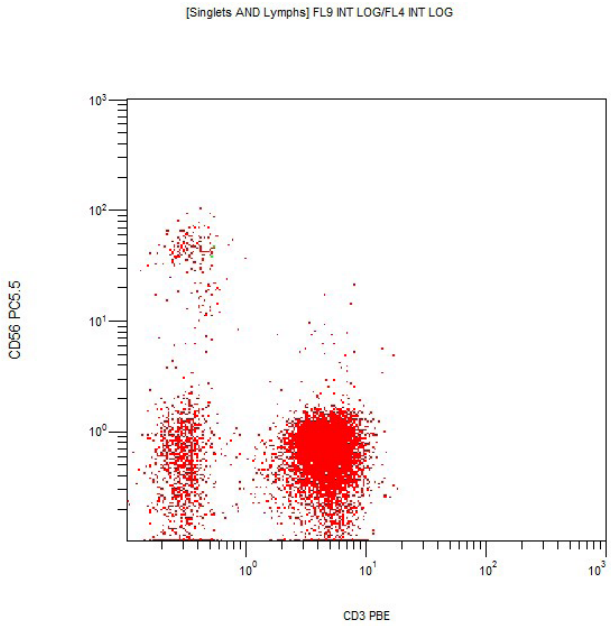
Applying the CD3+ gate to this dot plot permits “unstacking” of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



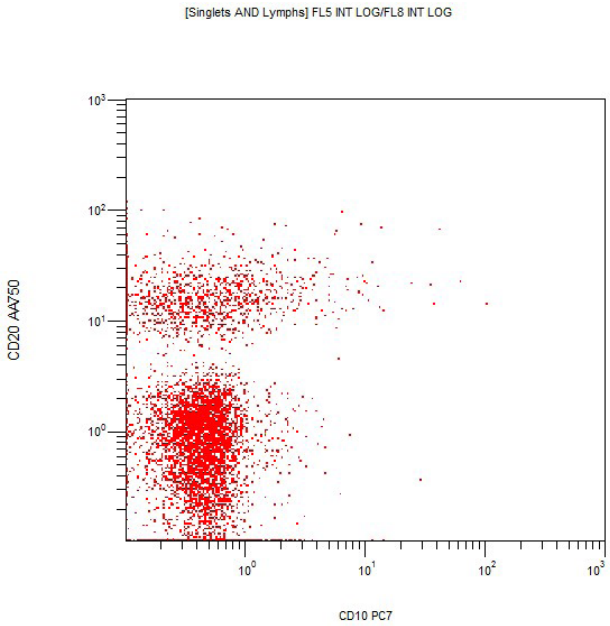
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations.



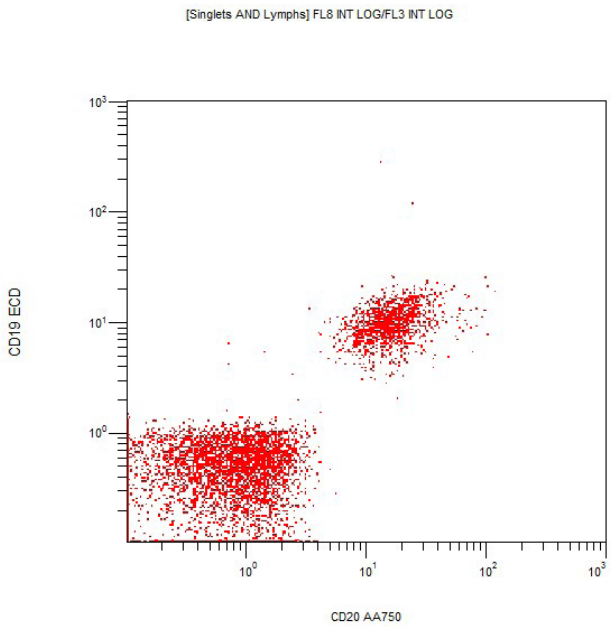
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable CD3 positive/CD5 positive T lymphocytes comprise the majority of cells. The remaining cells are predominantly B lymphocytes.



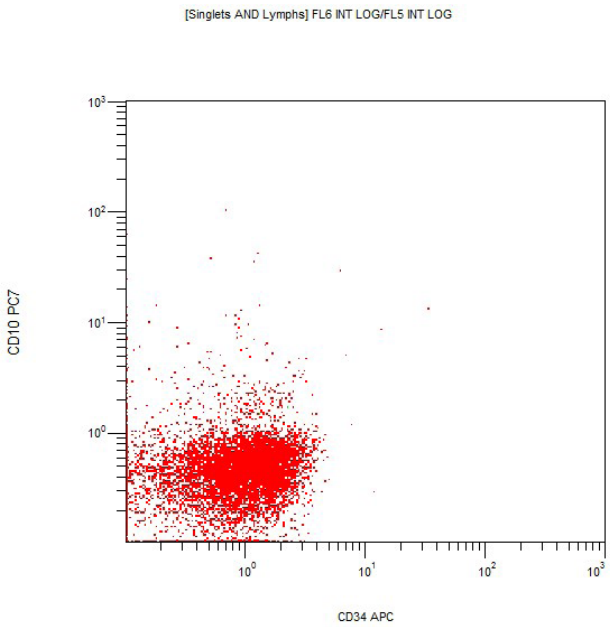
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and a small number of NK cells (CD56 positive, CD3 negative) are present. The CD3 and CD56 dual negative cells are B lymphocytes.



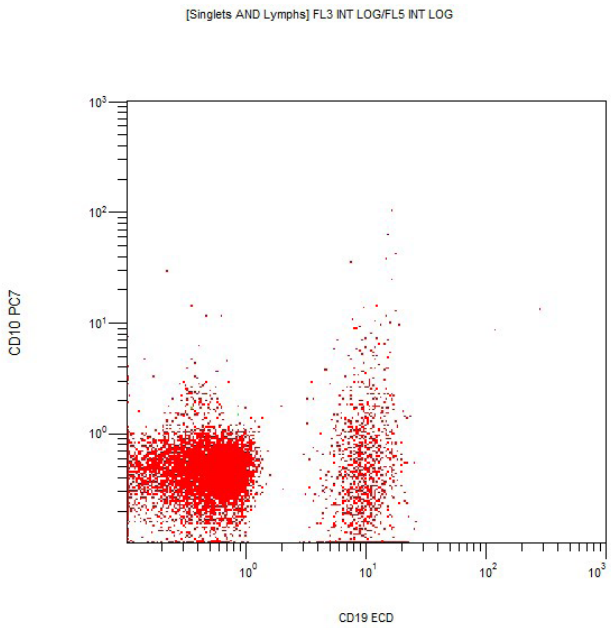
This CD10/CD20 dot plot is gated on Lymphocytes. No distinct population of B lymphocytes that co-express CD10 is noted. Note that normal germinal center cells do express low density CD10. In lymph nodes with prominent follicular hyperplasia this population may be very obvious.



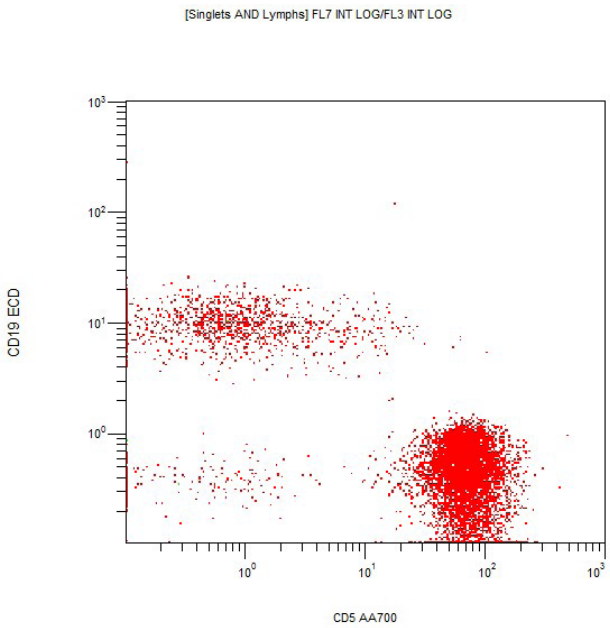
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display expected normal co-expression of CD19 and CD20.



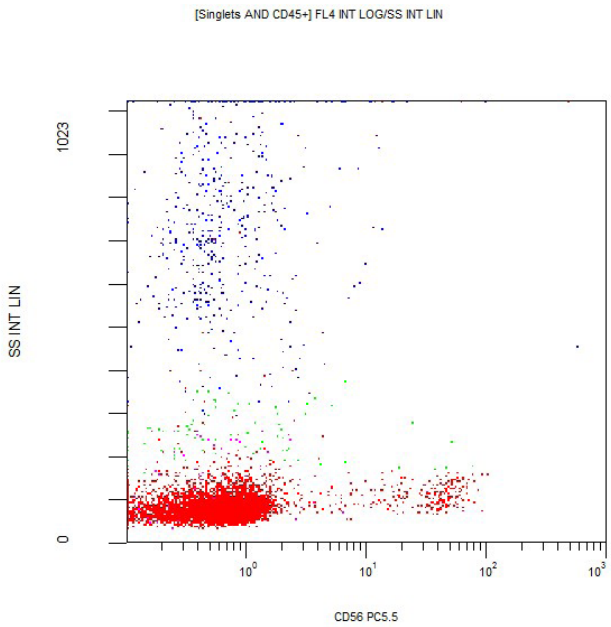
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, consistent with mature T and B lymphocytes. The scattered CD10 positive events are consistent with germinal center B lymphocytes.



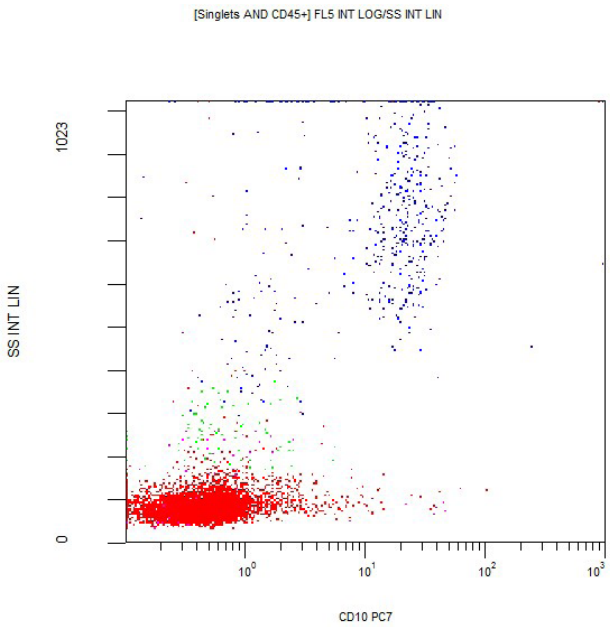
This CD19/CD10 dot plot is gated on Lymphocytes. T lymphocytes comprise the majority of cells and B lymphocytes show some co-expression of CD10.



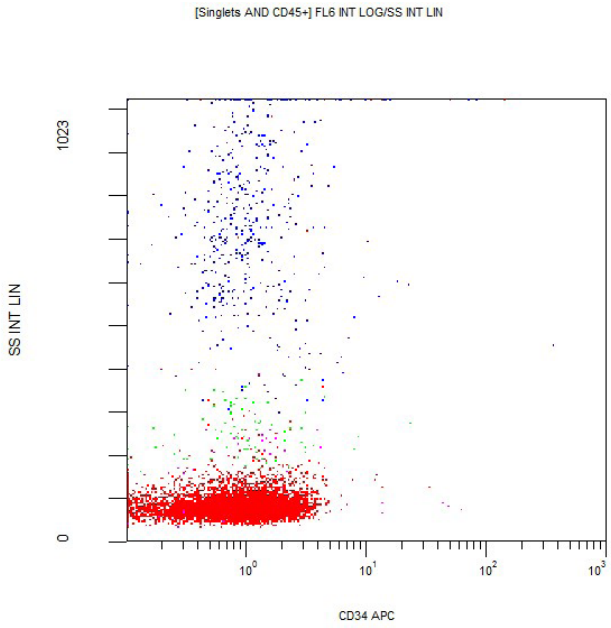
This CD5/CD19 dot plot is gated on Lymphocytes. Possible low density co-expression of CD5 and CD19 is identified on a small subset of B lymphocytes. Additional analysis of this population could be performed in order to establish or rule out immunoglobulin light chain restriction within this compartment.



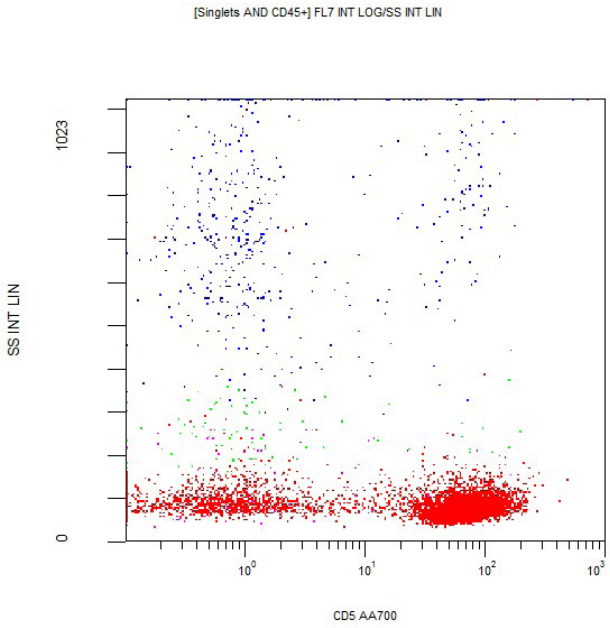
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells.



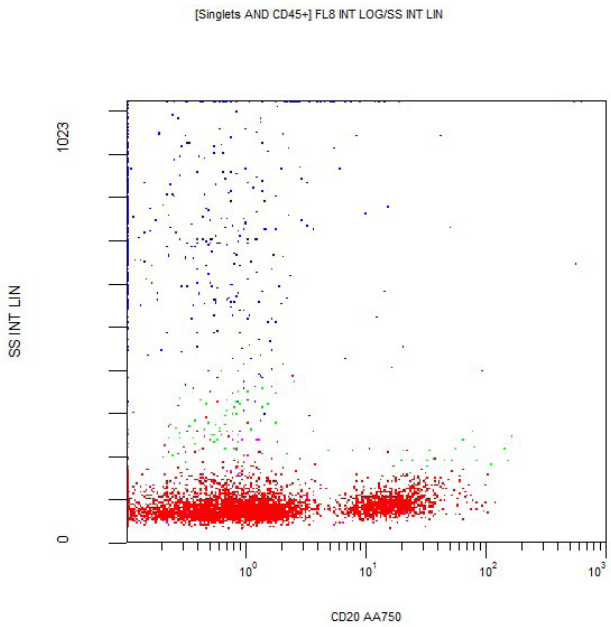
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are positive for CD10.



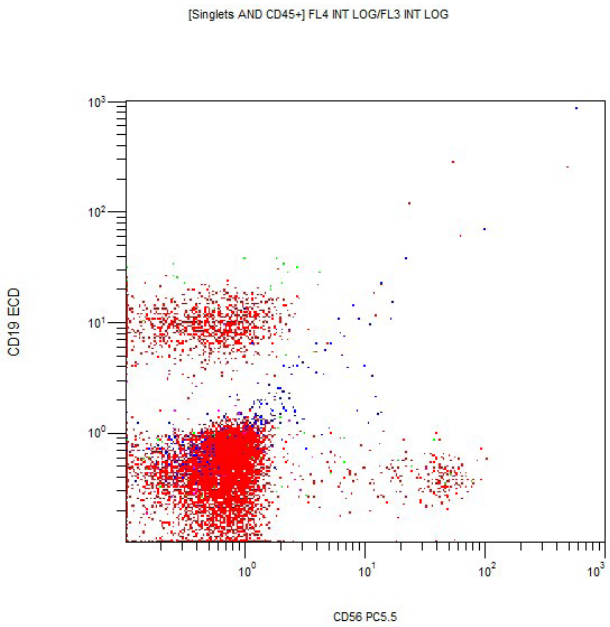
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No significant CD34 positive population is present.



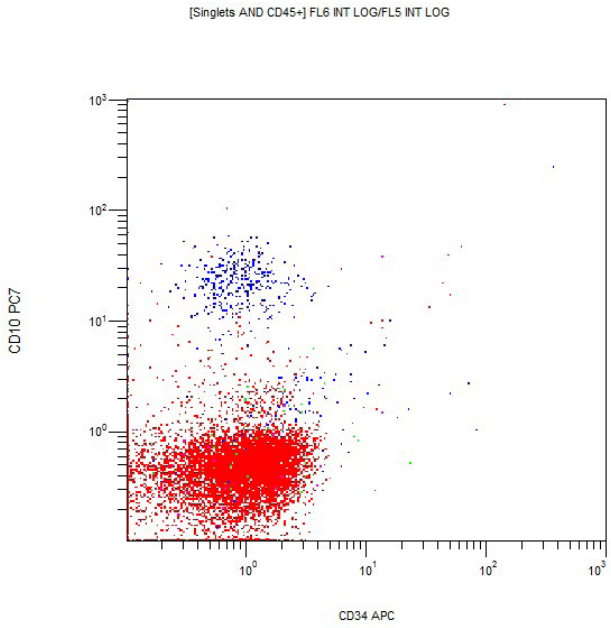
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The majority of the lymphocytes (red) express CD5, consistent with T lymphocytes.



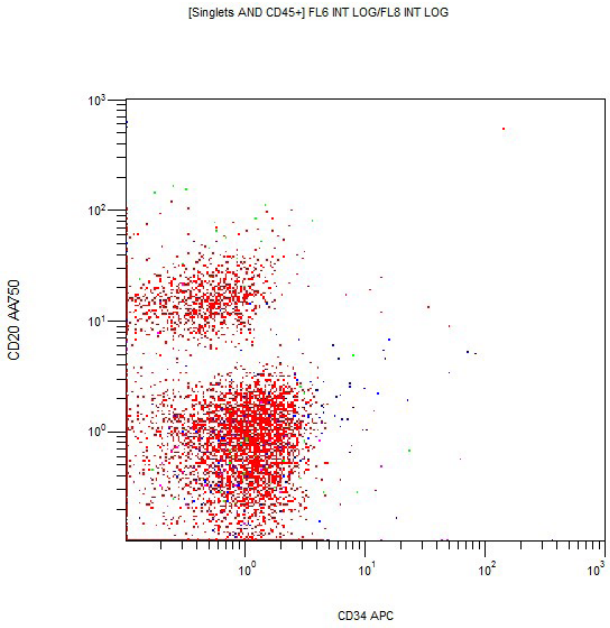
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). As noted elsewhere in this case, B lymphocytes are present.



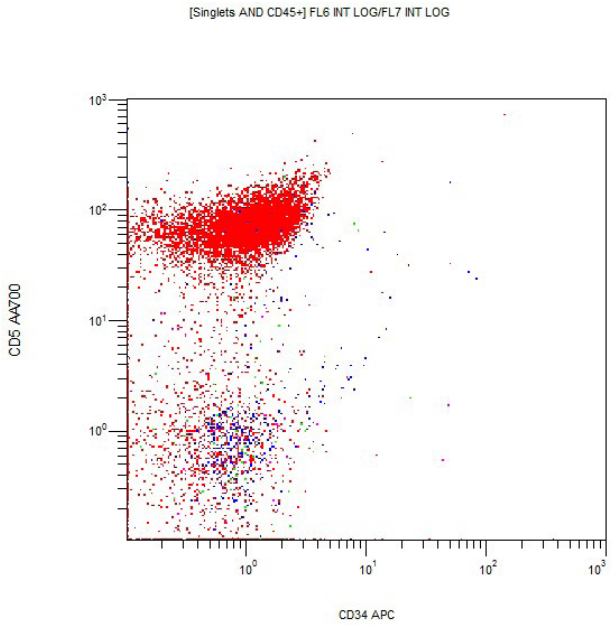
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes and NK cells are present. The blue 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). A small but distinct population of CD10 positive, CD34 negative events corresponds to granulocytes (blue). The blue events that appear to co-express CD34 and CD10 are consistent with high background fluorescence.



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.



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. Both Hodgkin lymphoma and metastatic malignancies should be considered in this type of sample.

NEOPLASTIC PROCESS OF B CELL ORIGIN

B cell neoplasms, which comprise the majority of all lymphoid neoplasms, are a diverse group of tumors that include acute lymphoblastic leukemias/lymphomas and mature B cell leukemias/lymphomas. To varying degrees, these neoplasms recapitulate normal stages of B cell differentiation and typically have distinctive immunophenotypes that permit classification according to their postulated cell of origin. In addition, cytogenetic profiles, genotype, and immunophenotype of the malignant cell have had considerable impact on prognostic and therapeutic stratifications of patients with B cell neoplasms.

B LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

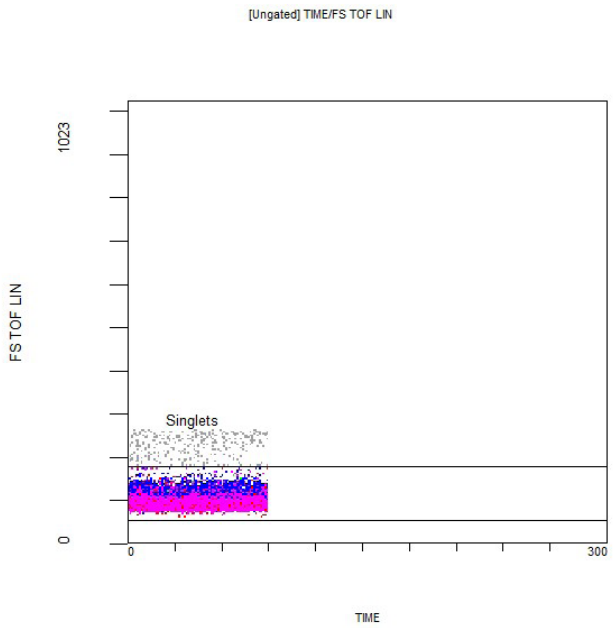
Case #4: B Acute Lymphoblastic Leukemia/Lymphoblastic Leukemia

Clinical Vignette

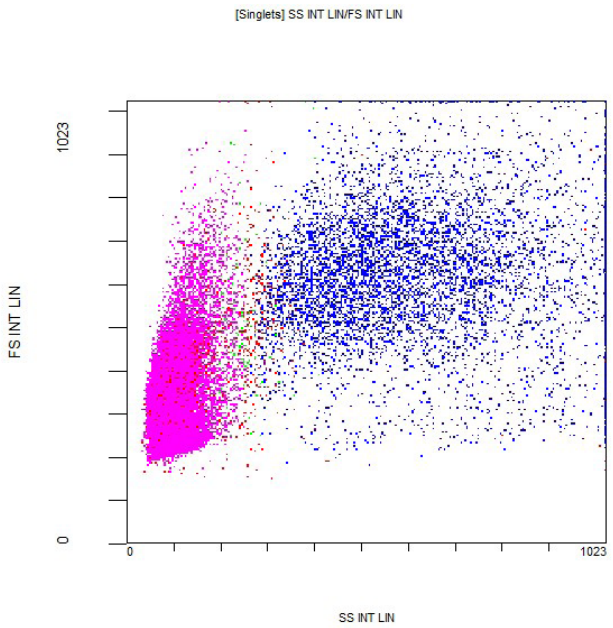
This 36-year-old female presents with anemia, thrombocytopenia, and neutropenia. Circulating atypical mononuclear cells are noted on microscopic examination. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

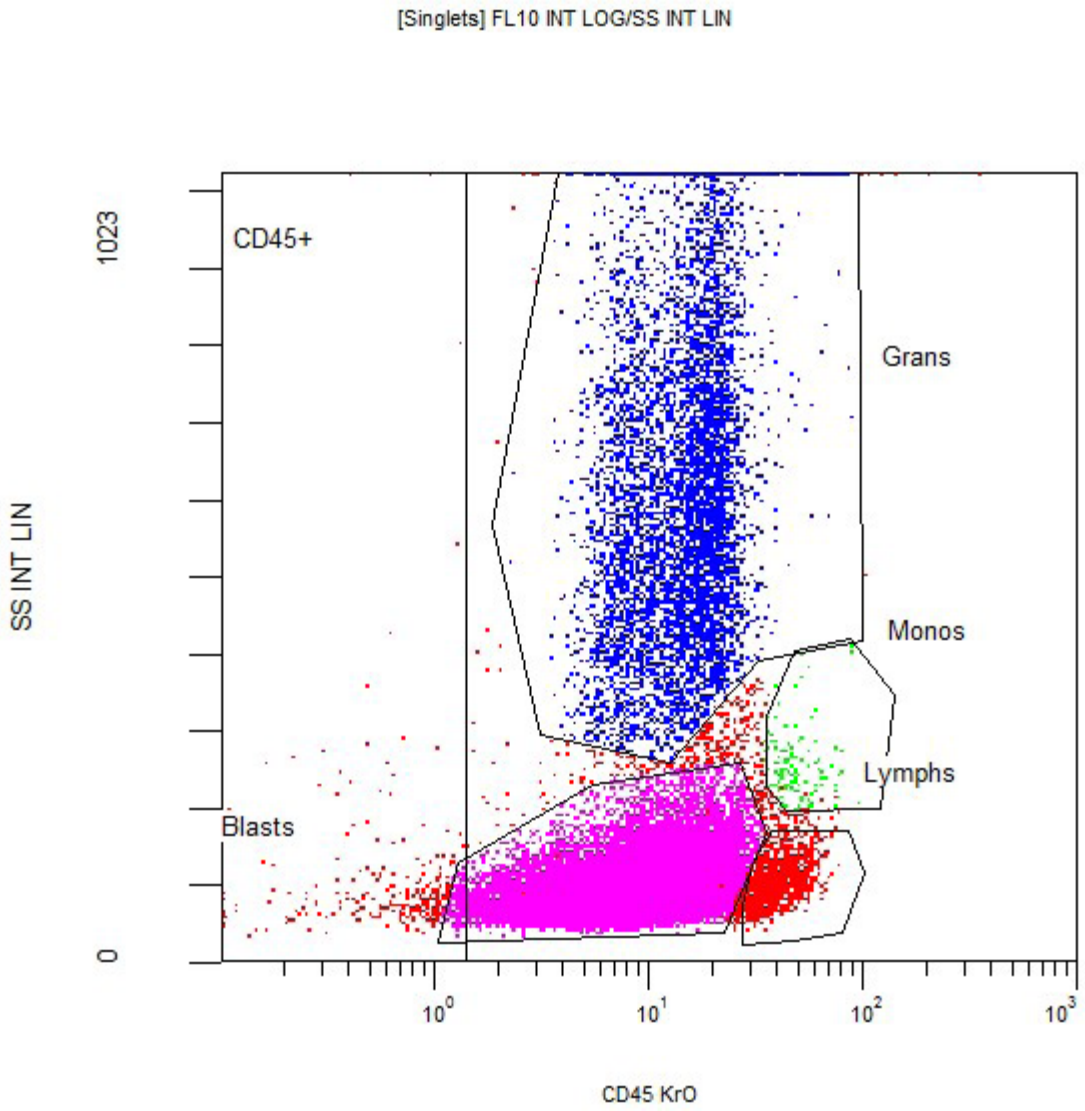
Access Case #4 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

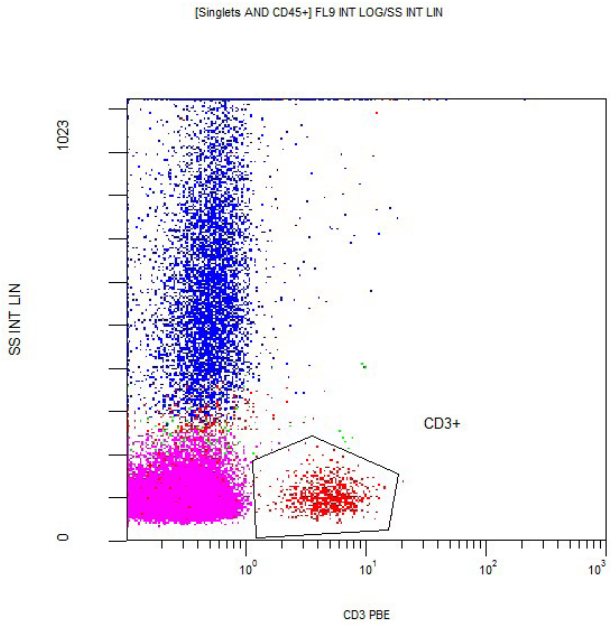


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of mononuclear cells and a relative paucity of apparent granulocytes.



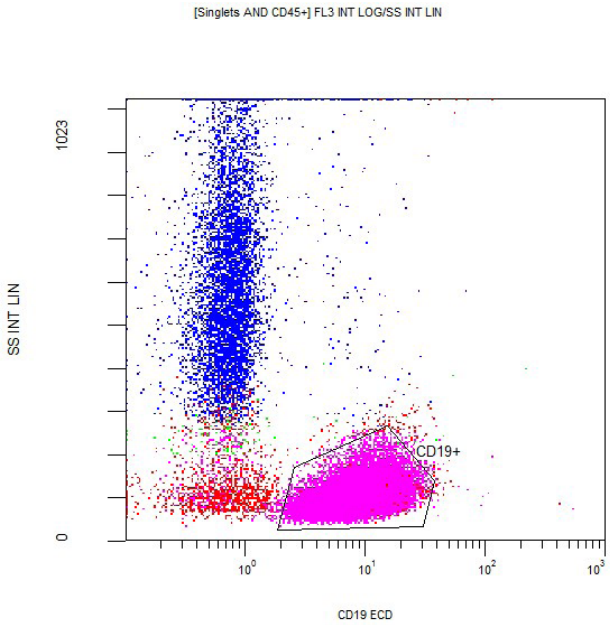
This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). Additionally, the "Blast" gate (pink) has been used to define a predominant population of mononuclear cells in this case. Note that the label for the Blast gate is not immediately adjacent to it---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.

Note that the overlap among the various populations in this particular case renders it somewhat difficult to distinguish among them. 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.

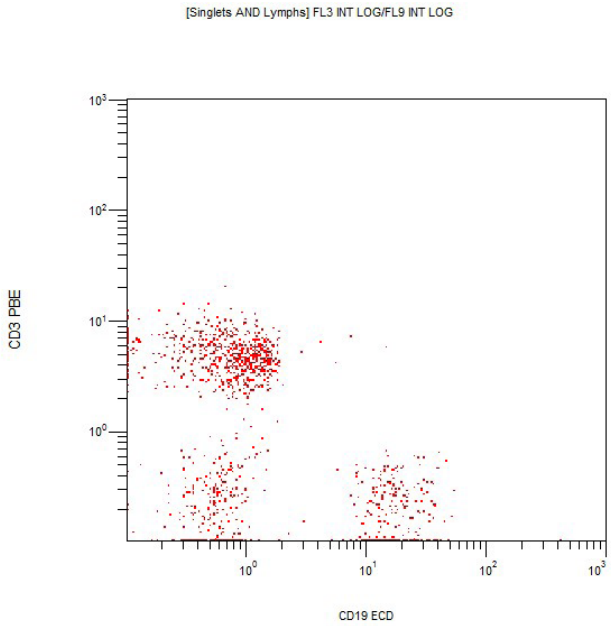


This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.

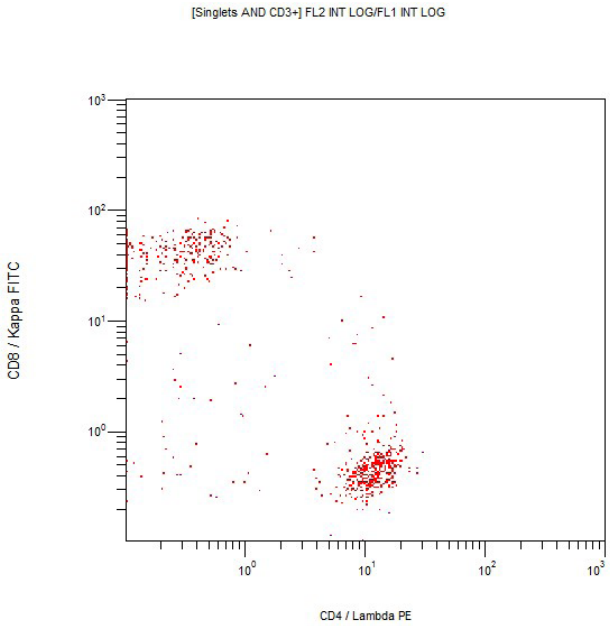
Note that the blasts (pink) are negative for CD3.



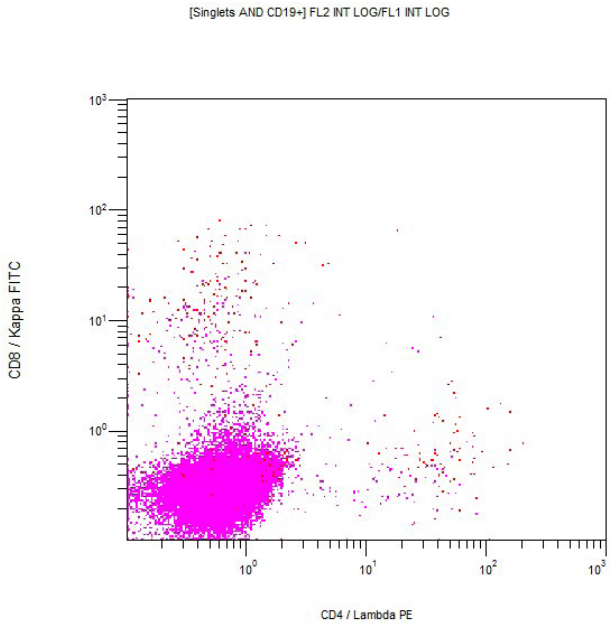
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that the blasts (pink) are CD19 positive.



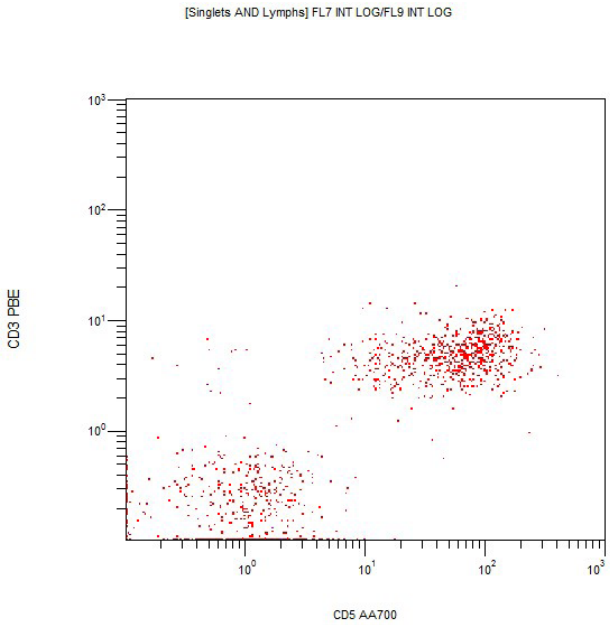
This CD19/CD3 dot plot gated on Lymphocytes demonstrates small numbers of T lymphocytes, B lymphocytes, and apparent NK cells (CD3 and CD19 dual negative).



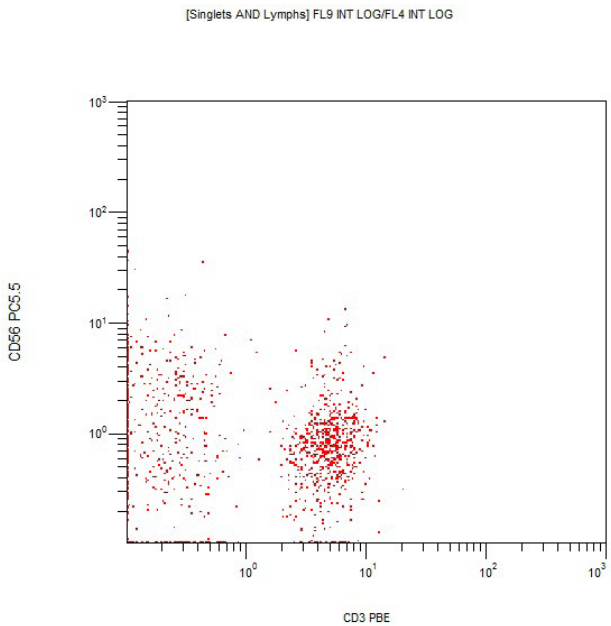
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



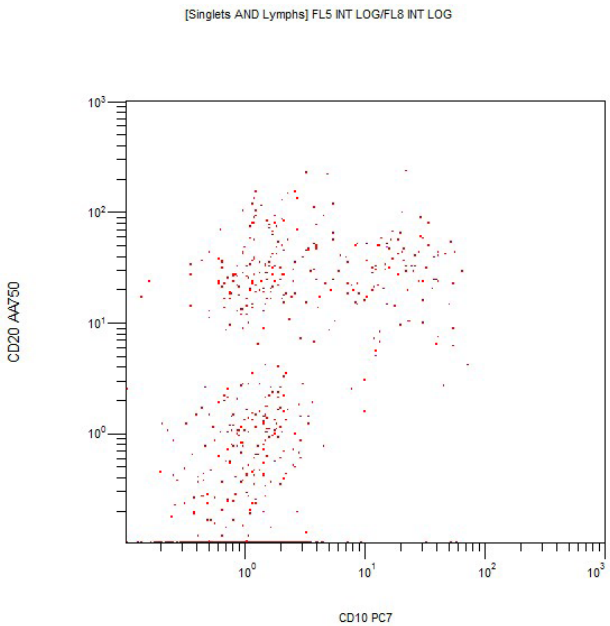
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. This sample contains a small number of normal polyclonal B lymphocytes as well as the predominant aberrant CD19 positive population which is negative for immunoglobulin light chains



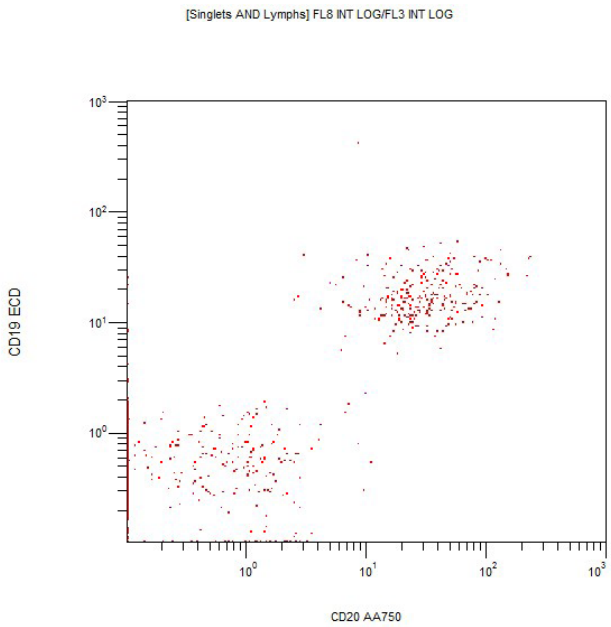
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable T lymphocytes cells and apparent NK cells (CD3 and CD5 dual negative) are also present.



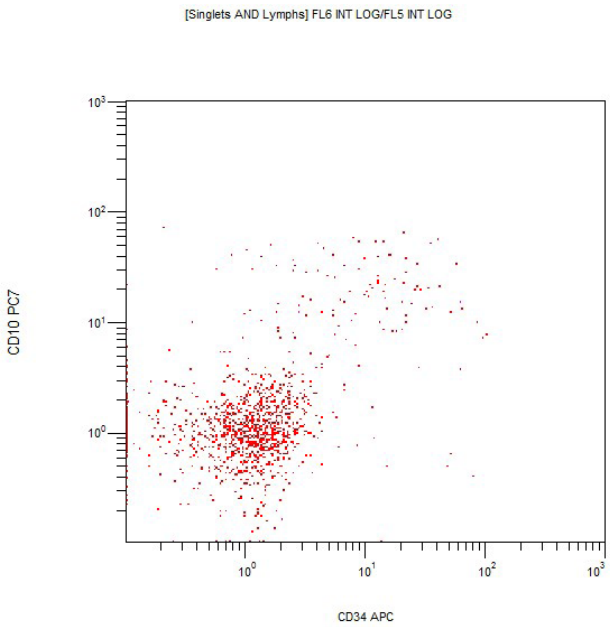
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present.



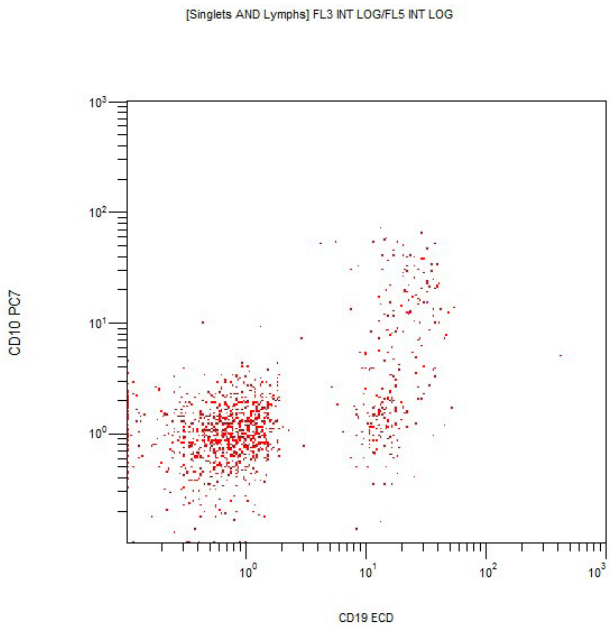
This CD10/CD20 dot plot is gated on Lymphocytes. Only a few mature B lymphocytes are present.



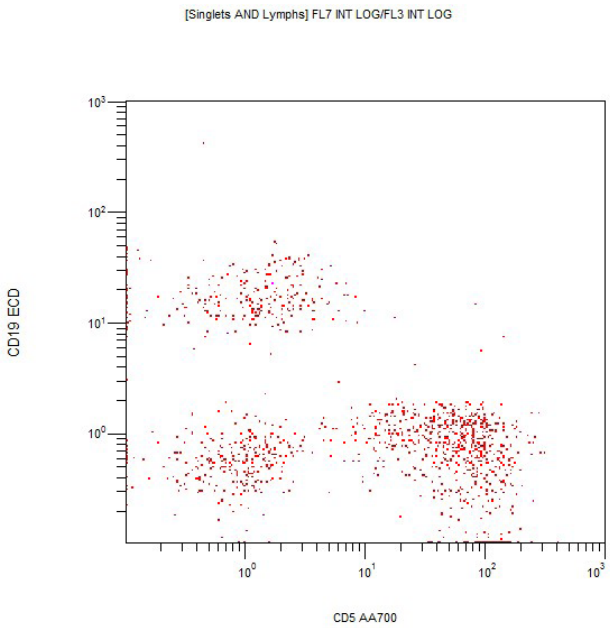
This CD20/CD19 dot plot is gated on Lymphocytes. The mature B lymphocytes display expected normal co-expression of CD19 and CD20.



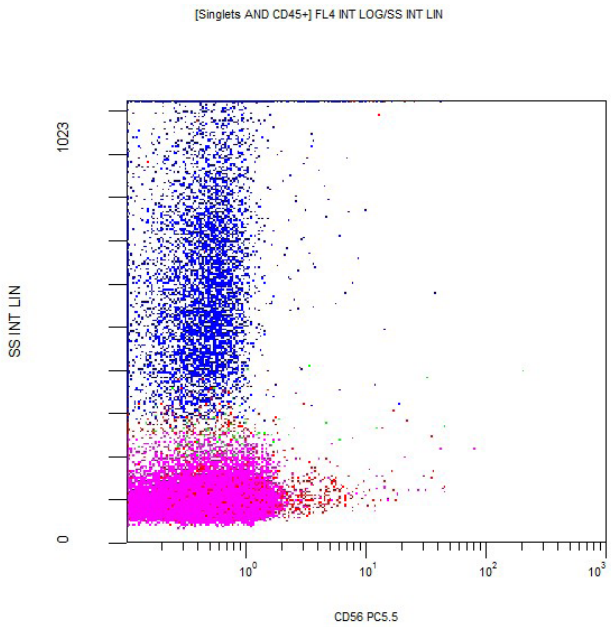
This CD34/CD10 dot plot is gated on Lymphocytes. The CD34 and CD10 dual positive events are blasts that have been included in the Lymphocyte gate.



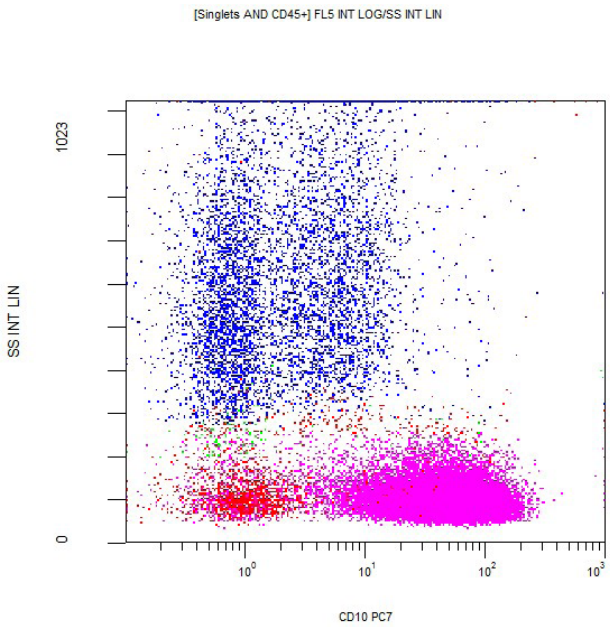
This CD19/CD10 dot plot is gated on Lymphocytes. The CD19 and CD10 dual positive events are blasts that have been included in the Lymphocyte gate.



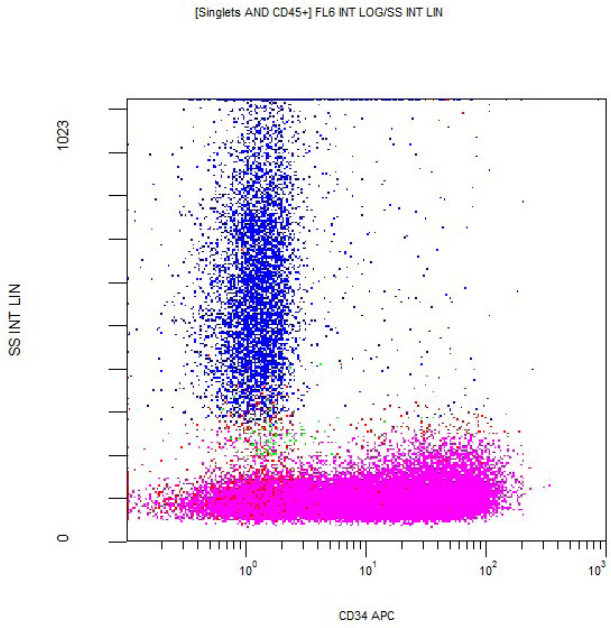
This CD5/CD19 dot plot is gated on Lymphocytes. No co-expression of CD5 and CD19 is identified.



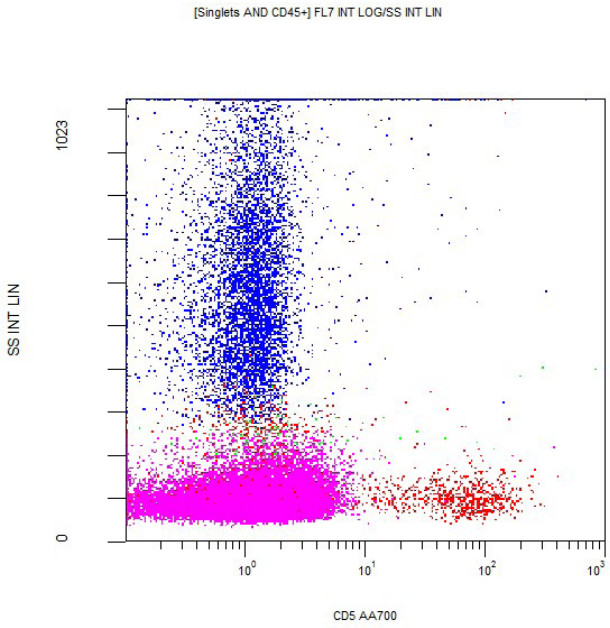
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells. The blasts (pink) do not express CD56.



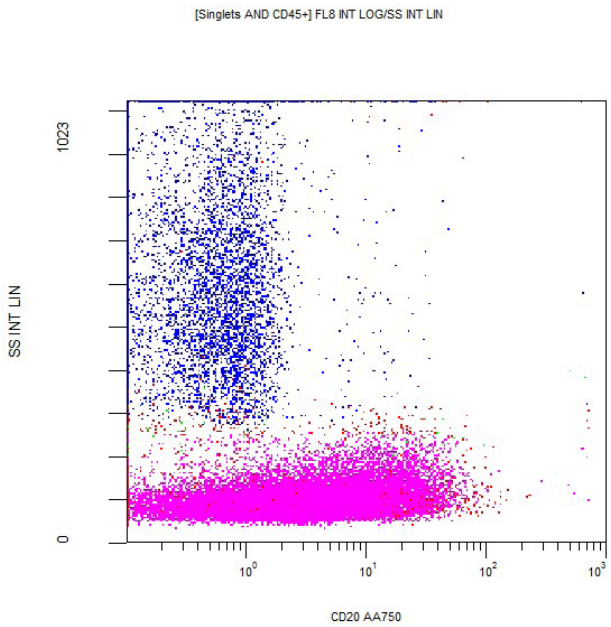
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) are positive for CD10. Some CD10 expression by granulocytes (blue) is noted.



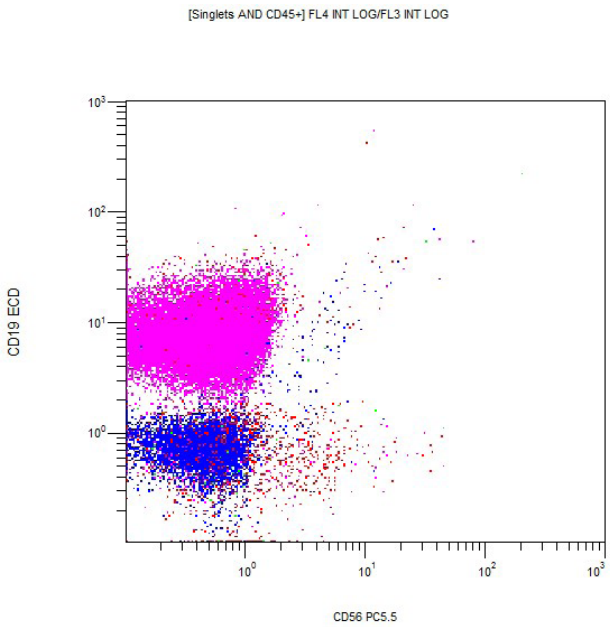
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Many of the blasts (pink) express CD34.



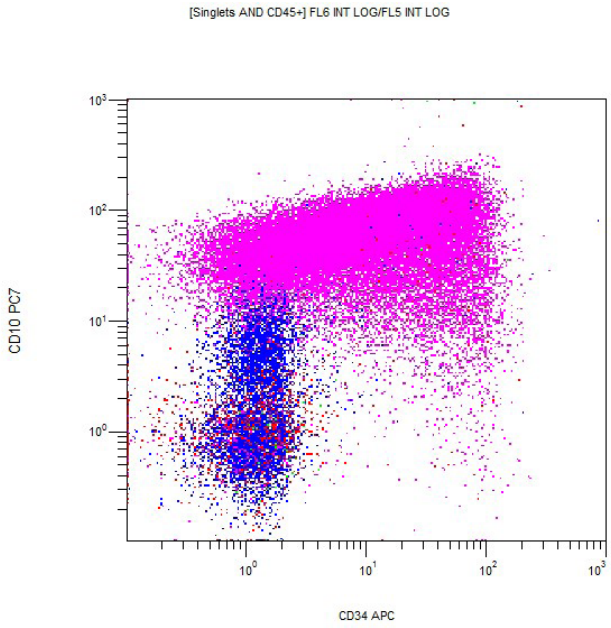
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) do not express CD5.



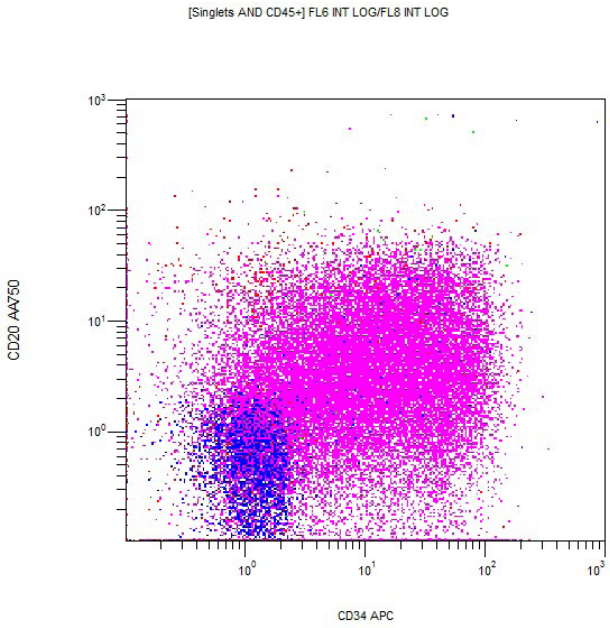
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A portion of the blasts (pink) express CD20.



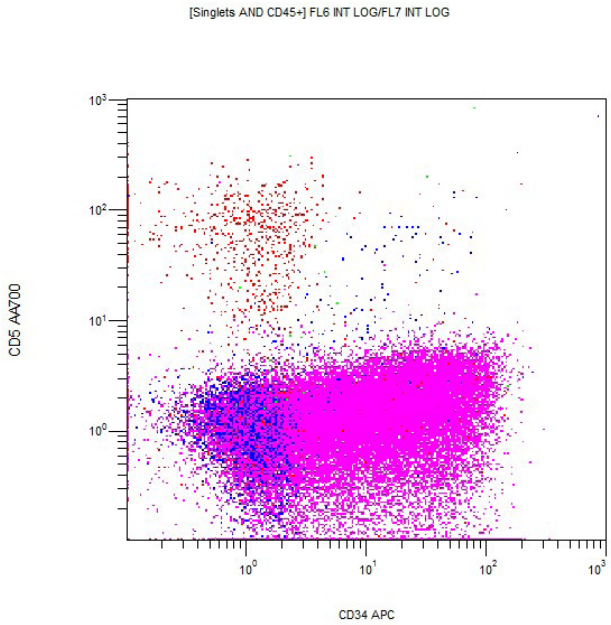
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) are negative for CD56 and positive for CD19.



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 the analysis, the blasts (pink) express CD10 and partial CD34.



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). As noted elsewhere the blasts (pink) express CD34 and partial CD20.



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). As noted elsewhere in the analysis, the blasts (pink) express CD34 but not CD5. CD5 positive T lymphocytes (red) are also present.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with increased light scatter properties that express CD10, CD19, partial CD20, partial CD34 and low density CD45. Surface immunoglobulin light chain expression is absent.

Taken together, the results are consistent with B acute lymphoblastic leukemia/lymphoblastic leukemia. Correlation with clinical and laboratory data is also recommended.

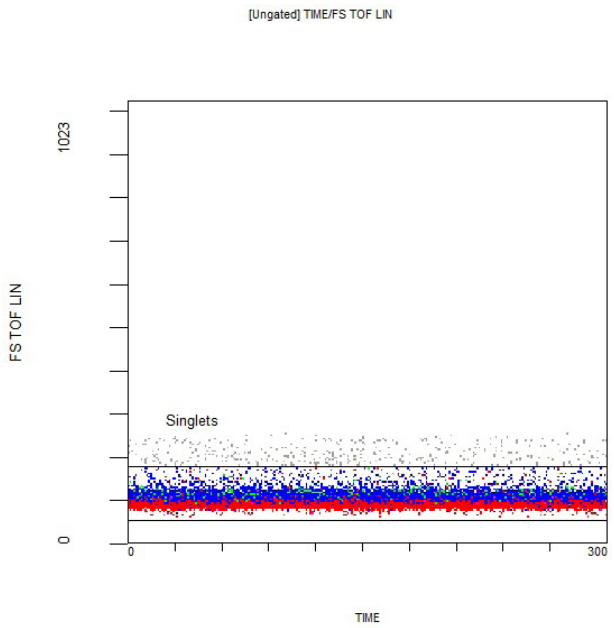
Case #5: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

Clinical Vignette

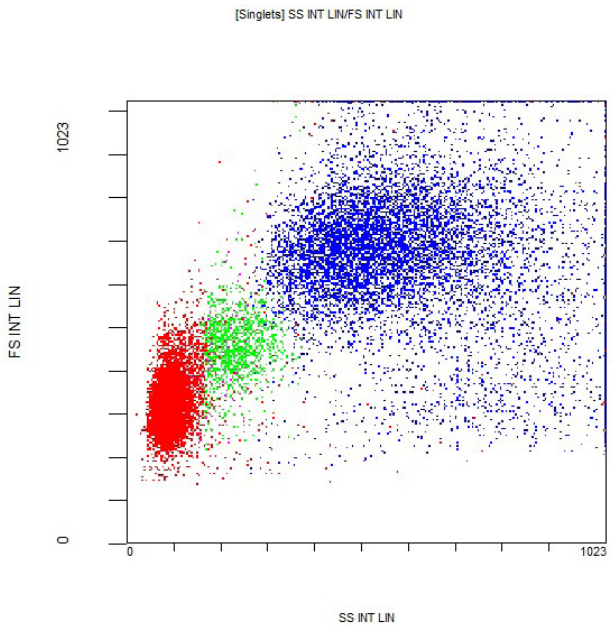
This 35-year-old male presents with lymphocytosis. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

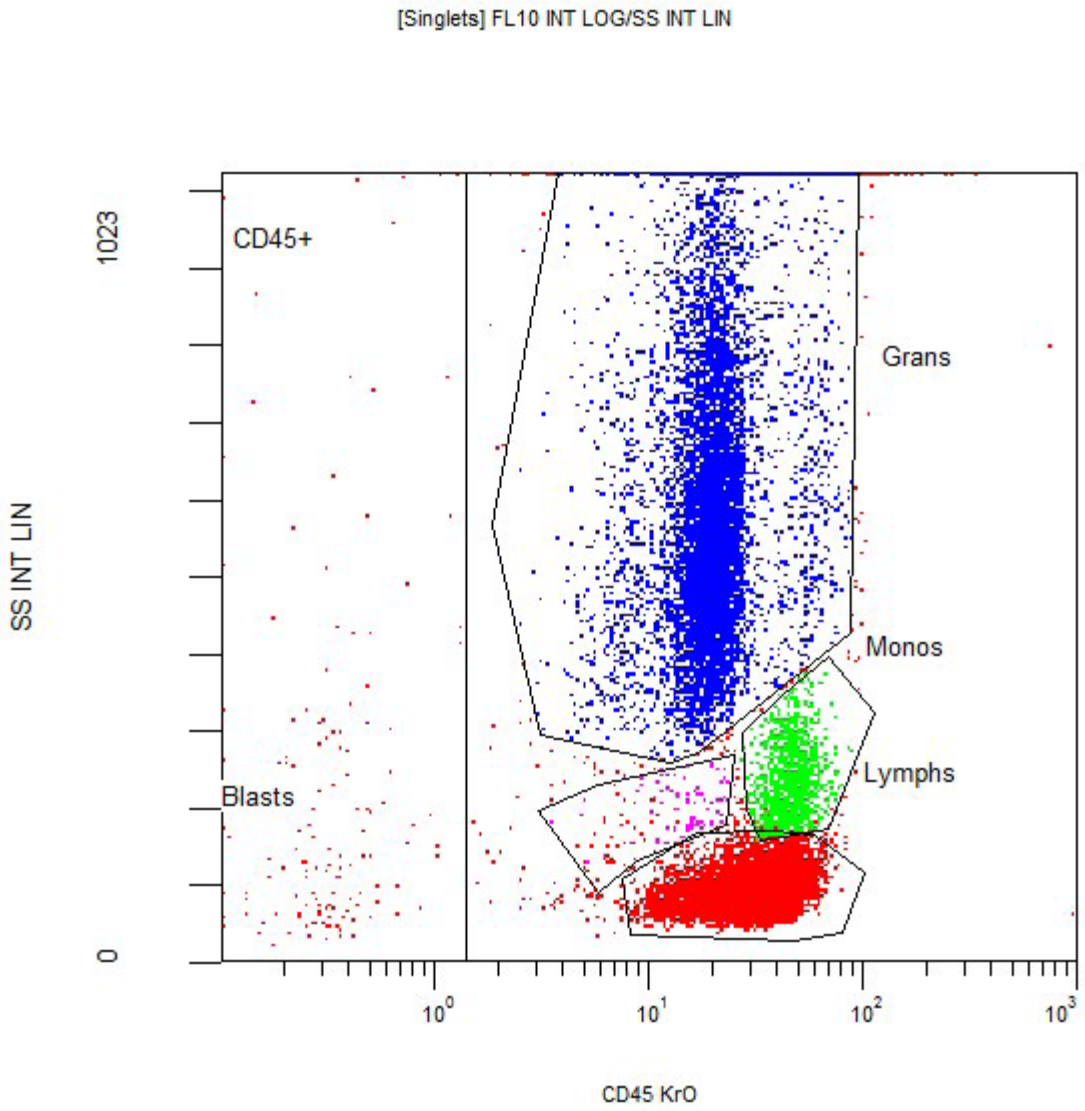
Access Case #5 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

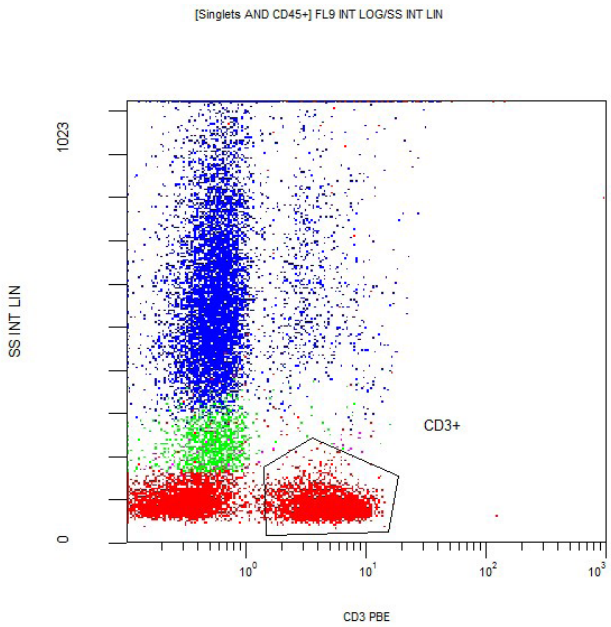


This Side Scatter/Forward Scatter dot plot demonstrates lymphocytes (red), monocytes (green), and granulocytes (blue).

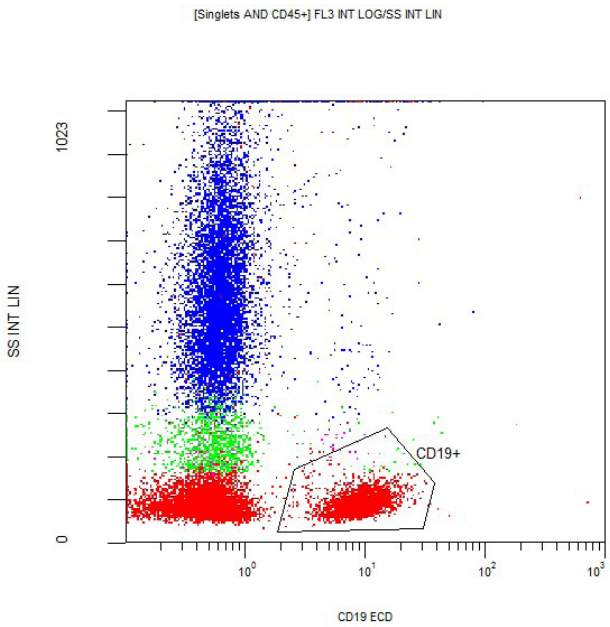


This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). The "Blast" gate contains only a few events. Note that the 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.

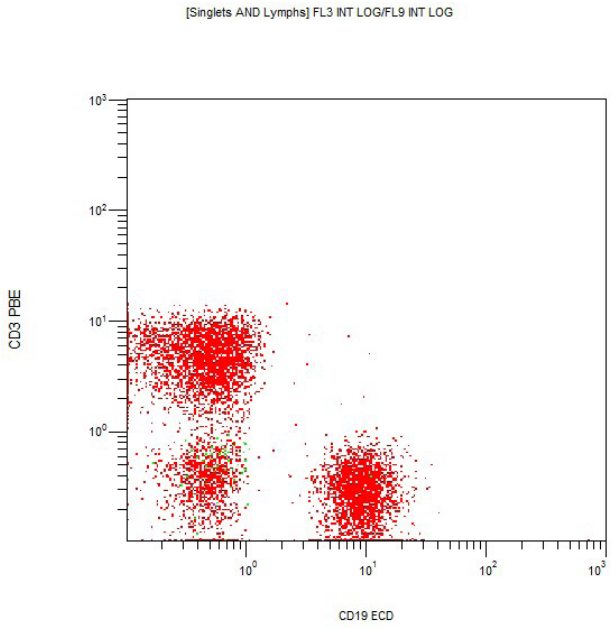
Note the mild degree of overlap in this case between apparent monocytes and lymphocytes. The gates should be adjusted such that they conform to the naturally occurring separations among the populations, but where no separation is observed an estimate based on experience may be used.



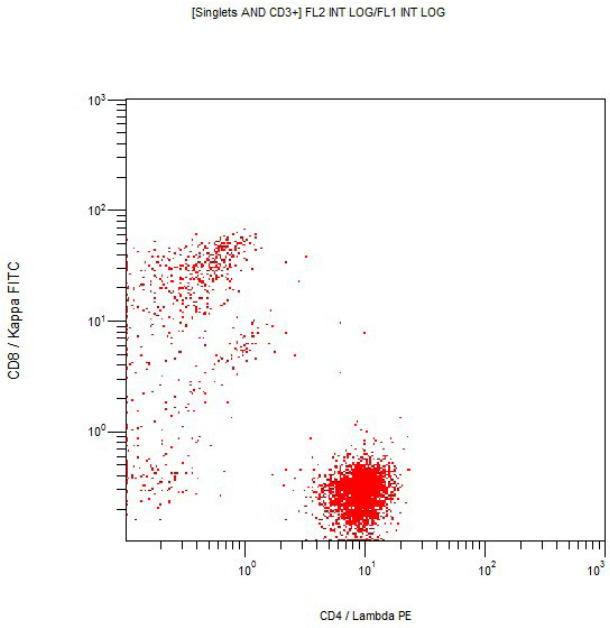
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.



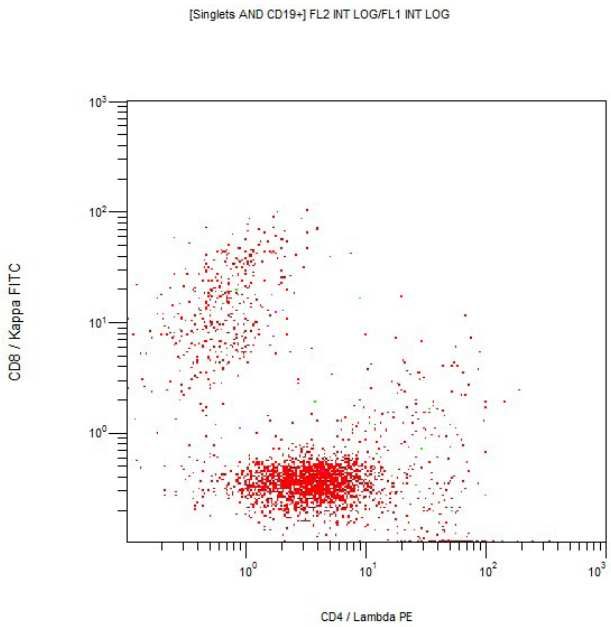
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only.



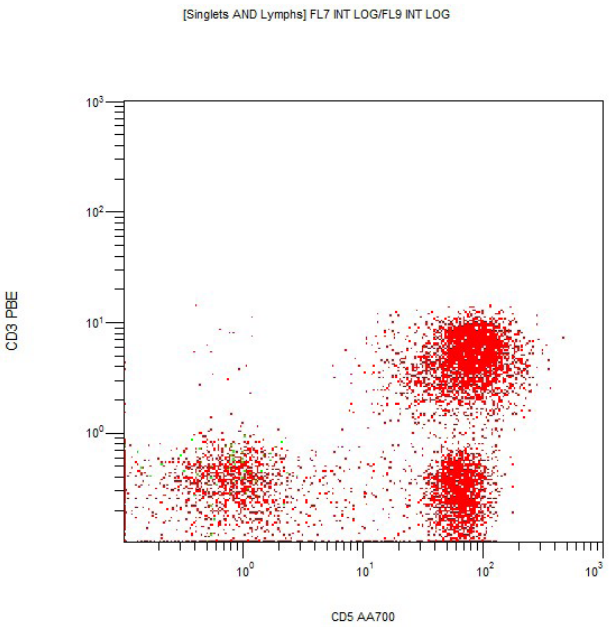
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present.



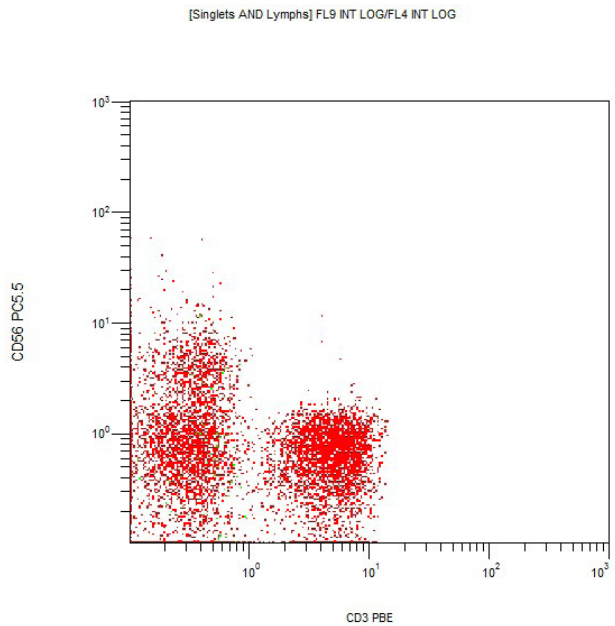
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes. A small population of CD4 and CD8 dual negative T lymphocytes is noted.



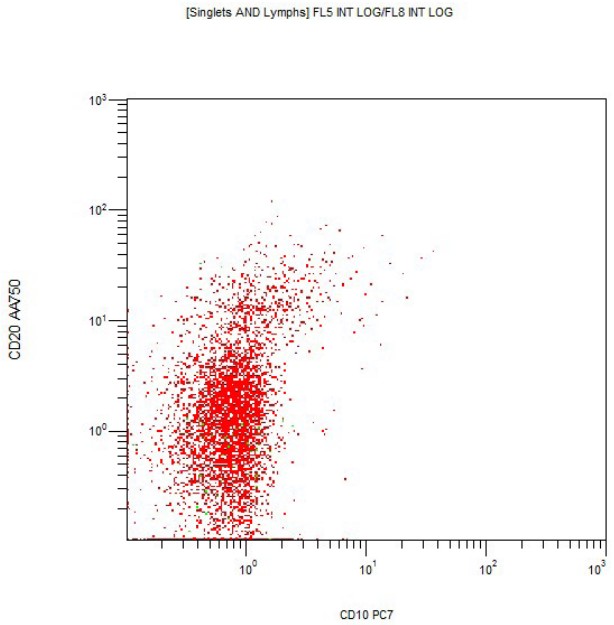
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. Note that the majority of the B lymphocytes in this case display Lambda immunoglobulin light chain restriction, an aberrant finding consistent with a B-cell lymphoproliferative disorder. A polyclonal population is also present, with approximately equal numbers of Kappa and Lambda-expressing cells. Note that the Lambda expression density for the aberrant population is lower density than that of the polyclonal cells. This finding is typical of chronic lymphocytic leukemia/small lymphocytic lymphoma.



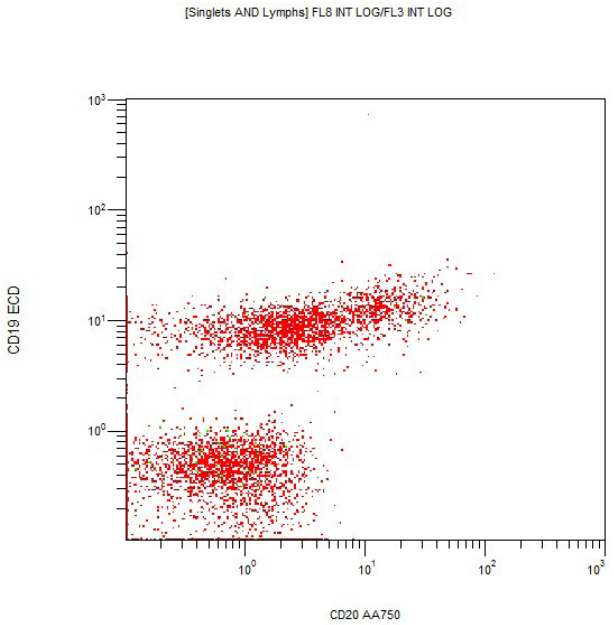
This CD5/CD3 dot plot is gated on Lymphocytes. In addition to unremarkable CD3 positive, CD5 positive T lymphocytes, a population of CD5 positive, CD3 negative cells is noted.



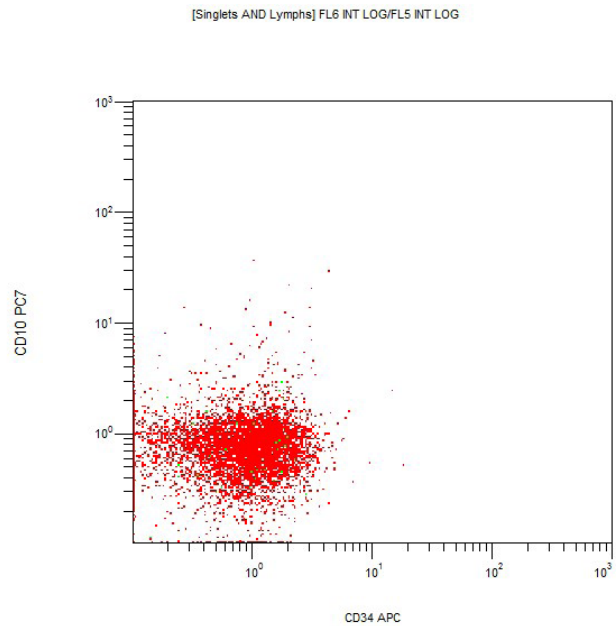
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. A small population of CD56 positive T lymphocytes is noted. The CD3 and CD56 dual negative cells are B lymphocytes (including the aberrant population).



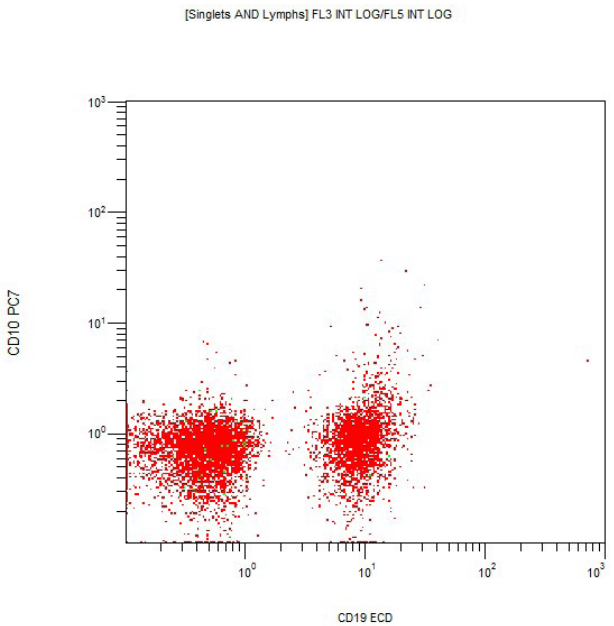
This CD10/CD20 dot plot is gated on Lymphocytes. Note that no distinct CD20 positive population is discernible in this display.



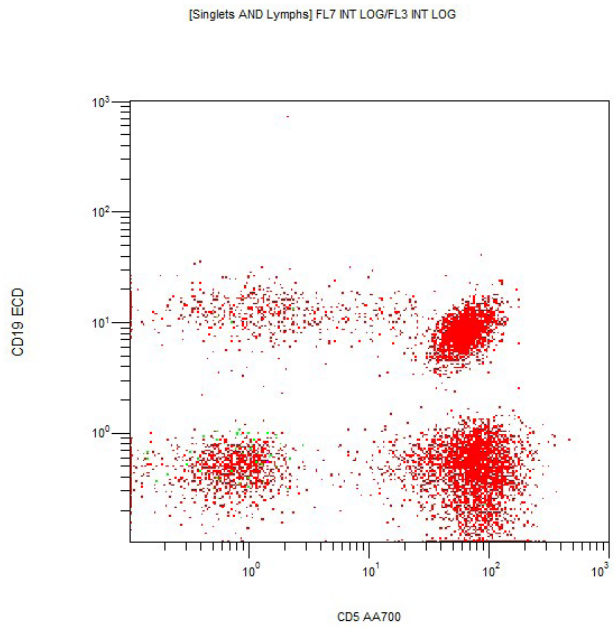
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes do not display the expected normal co-expression of CD19 and CD20. Instead, there are two populations, with one displaying higher density CD20 expression (as well as slightly higher density CD19 expression) and the other displaying lower CD20 expression. This finding is typical of chronic lymphocytic leukemia/small lymphocytic lymphoma.



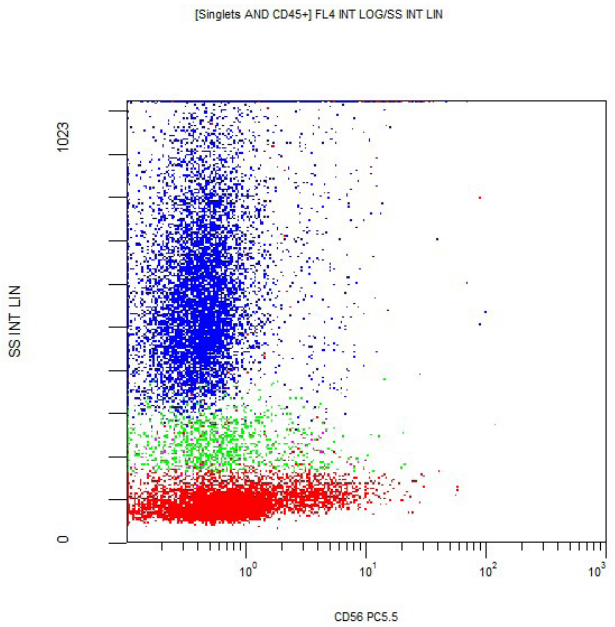
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, as expected for both normal mature peripheral blood lymphocytes as well as mature lymphoproliferative disorders such as chronic lymphocytic leukemia/small lymphocytic lymphoma.



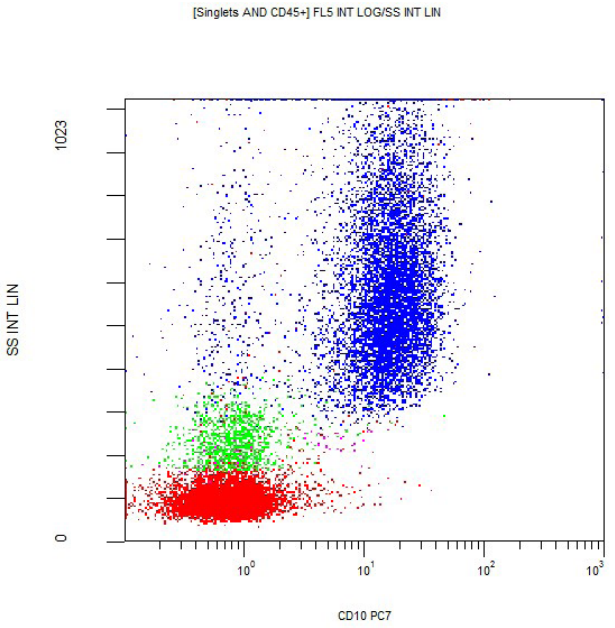
This CD19/CD10 dot plot is gated on Lymphocytes. No aberrant population is apparent in this display, although it is present and comprises the majority of the CD19 positive cells in this case.



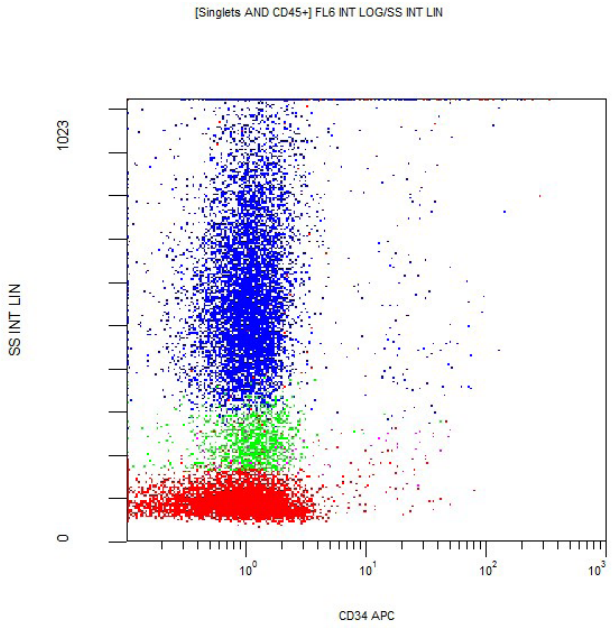
This CD5/CD19 dot plot is gated on Lymphocytes. In addition to CD5 positive, CD19 negative T lymphocytes and CD5 negative, CD19 positive normal B lymphocytes, an aberrant CD5 and CD19 dual positive population is present. This aberrant population corresponds to the Lambda immunoglobulin-restricted population noted earlier in this analysis, whereas the other B lymphocytes correspond to the polyclonal population.



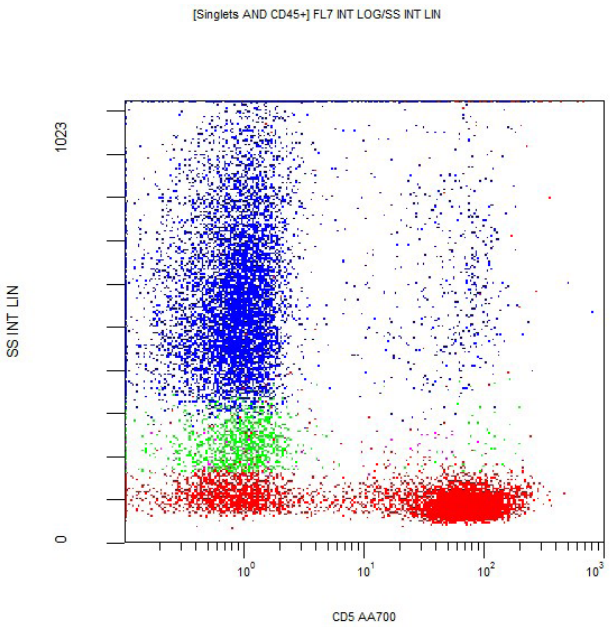
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells.



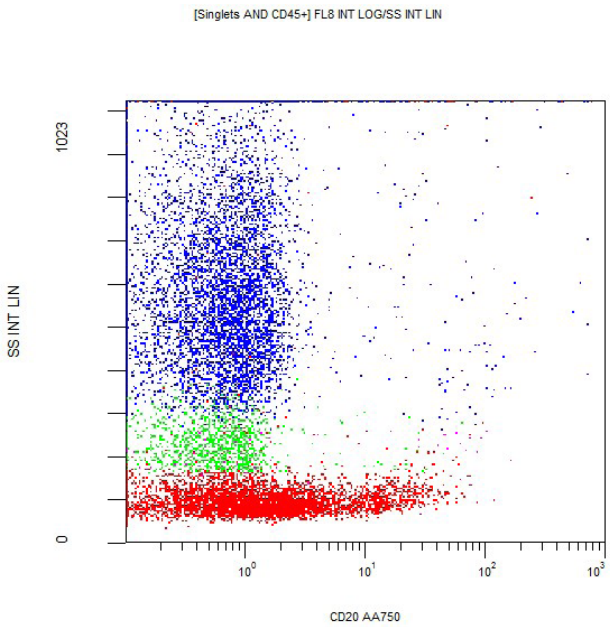
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are positive for CD10.



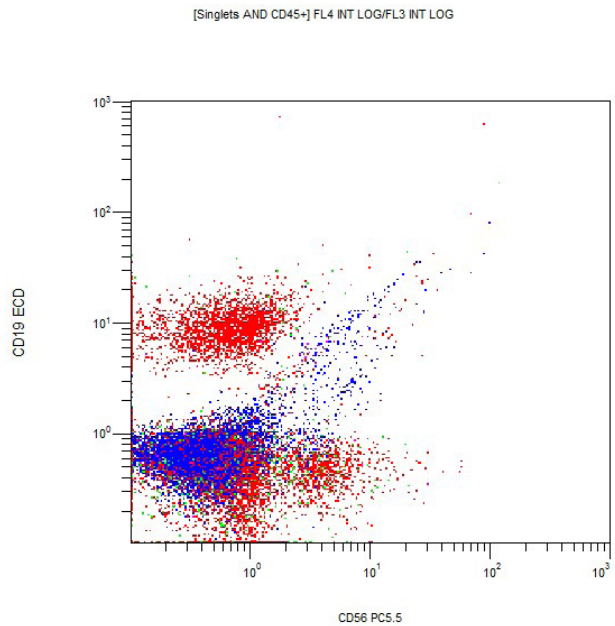
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No significant CD34 positive population is present.



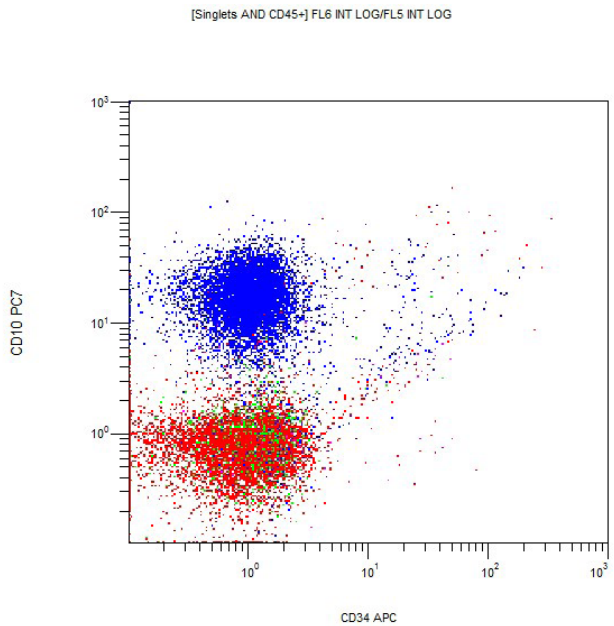
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The CD5 positive events depicted here include both normal T lymphocytes and the aberrant B lymphocyte population.



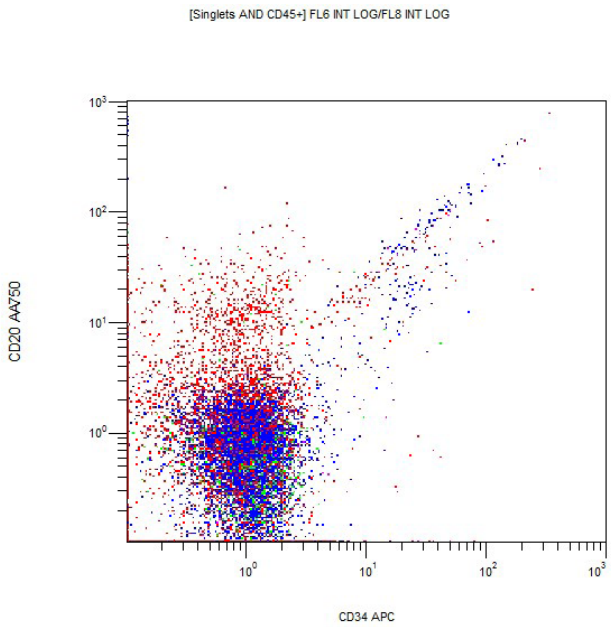
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). As noted elsewhere in this case, the normal B lymphocytes in this case express higher density CD20 than the aberrant population. In this case the aberrant B lymphocyte population cannot be easily distinguished from the T lymphocytes due to their low density CD20 expression.



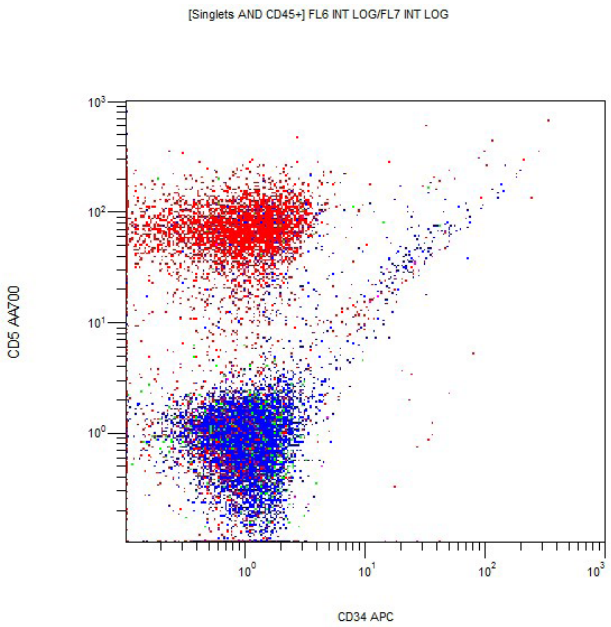
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes and NK cells are present. The blue 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. The blue events noted on the diagonal are consistent with high background fluorescence.



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. The blue events noted on the diagonal are consistent with high background fluorescence.



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. The blue events noted on the diagonal are consistent with high background fluorescence.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with low light scatter properties that express CD19, low density CD20, CD5, and CD45 and display Lambda immunoglobulin light chain restriction.

Taken together, the findings in this case are most consistent with chronic lymphocytic leukemia/small lymphocytic lymphoma. Note that correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

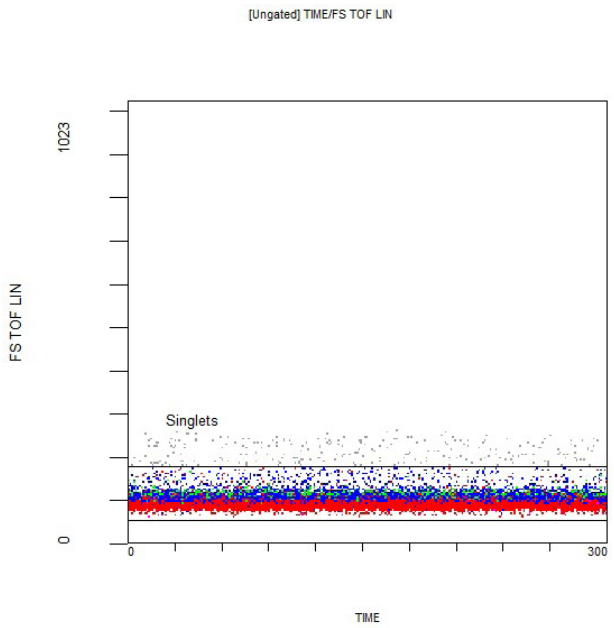
Case #6: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

Clinical Vignette

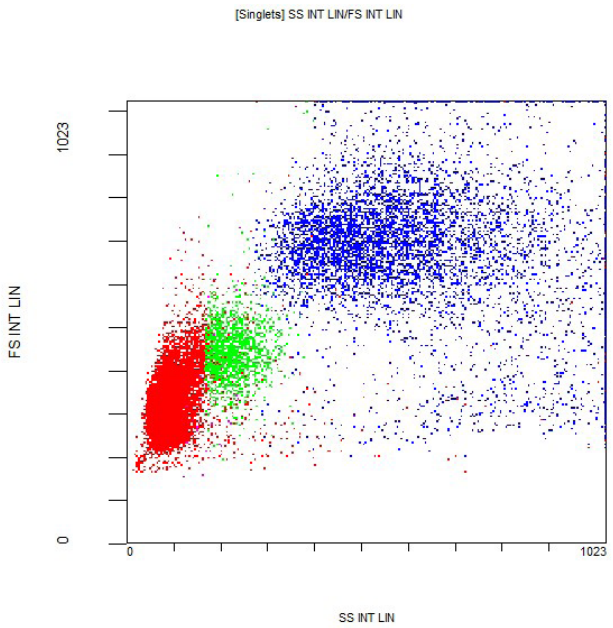
This 89-year-old female presents with lymphocytosis. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

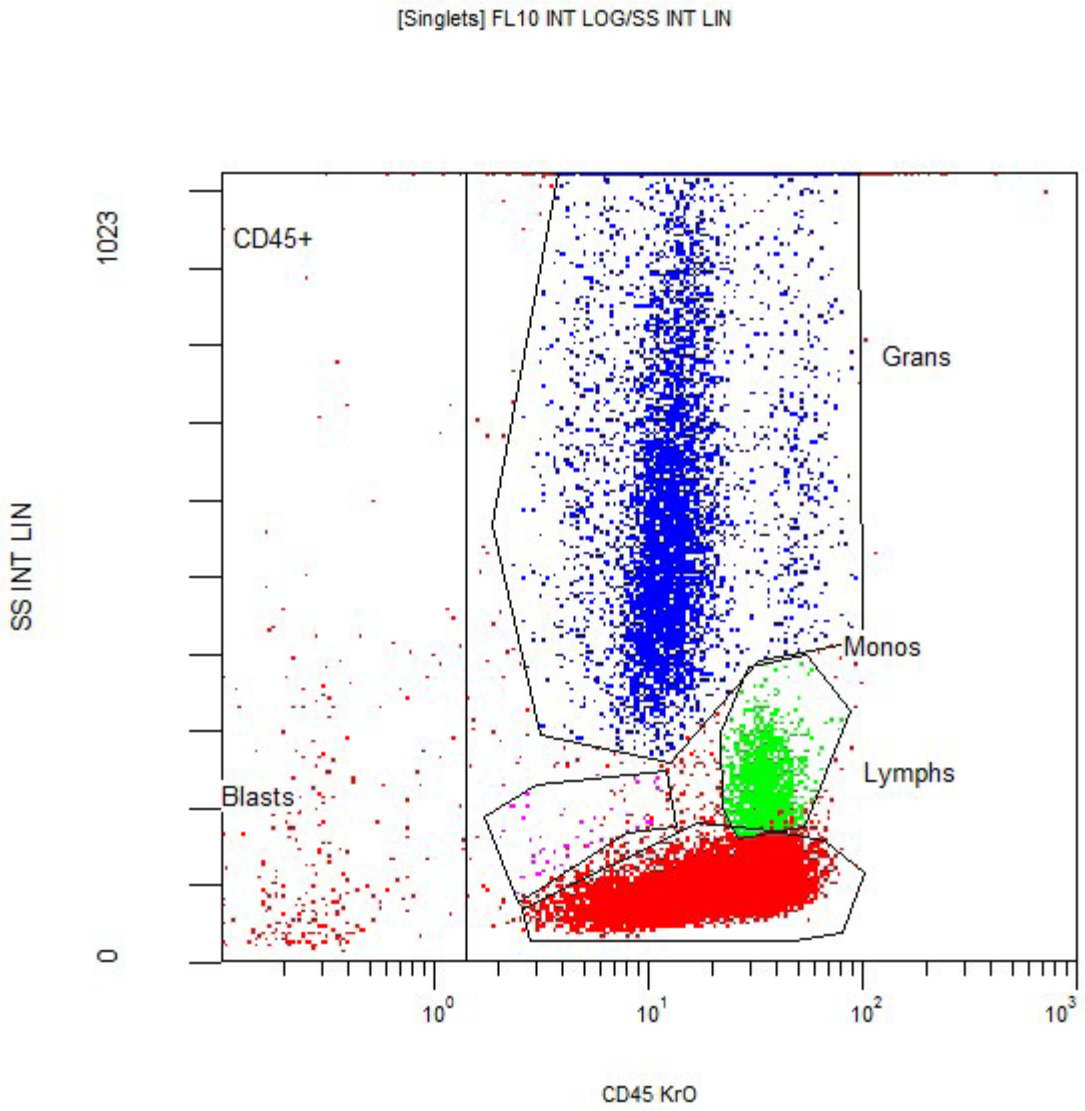
Access Case #6 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

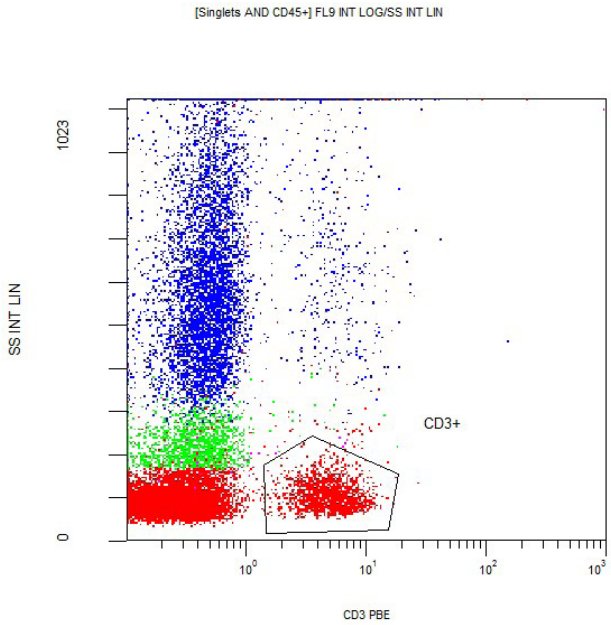


This Side Scatter/Forward Scatter dot plot demonstrates lymphocytes (red), monocytes (green), and granulocytes (blue).

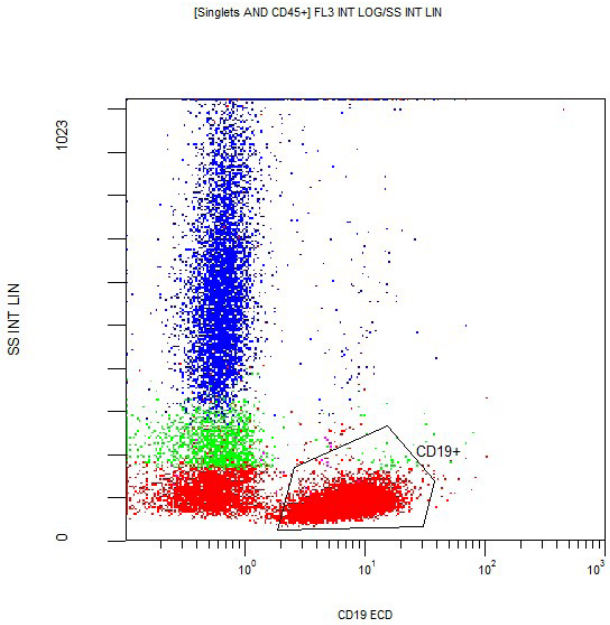


This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). The "Blast" gate contains only a few events. Note that the 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.

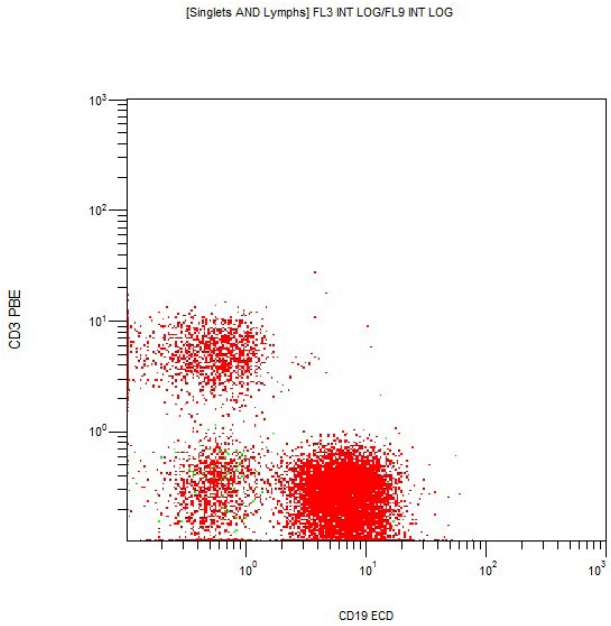
Note the mild degree of overlap in this case between apparent monocytes and lymphocytes. The gates should be adjusted such that they conform to the naturally occurring separations among the populations, but where no separation is observed an estimate based on experience may be used.



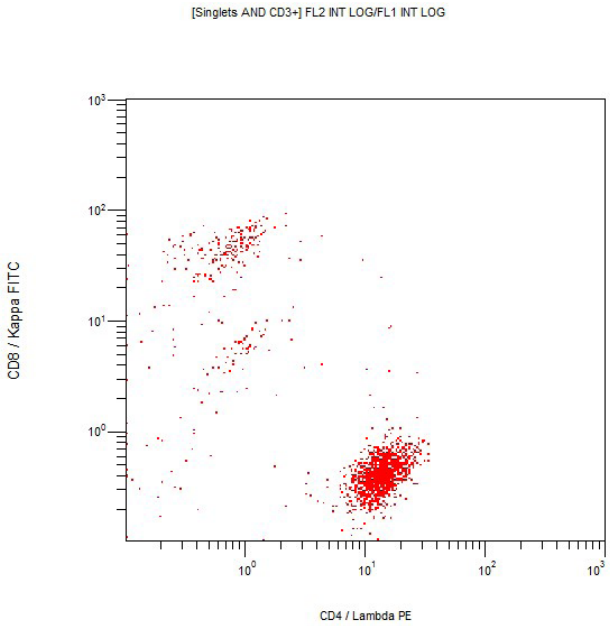
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.



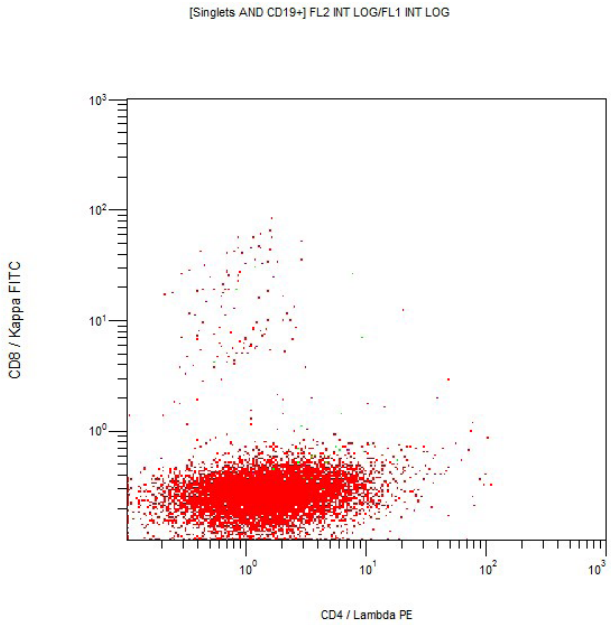
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only.



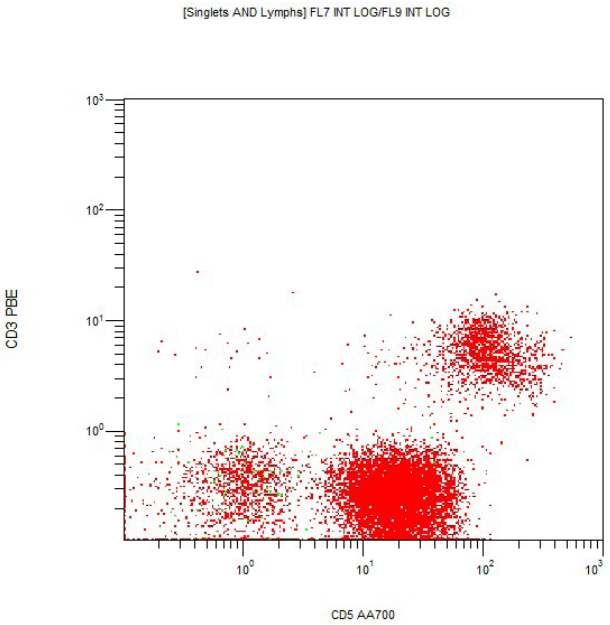
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present. Note that there are clearly more B lymphocytes than T lymphocytes in this sample, an unusual finding in peripheral blood.



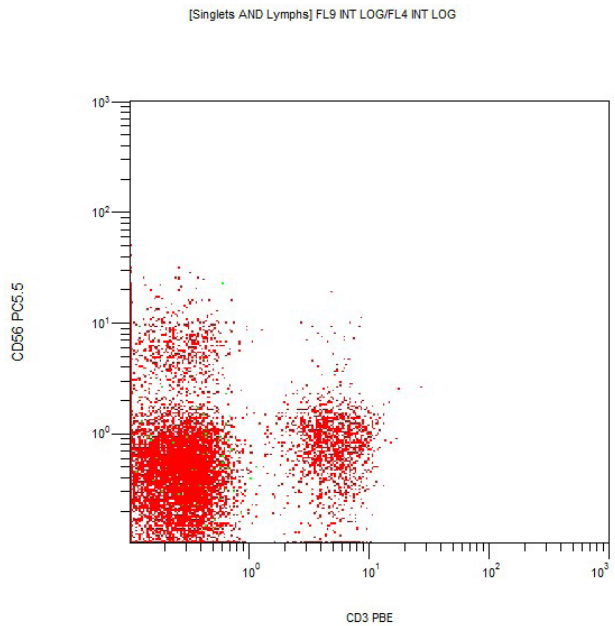
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes. A small population of CD4 and CD8 dual negative T lymphocytes is noted.



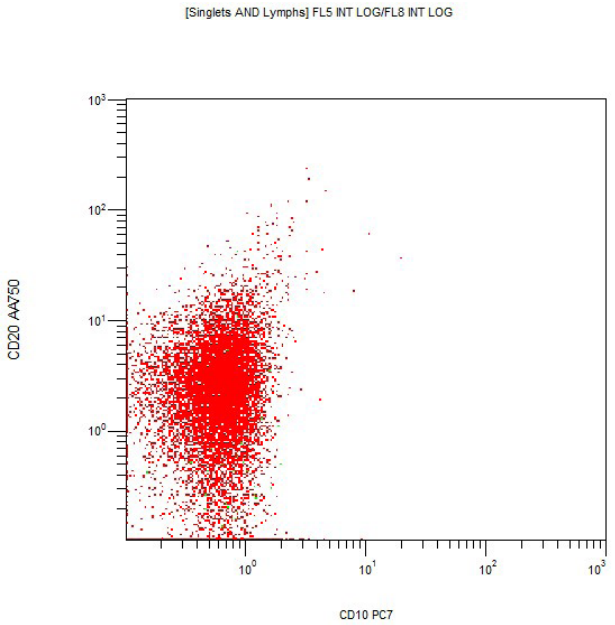
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. Note that the majority of the B lymphocytes in this case display Lambda immunoglobulin light chain restriction, an aberrant finding consistent with a B-cell lymphoproliferative disorder. A polyclonal population is also present, with approximately equal numbers of Kappa and Lambda-expressing cells. Note that the Lambda expression density for the aberrant population is lower density than that of the polyclonal cells. This finding is typical of chronic lymphocytic leukemia/small lymphocytic lymphoma.



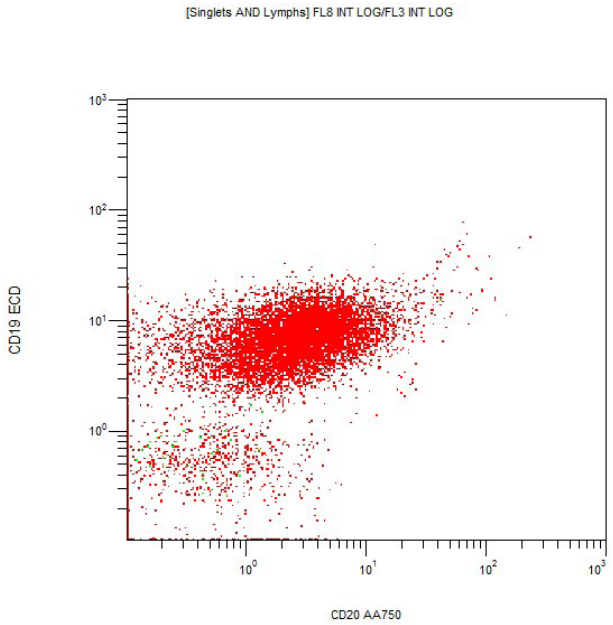
This CD5/CD3 dot plot is gated on Lymphocytes. In addition to unremarkable CD3 positive, CD5 positive T lymphocytes, a population of CD5 positive, CD3 negative cells is noted.



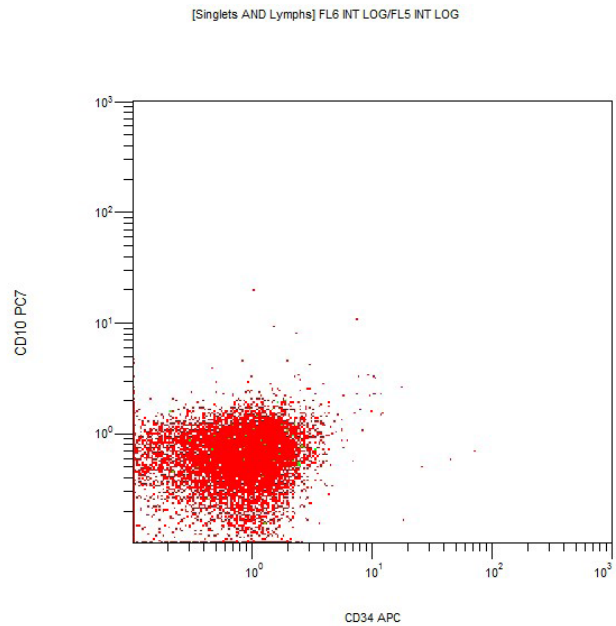
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. A small population of CD56 positive T lymphocytes is noted. The CD3 and CD56 dual negative cells are B lymphocytes (including the aberrant population).



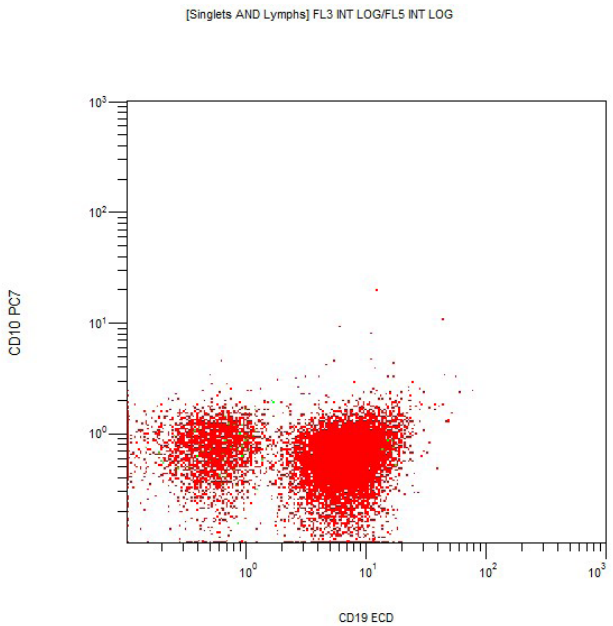
This CD10/CD20 dot plot is gated on Lymphocytes. Note that no distinct CD20 positive population is discernible in this display, consistent with the low density CD20 expression that is typical of chronic lymphocytic leukemia.



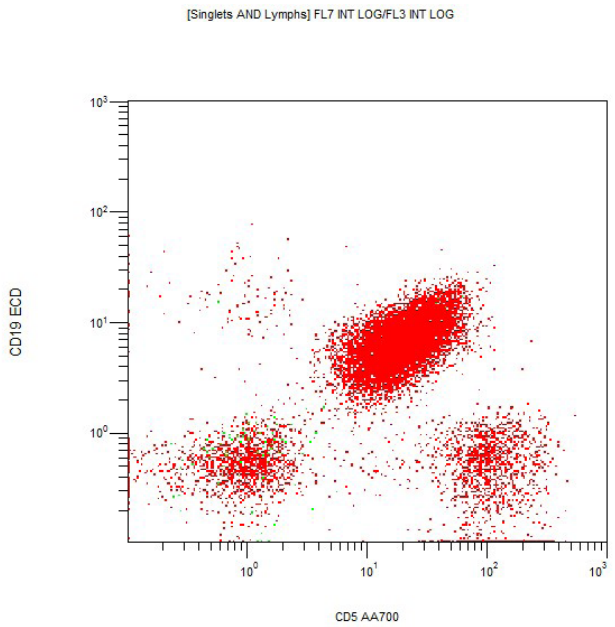
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes do not display the expected normal co-expression of CD19 and CD20. Instead, virtually all of the B lymphocytes display lower than usual CD20 expression. This finding is typical of chronic lymphocytic leukemia/small lymphocytic lymphoma.



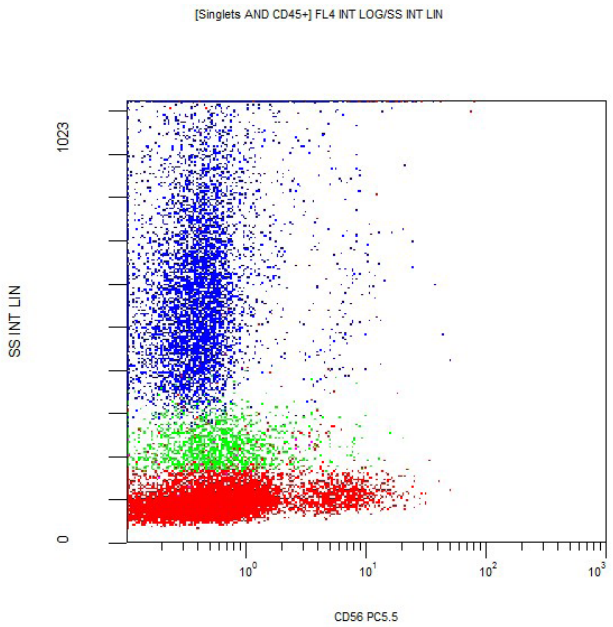
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, as expected for both normal mature peripheral blood lymphocytes as well as mature lymphoproliferative disorders such as chronic lymphocytic leukemia/small lymphocytic lymphoma.



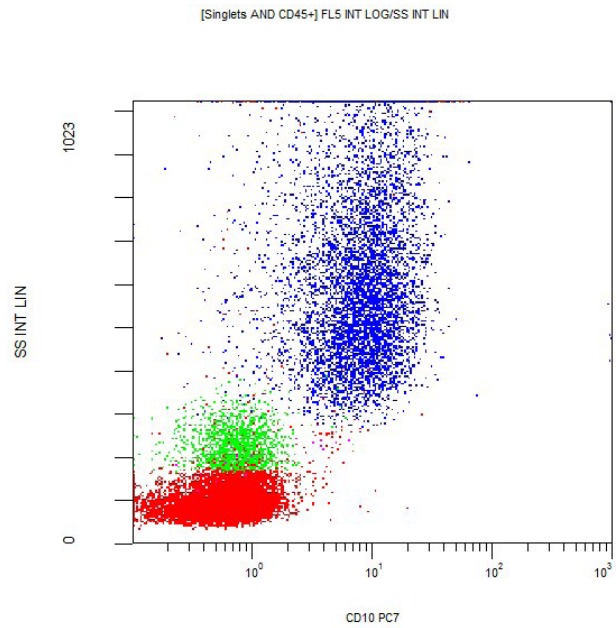
This CD19/CD10 dot plot is gated on Lymphocytes. No aberrant population is apparent in this display, although it is present and comprises the majority of the CD19 positive cells in this case.



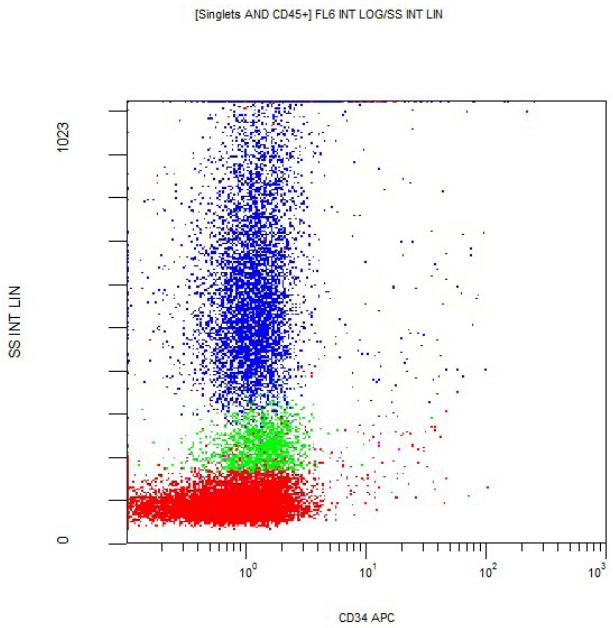
This CD5/CD19 dot plot is gated on Lymphocytes. In addition to CD5 positive, CD19 negative T lymphocytes and a very small population of CD5 negative, CD19 positive normal B lymphocytes, a prominent aberrant CD5 and CD19 dual positive population is present. This aberrant population corresponds to the Lambda immunoglobulin-restricted population noted earlier in this analysis, whereas the other B lymphocytes correspond to the polyclonal population.



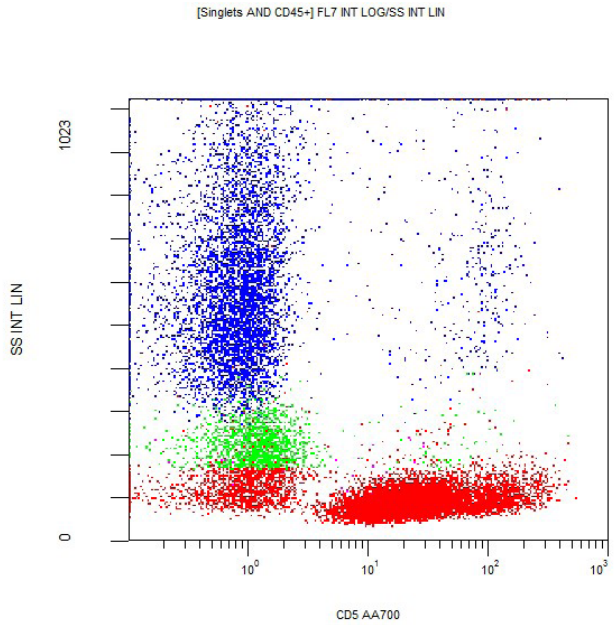
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells.



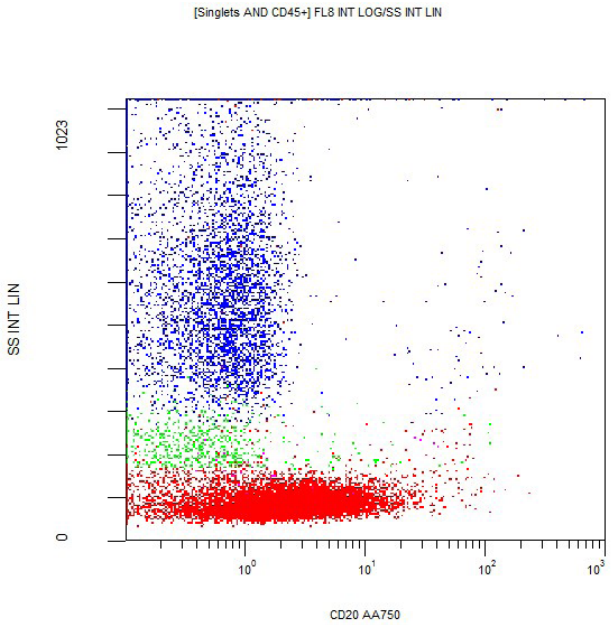
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are positive for CD10.



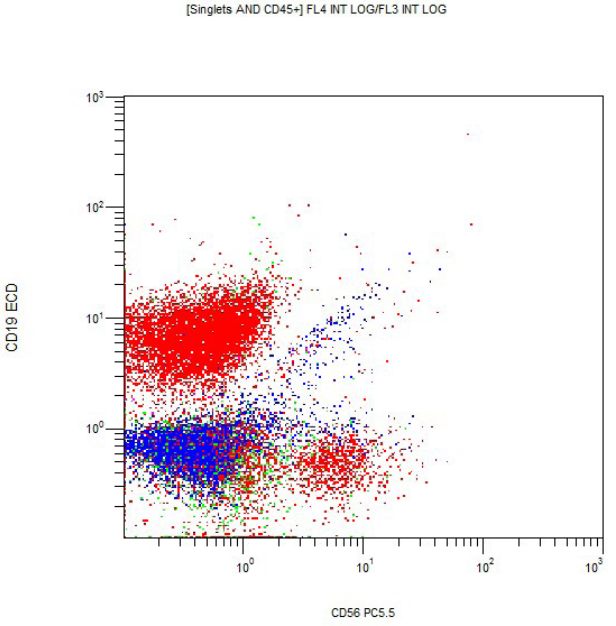
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No significant CD34 positive population is present.



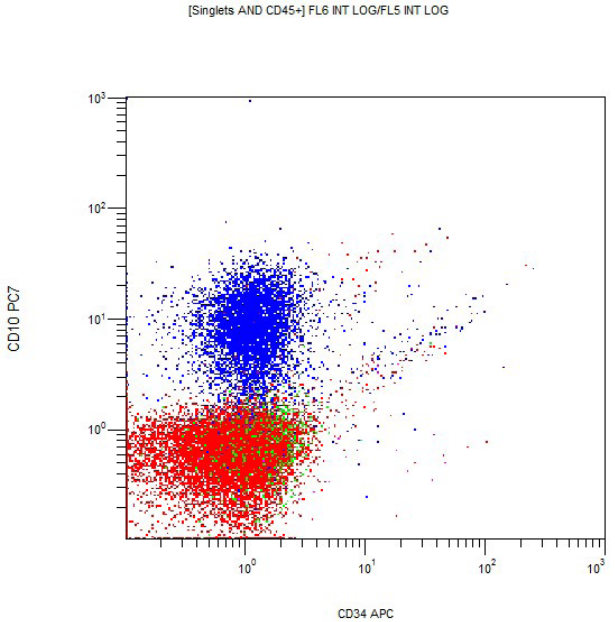
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The CD5 positive events depicted here include both normal T lymphocytes and the aberrant B lymphocyte population. Two populations can be discerned, with the higher density CD5 population being T lymphocytes and the lower density CD5 population being the aberrant B lymphocyte population.



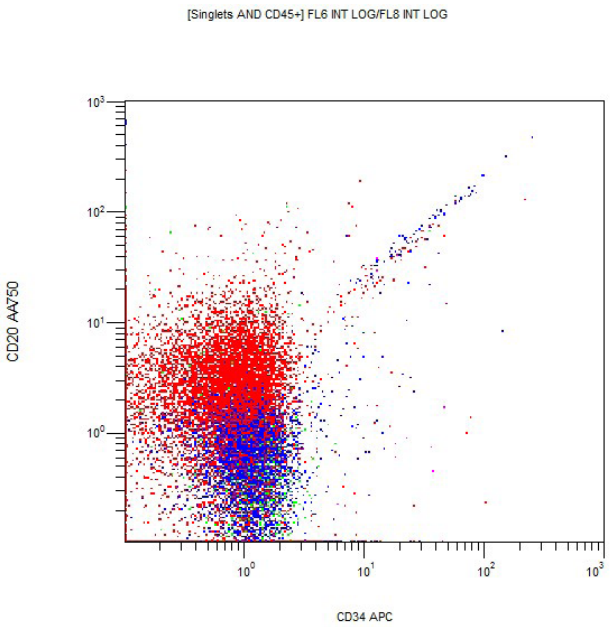
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). As noted elsewhere in this case, the normal B lymphocytes in this case express higher density CD20 than the aberrant population. In this case the aberrant B lymphocyte population cannot be easily distinguished from the T lymphocytes due to their low density CD20 expression.



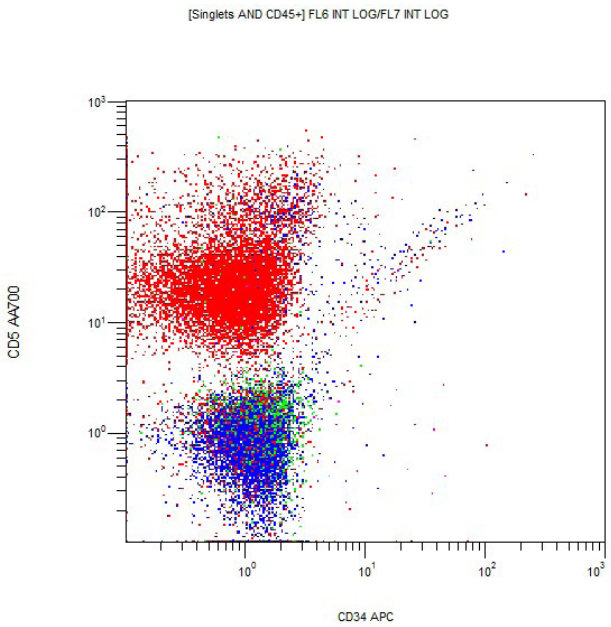
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes and NK cells are present. The blue 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. The blue events noted on the diagonal are consistent with high background fluorescence.



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. The blue events noted on the diagonal are consistent with high background fluorescence.



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. The higher density CD5 T lymphocytes and lower density CD5 aberrant B lymphocytes are distinguishable here. The blue events noted on the diagonal are consistent with high background fluorescence.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with low light scatter properties that express CD19, low density CD20, CD5, and CD45 and display Lambda immunoglobulin light chain restriction.

Taken together, the findings in this case are most consistent with chronic lymphocytic leukemia/small lymphocytic lymphoma. Note that correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

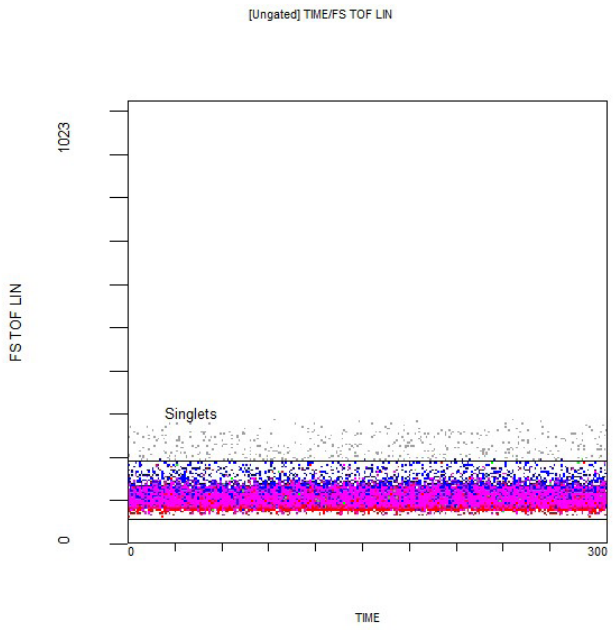
Case #7: Plasma Cell Myeloma

Clinical Vignette

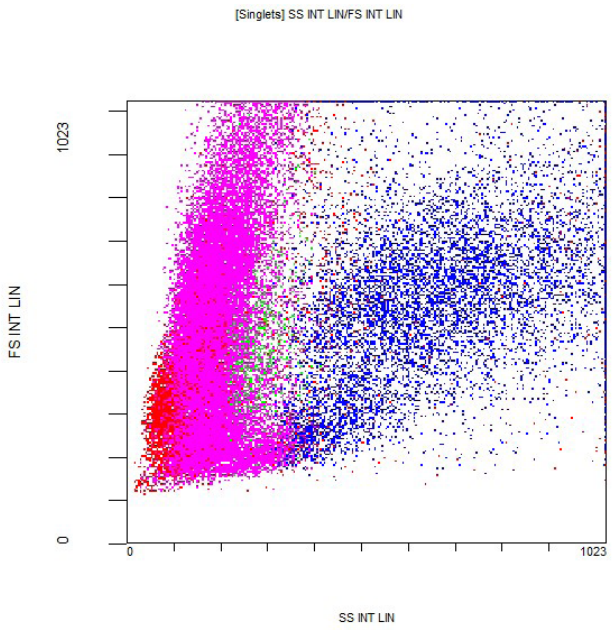
This 52-year-old female presents with unknown findings (i.e. no clinical or laboratory data are available). Atypical mononuclear cells are noted on microscopic examination of the bone marrow aspirate. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

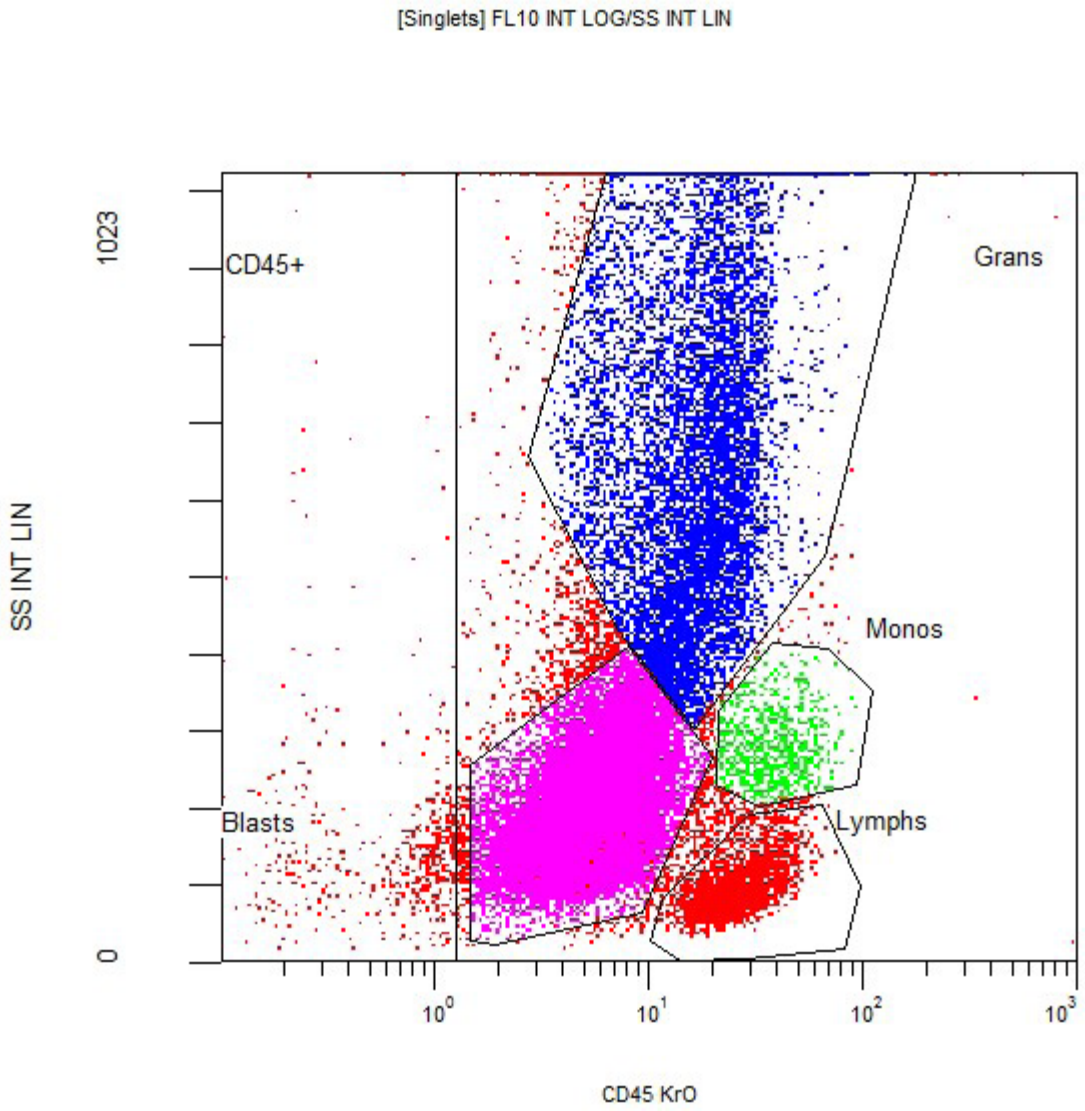
Access Case #7 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

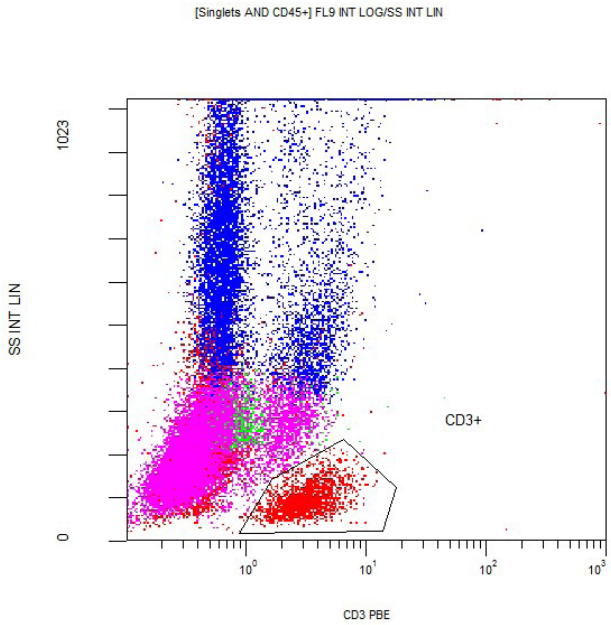


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of mononuclear cells and a relative paucity of apparent granulocytes.



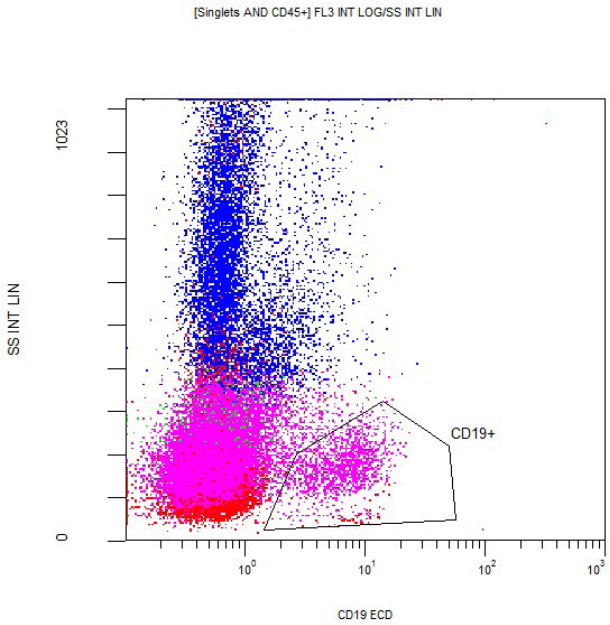
This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). Additionally, the "Blast" gate (pink) has been used to define a predominant population of mononuclear cells in this case. Note that the label for the Blast gate is not immediately adjacent to it---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.

Note that the overlap among the various populations in this particular case renders it somewhat difficult to distinguish among them. 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.

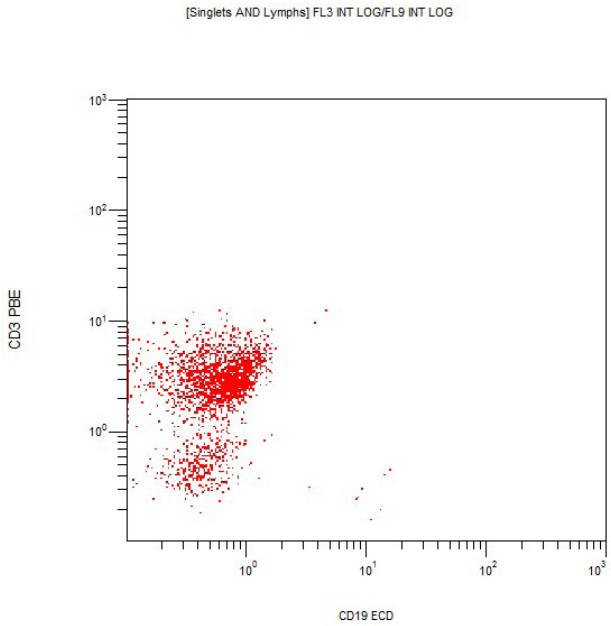


This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.

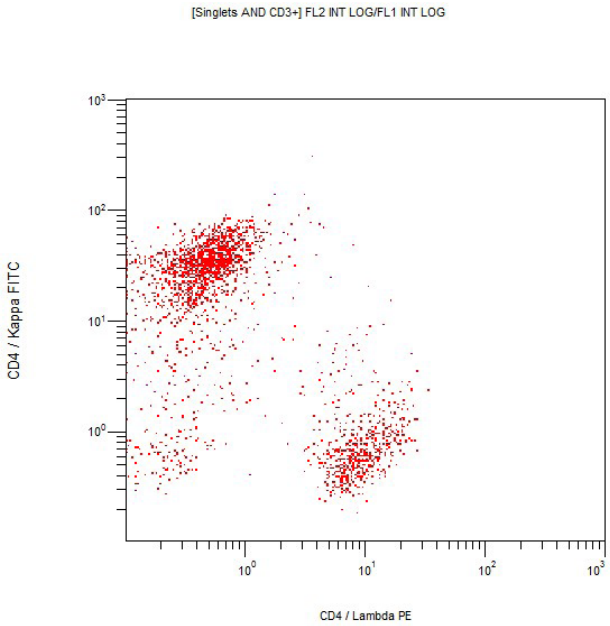
Note that the blasts (pink) are negative for CD3



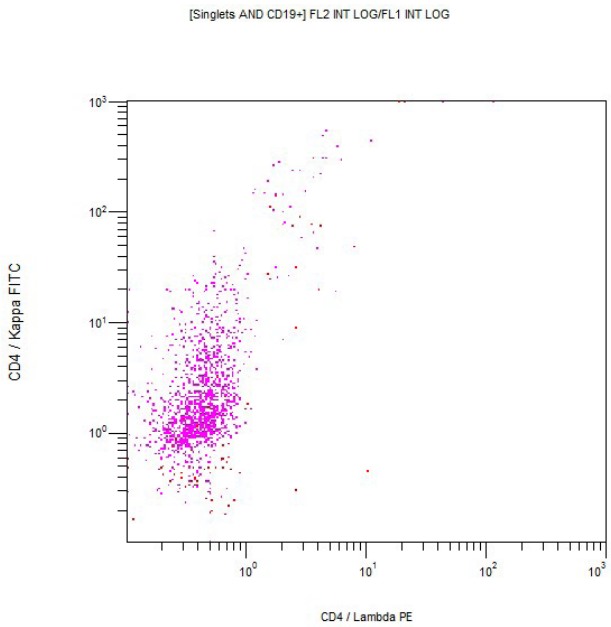
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. The findings in this display are interesting in that very few of these CD19 positive events are included in the Lymphocytes gate (red) and only a subset of the blasts (pink) are included in the CD19+ gate. The light scatter properties of this latter population are not consistent with normal B lymphocytes.



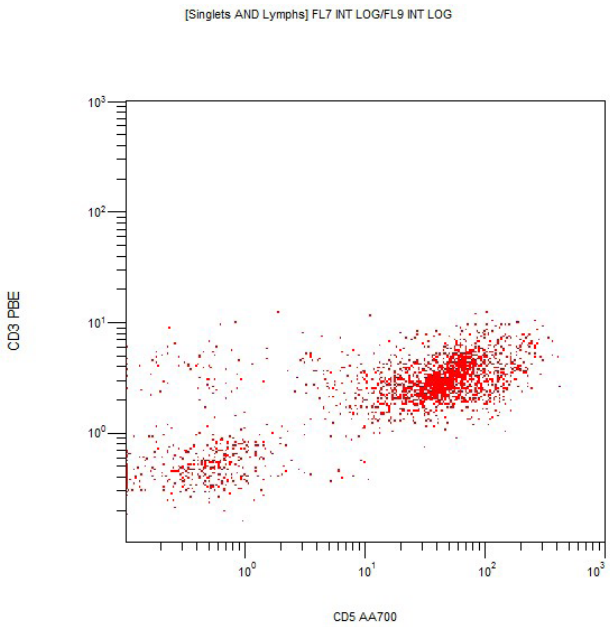
This CD19/CD3 dot plot gated on Lymphocytes confirms the paucity of normal B lymphocytes in this sample.



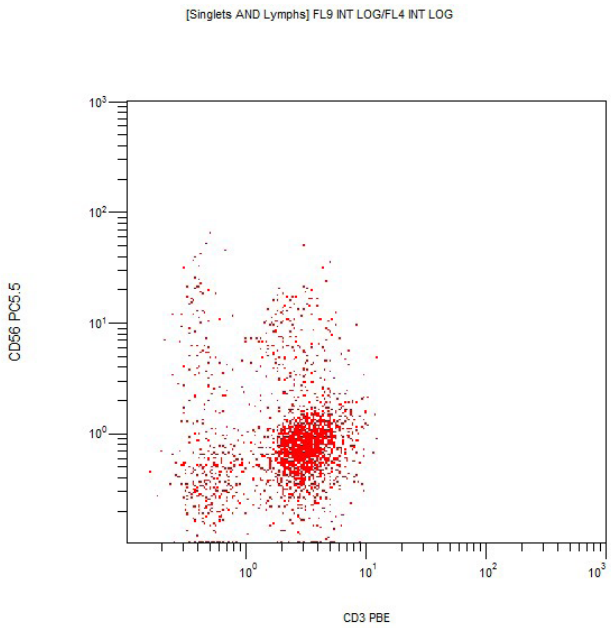
Applying the CD3+ gate to this dot plot permits “unstacking” of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



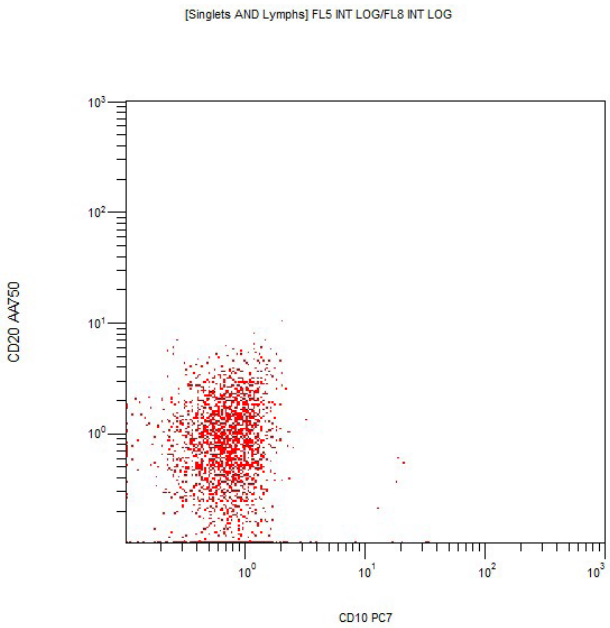
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. Note that the blasts (pink) appear to display Kappa immunoglobulin light chains.



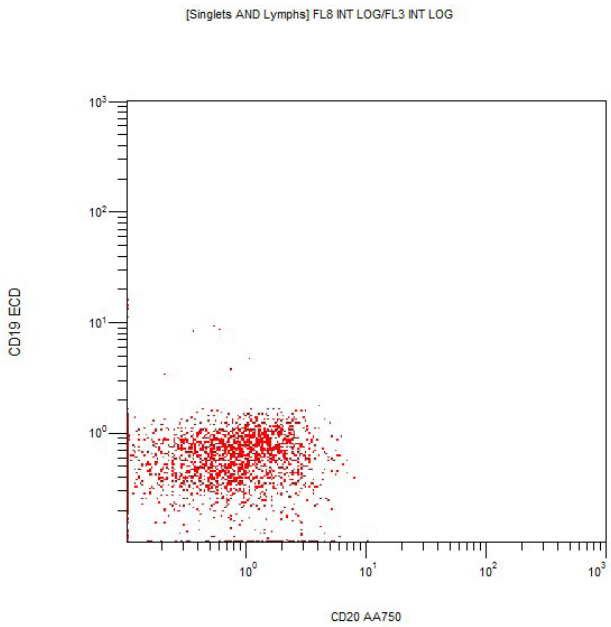
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable T lymphocytes comprise the majority of cells. Apparent NK cells (CD3 and CD5 dual negative) are also present.



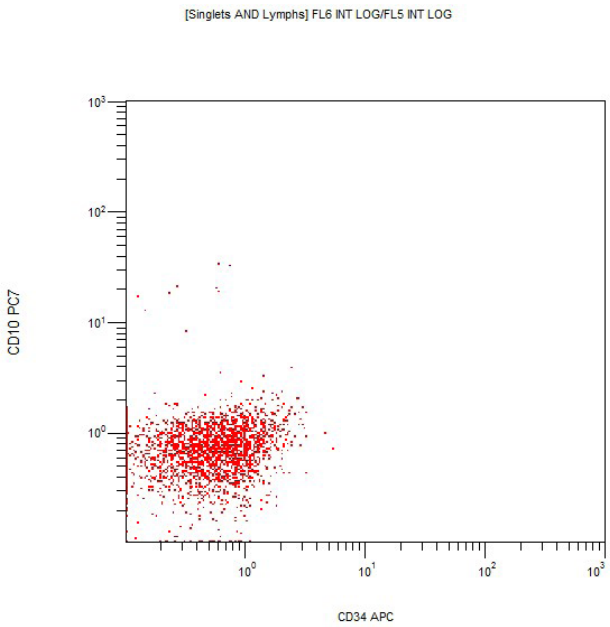
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present.



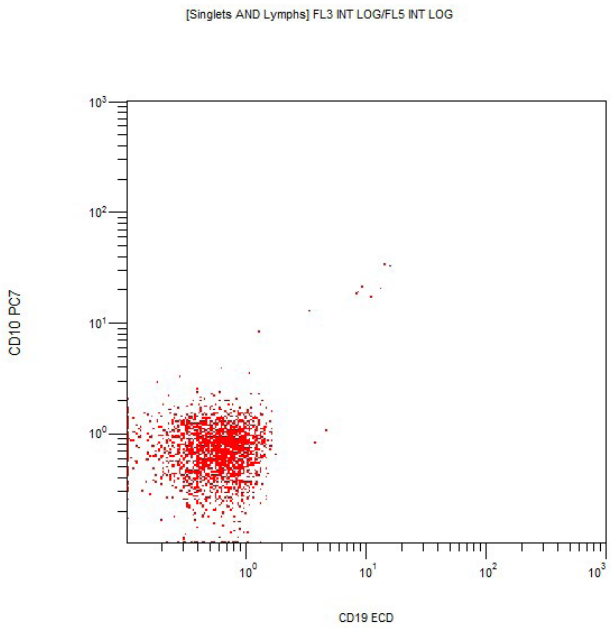
This CD10/CD20 dot plot is gated on Lymphocytes. No B lymphocytes that co-express CD10 are present.



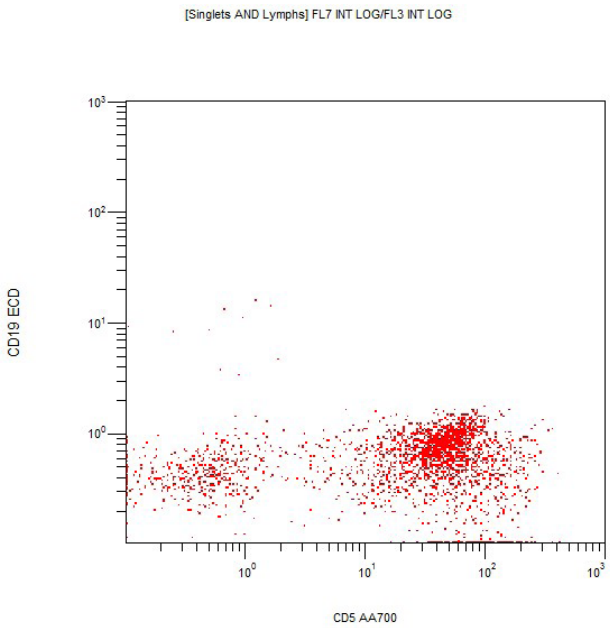
This CD20/CD19 dot plot is gated on Lymphocytes. Very few B lymphocytes are present.



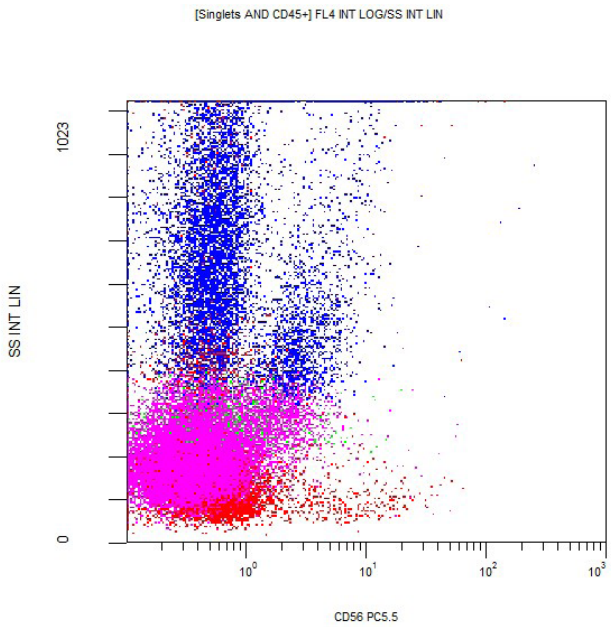
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are negative for both markers, consistent with the expected presence of mature T and B lymphocytes and NK cells.



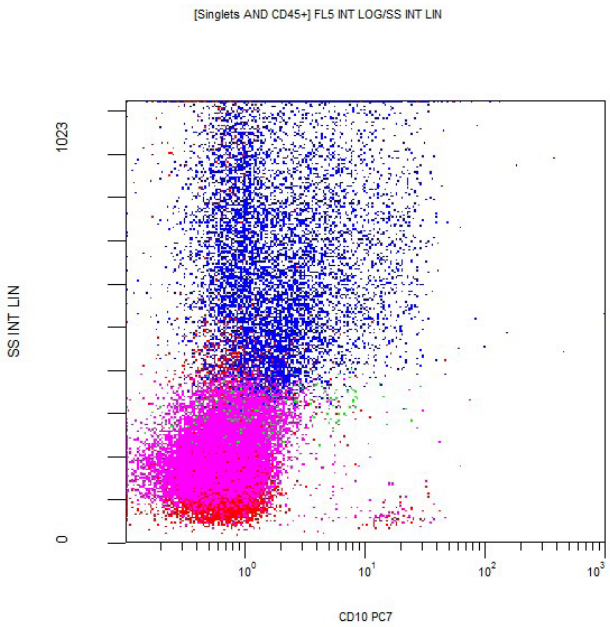
This CD19/CD10 dot plot is gated on Lymphocytes. The small population of CD19 and CD10 dual positive cells may represent hematogones.



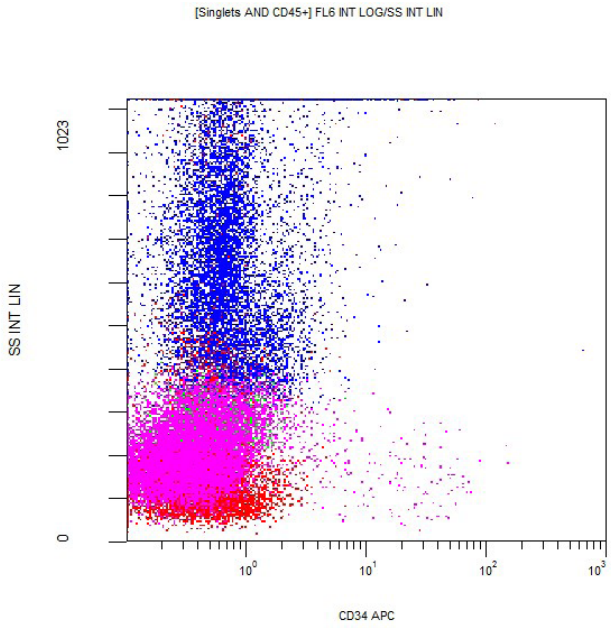
This CD5/CD19 dot plot is gated on Lymphocytes. No co-expression of CD5 and CD19 is identified.



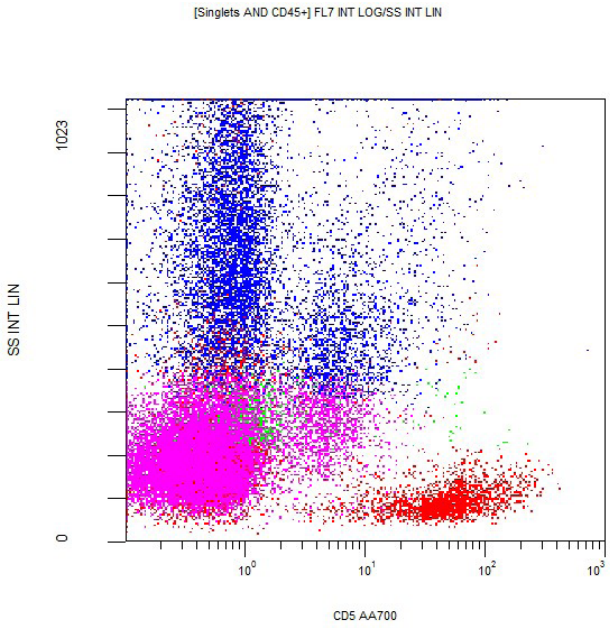
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells. The blasts (pink) do not express CD56.



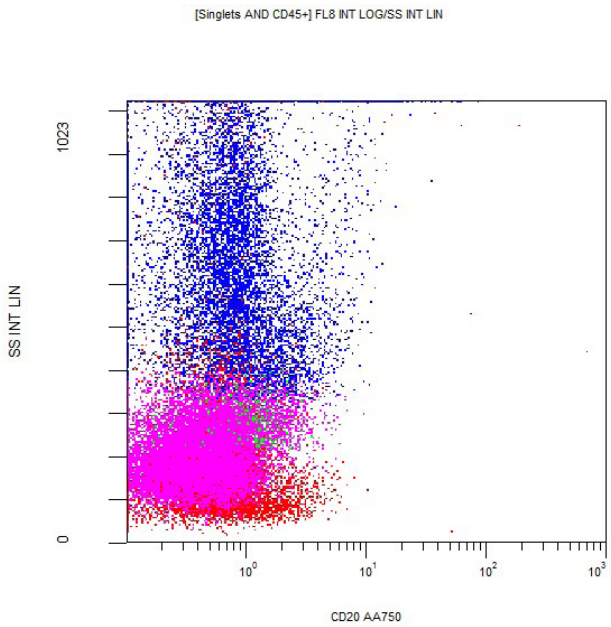
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Blasts (pink) are negative for CD10.



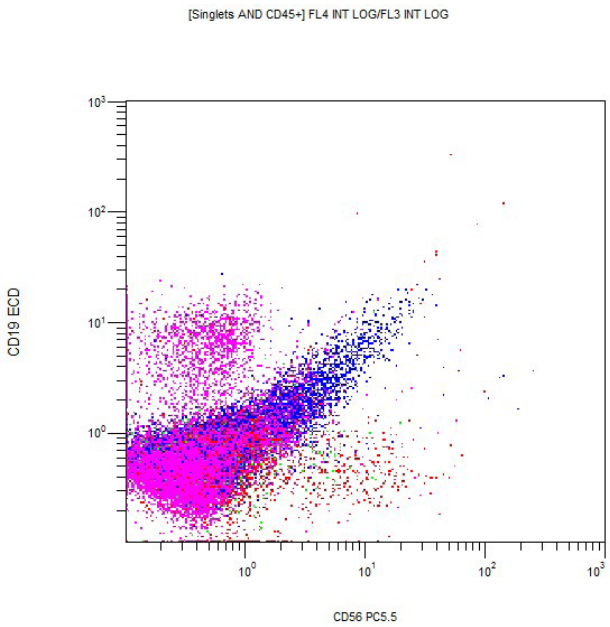
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Scattered CD34 positive events are noted, but that vast majority of the blasts (pink) are negative, and apparently positive events may represent debris.



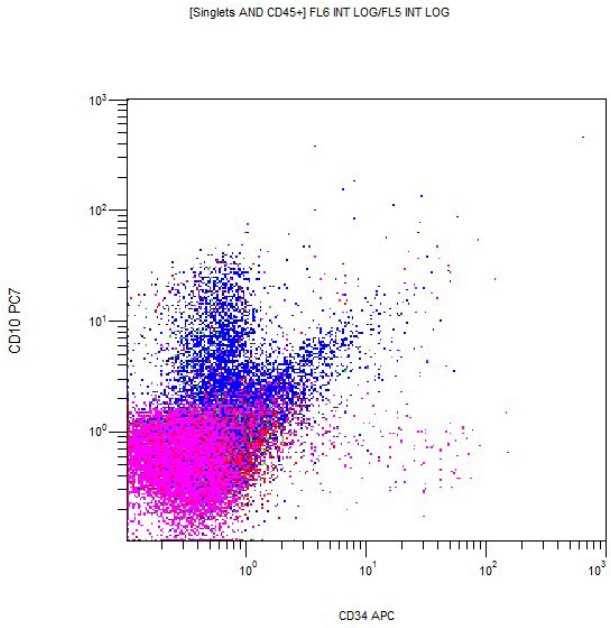
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) do not express CD5. The majority of the lymphocytes (red) do express CD5, consistent with T lymphocytes.



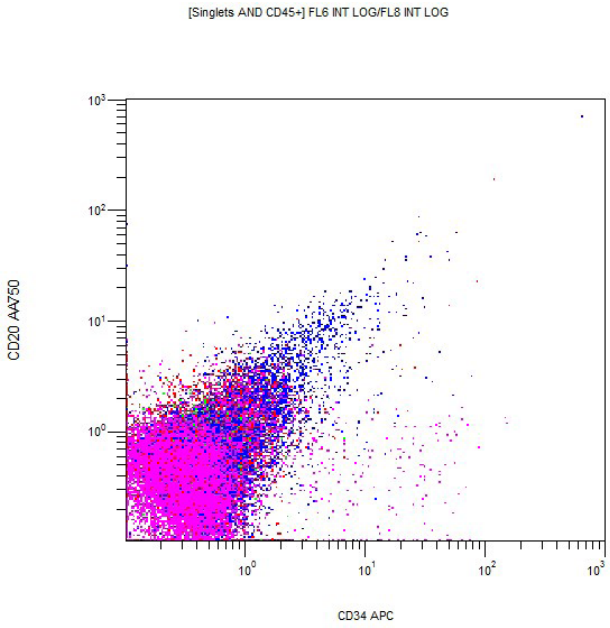
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) do not express CD20. As noted elsewhere in this analysis, very few B lymphocytes are present in this sample.



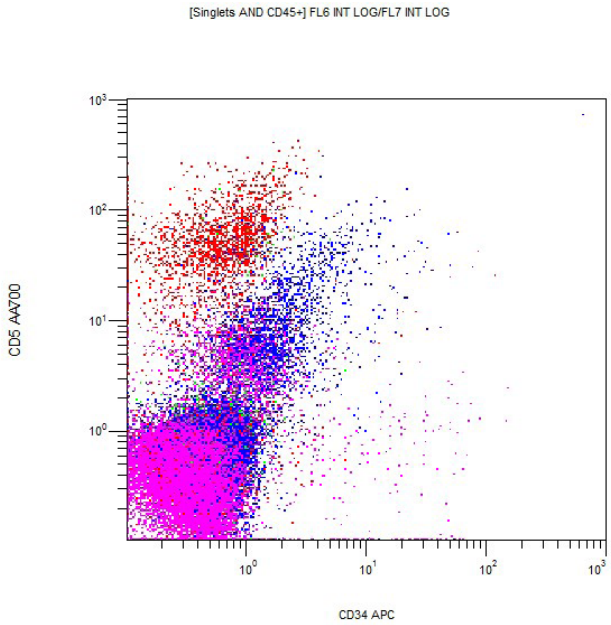
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A subset of the blasts (pink) express CD19 but not CD56 or CD19. 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 the blasts (pink) are negative for CD34. A small but distinct population of CD10 positive/CD34 negative events corresponds to granulocytes (blue). The blue events that appear to co-express CD34 and CD10 are consistent with high background fluorescence.



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). As noted elsewhere the blasts (pink) are negative for CD34. The blue events that appear to co-express CD34 and CD20 are consistent with high background fluorescence.



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). As noted elsewhere in this analysis the blasts (pink) are negative for both CD34 and CD5. CD5 positive T lymphocytes (red) are also present. The blue events that appear to co-express CD34 and CD5 are consistent with high background fluorescence.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with increased light scatter properties that express partial CD19 and low density CD45.The CD19 positive compartment displays Kappa immunoglobulin light chain restriction. These cells fail to express CD3, CD5, CD10, CD19, CD20, and CD56. In order to assess expression of CD4 and CD8 by these cells, additional dot plots may be analyzed. Light chain expression by the non-CD19 positive subset of the aberrant population may also be assessed with additional dot plots.

Taken together, the results indicate the presence of a phenotypically distinct population of cell of apparent hematolymphoid lineage. Diagnostic considerations are broad and additional flow cytometric immunophenotyping of this sample (data not shown) confirms an immunophenotype consistent with plasma cell myeloma. Correlation with clinical and laboratory data is also recommended.

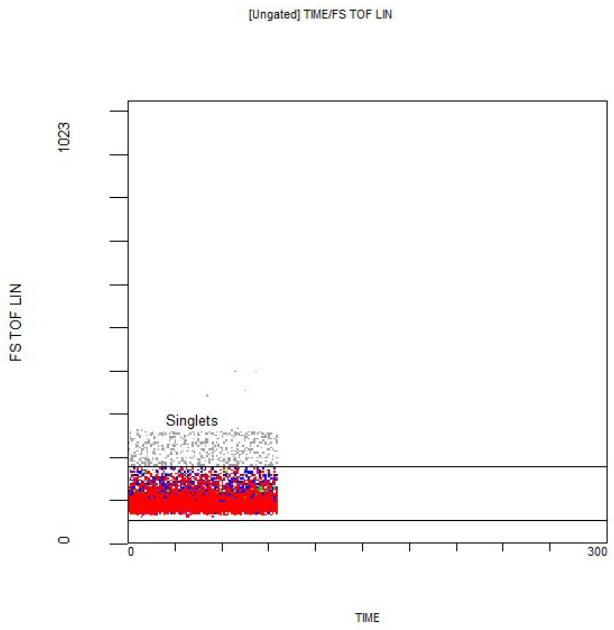
Case #8: Follicular Lymphoma

Clinical Vignette

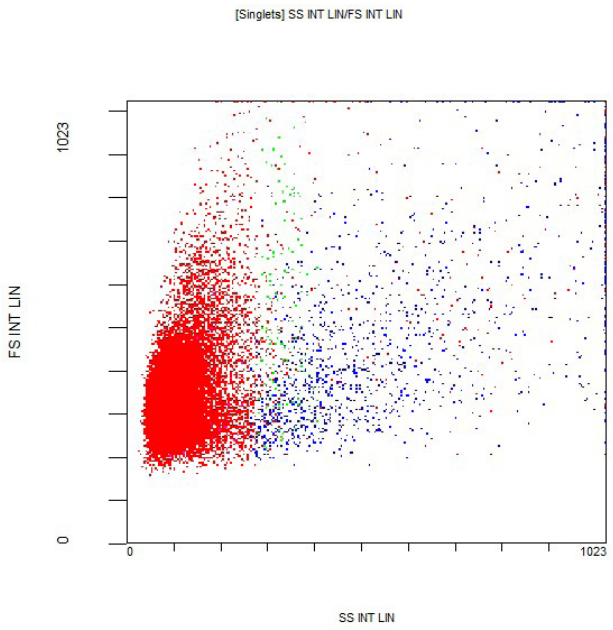
This 47-year-old male presents with lymphadenopathy. A lymph node biopsy sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

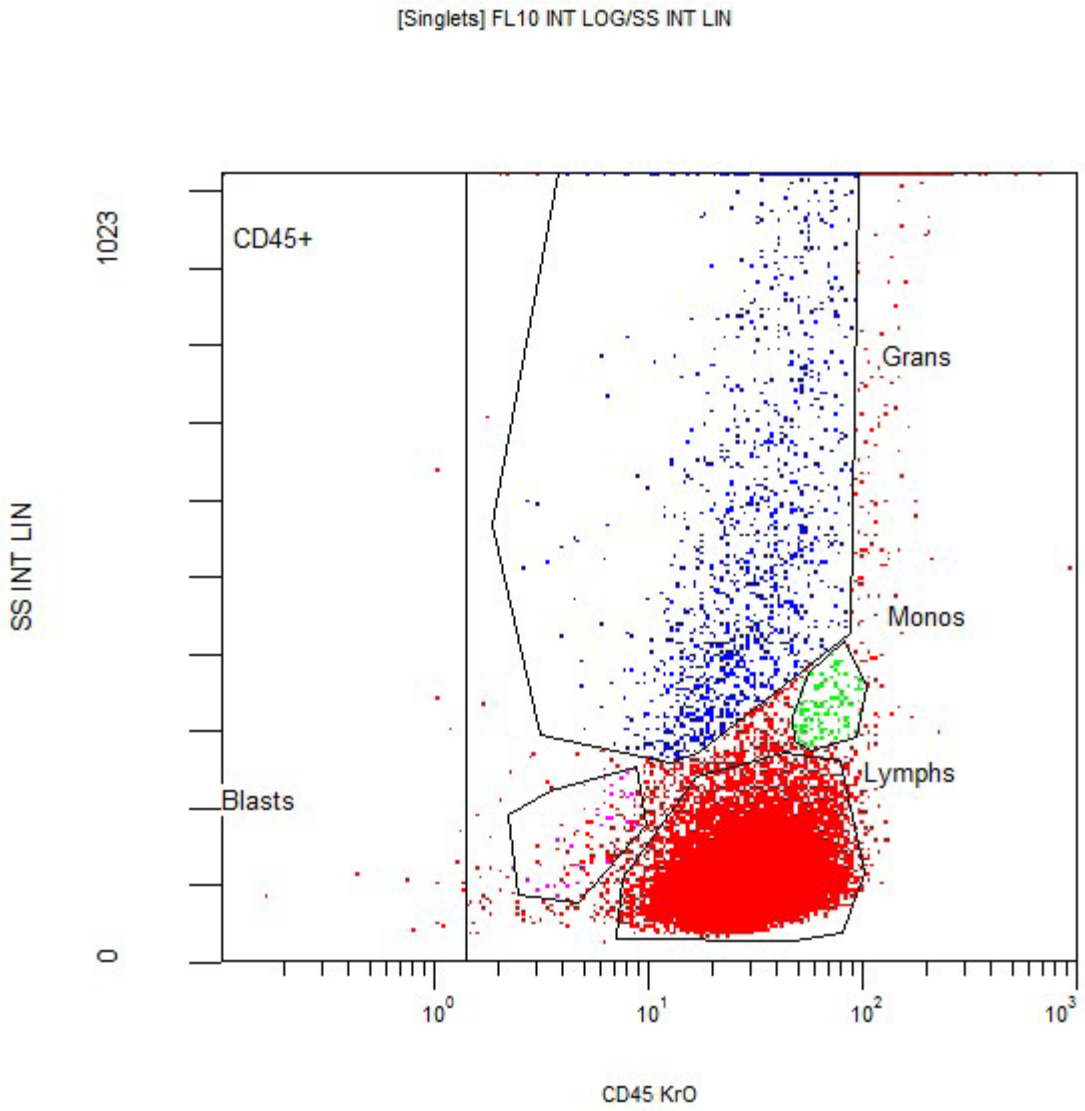
Access Case #8 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

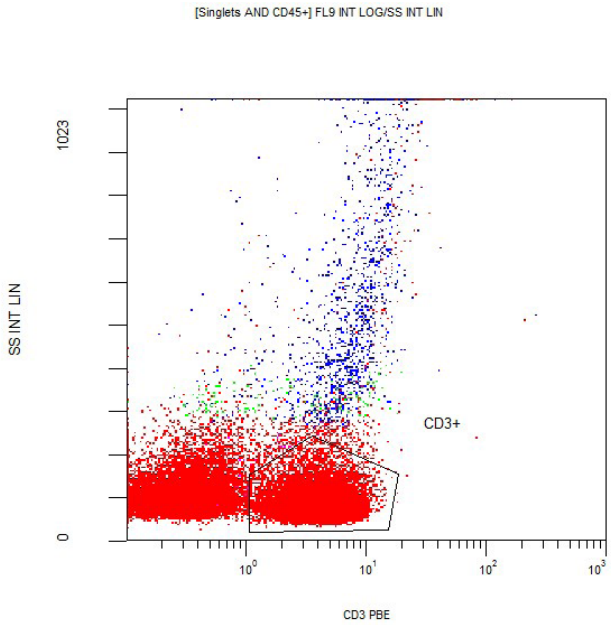


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of apparent lymphocytes (red) as well as a few monocytes (green) and possible granulocytes (blue).

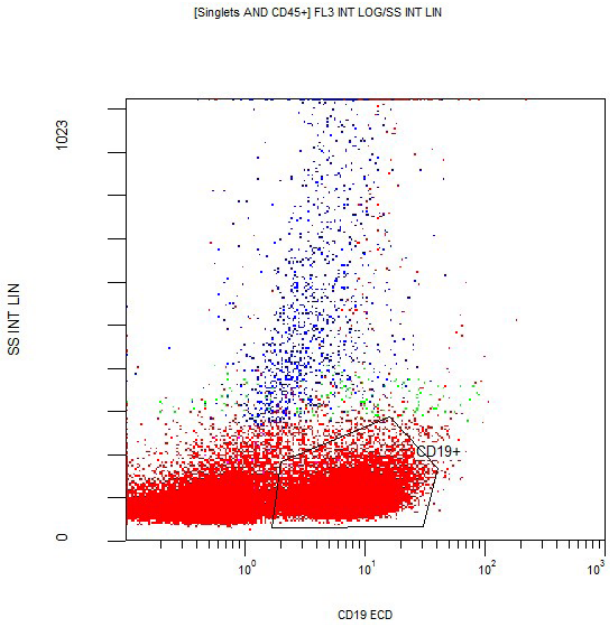


This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), granulocytes (blue) and blasts (pink). Note that the label for the Blast gate is not 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.

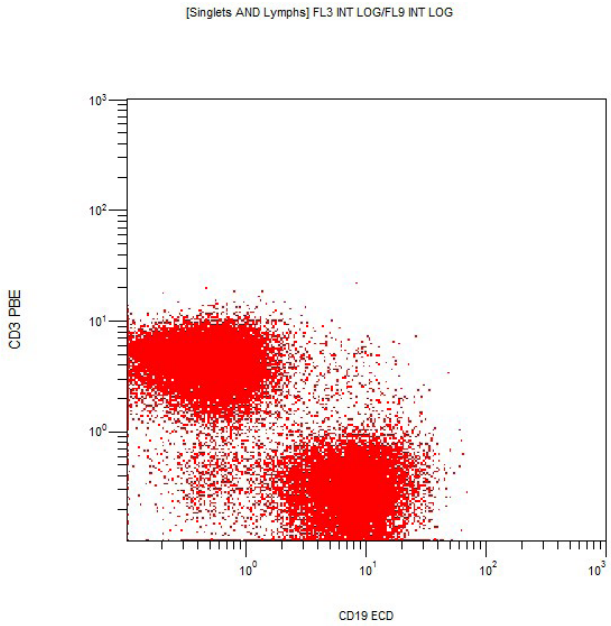
Note that although gates for monocytes (green), granulocytes (blue), and blasts (pink) have been are present here, the events within them may not represent those cells (and may in fact be debris).



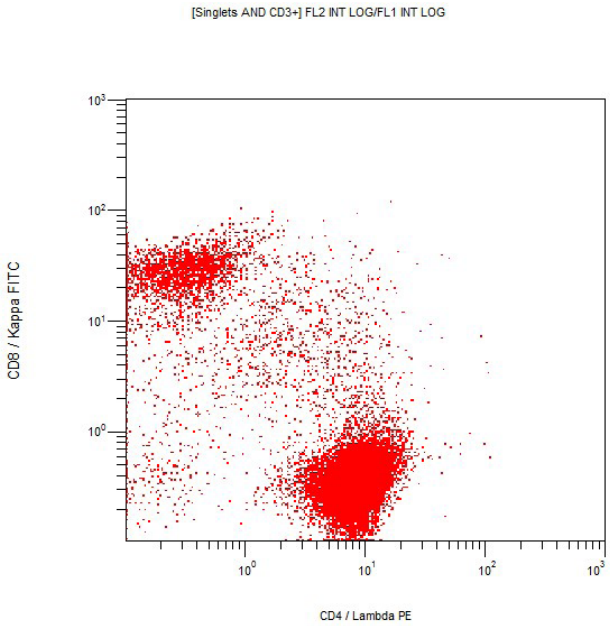
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.



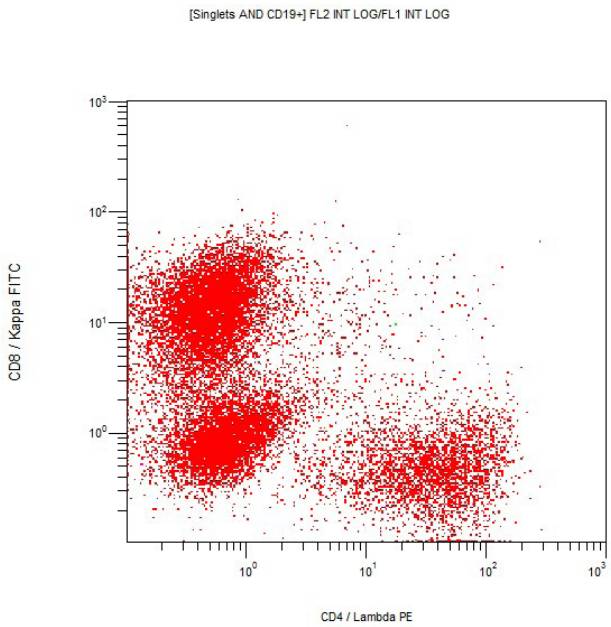
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only, but in this case it's difficult to find a clear break between the CD19 positive and CD19 negative events.



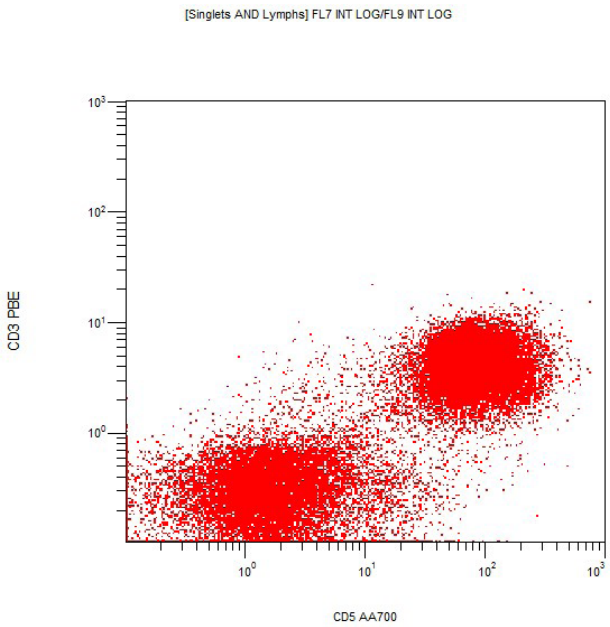
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis.



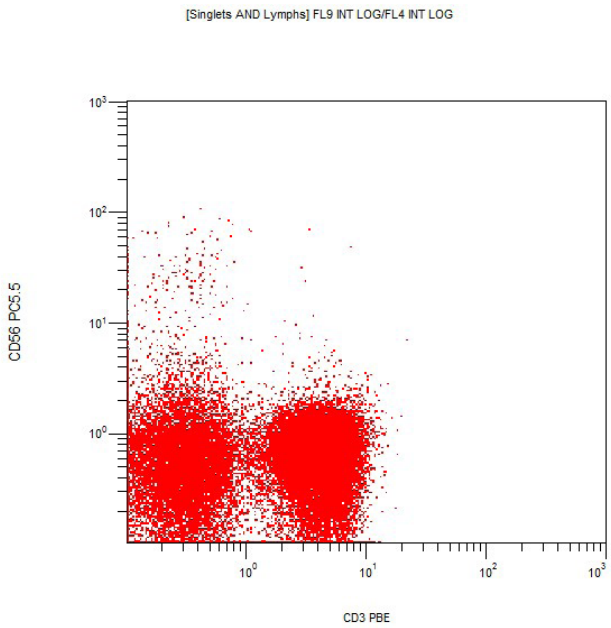
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



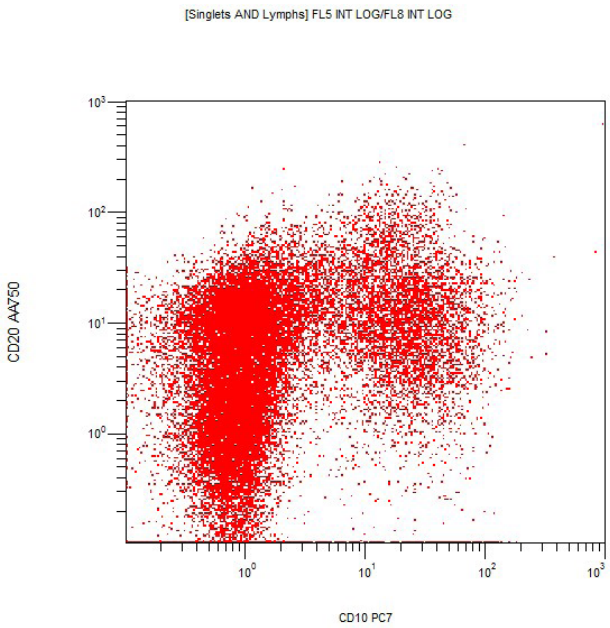
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. Note that there are three populations here: Lambda, Kappa, and a third population that appears to express neither Kappa nor Lambda immunoglobulin light chains.



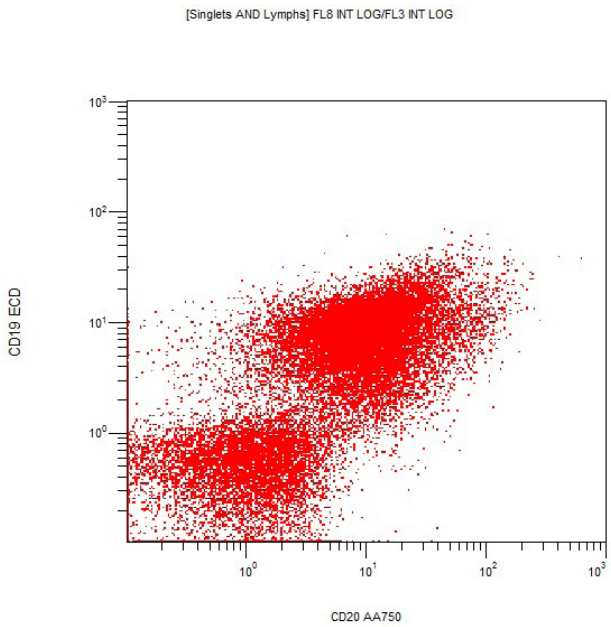
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable CD3 positive/CD5 positive T lymphocytes are present. The remaining cells are presumably B lymphocytes that do not co-express CD5.



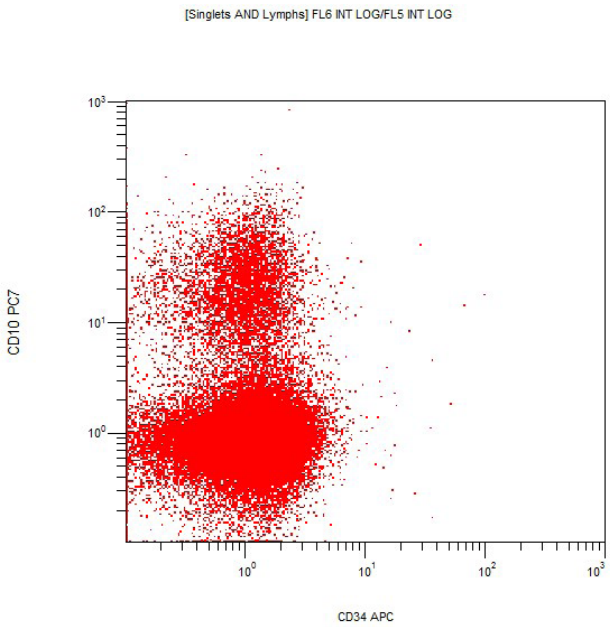
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and a small number of NK cells (CD56 positive, CD3 negative) are present. The CD3 and CD56 dual negative cells are presumably B lymphocytes.



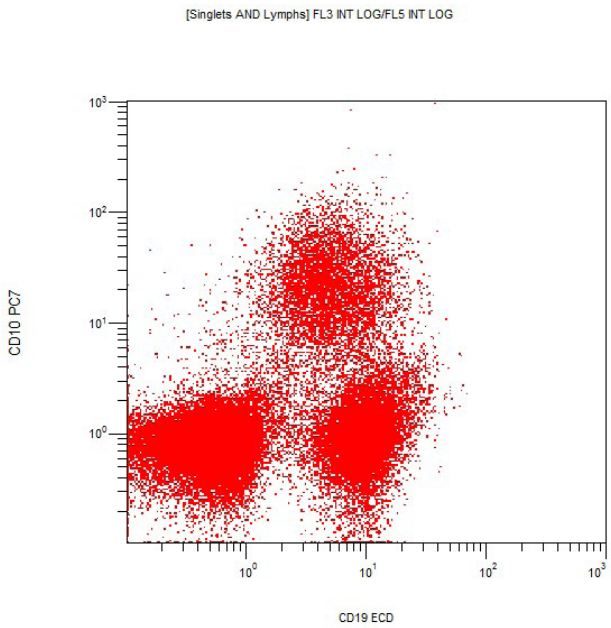
This CD10/CD20 dot plot is gated on Lymphocytes. A distinct population of B lymphocytes that co-express CD10 is noted. CD10 negative, CD20 positive B lymphocytes are also noted.



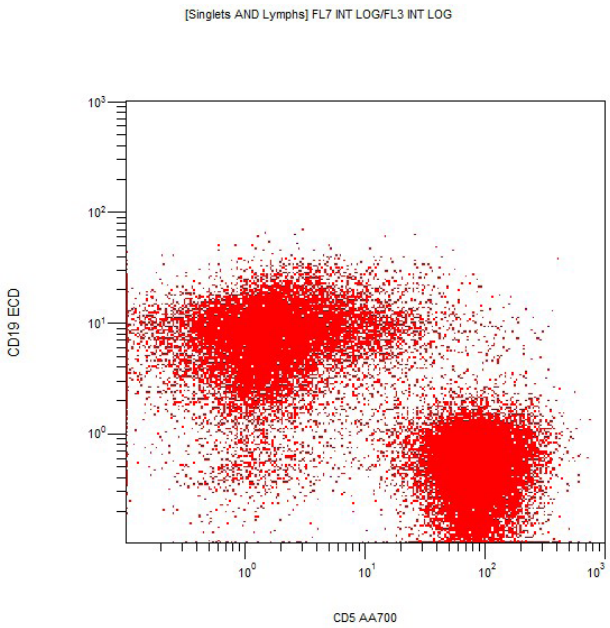
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display co-expression of CD19 and CD20.



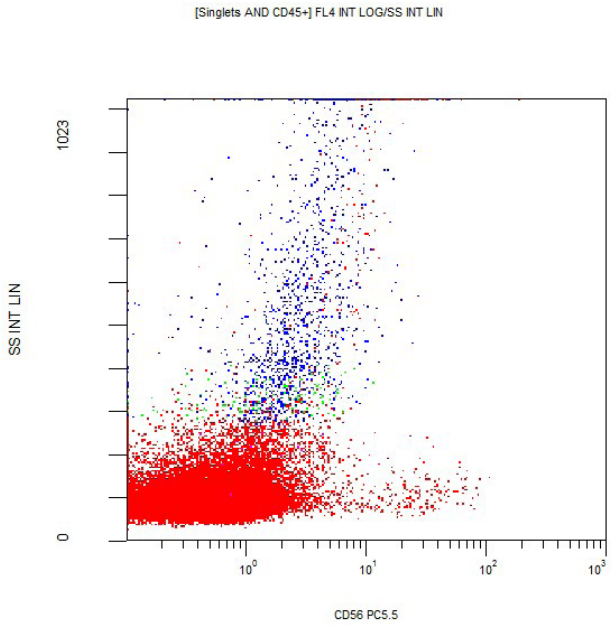
This CD34/CD10 dot plot is gated on Lymphocytes. The CD10 positive population is consistent with that noted elsewhere in this analysis. CD34 expression is absent.



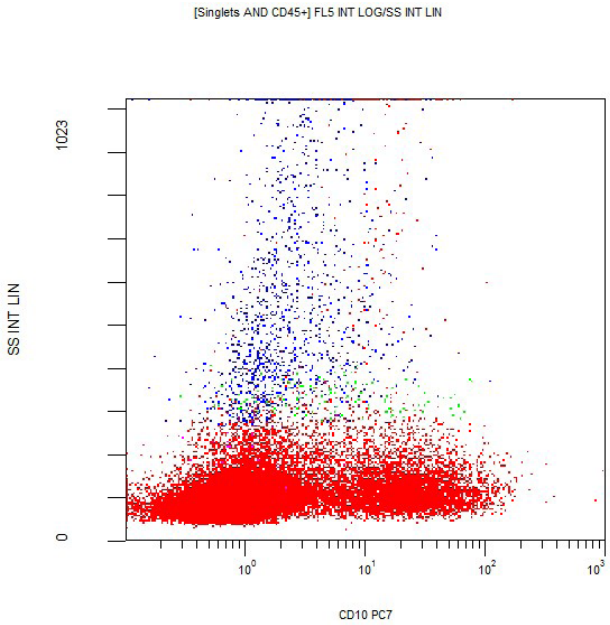
This CD19/CD10 dot plot is gated on Lymphocytes. The CD10 and CD19 dual positive population is aberrant, whereas the CD19 positive, CD10 negative cells are consistent with normal B lymphocytes. The third population that is CD10 and CD19 dual negative is comprised of T cells.



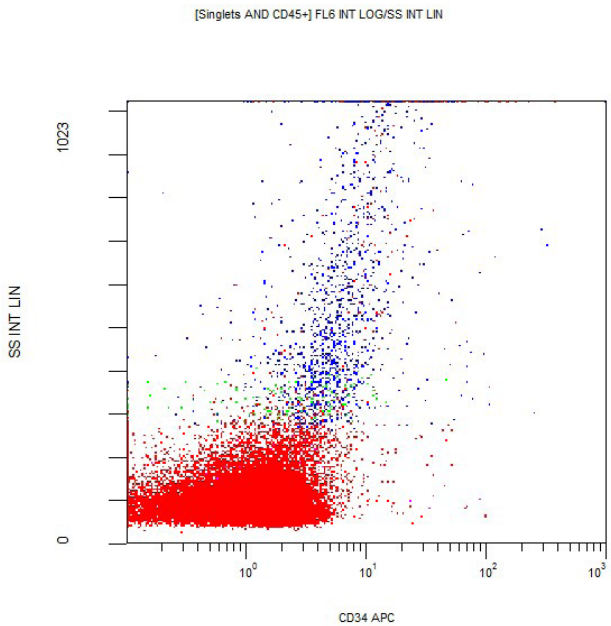
This CD5/CD19 dot plot is gated on Lymphocytes. T and B lymphocytes are clearly distinguishable and no significant CD5 and CD10 co-expression is present.



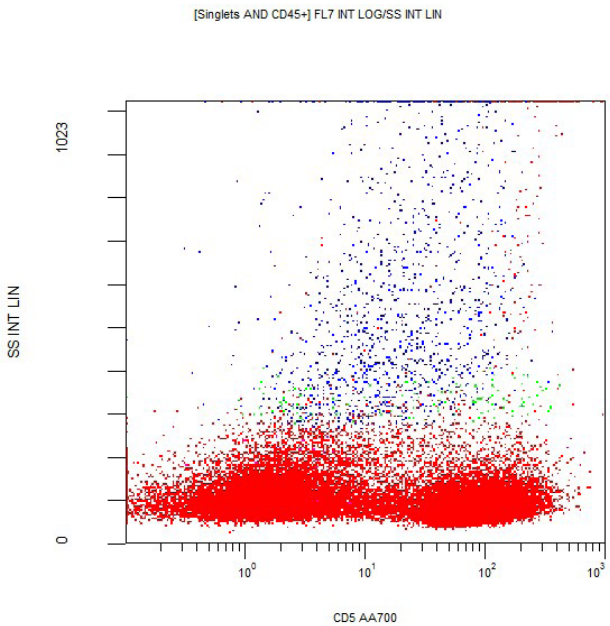
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells.



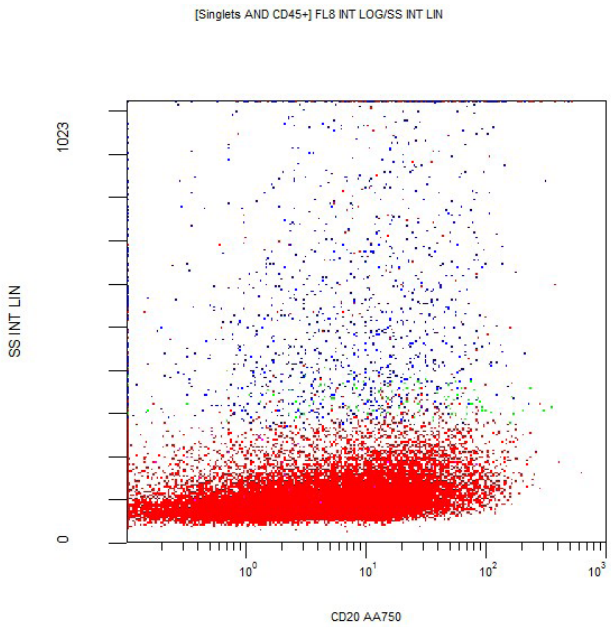
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The CD10 positive events in red represent the aberrant population in this case. The blue events are negative for CD10 and consistent with debris.



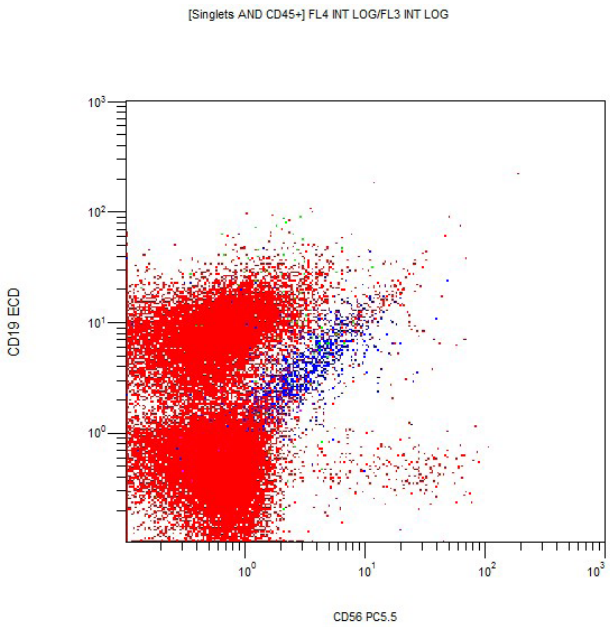
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). No significant CD34 positive population is present.



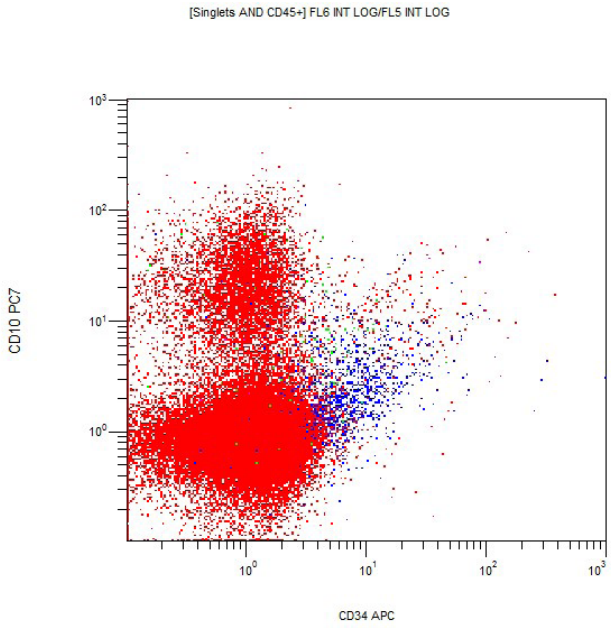
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A portion of the lymphocytes (red) express CD5, consistent with T lymphocytes.



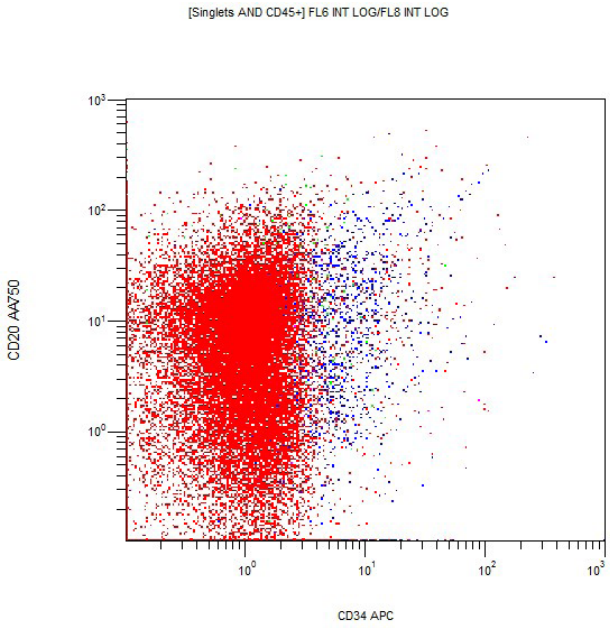
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Clear delineation between the CD20 positive (B lymphocytes) and CD10 negative (T lymphocytes) is not possible.



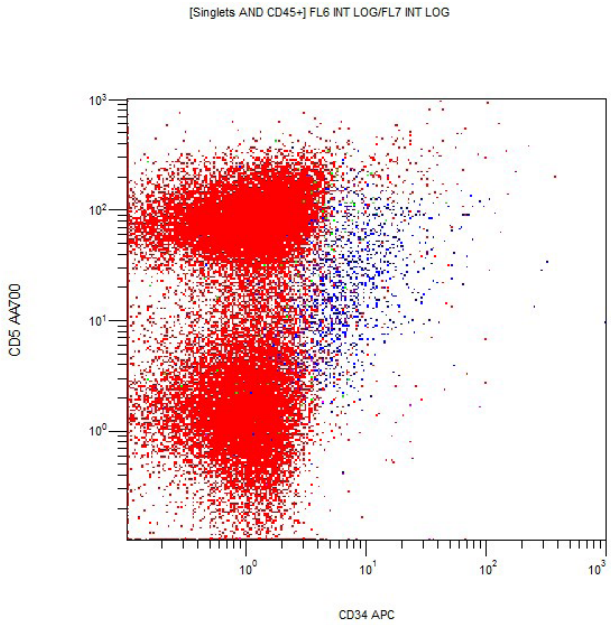
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes and NK cells are present. The blue 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). A distinct population of CD10 positive, CD34 negative events corresponds to aberrant B lymphocytes (red). The blue events that appear to co-express CD34 and CD10 are consistent with high background fluorescence.



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). The blue events are consistent with debris.



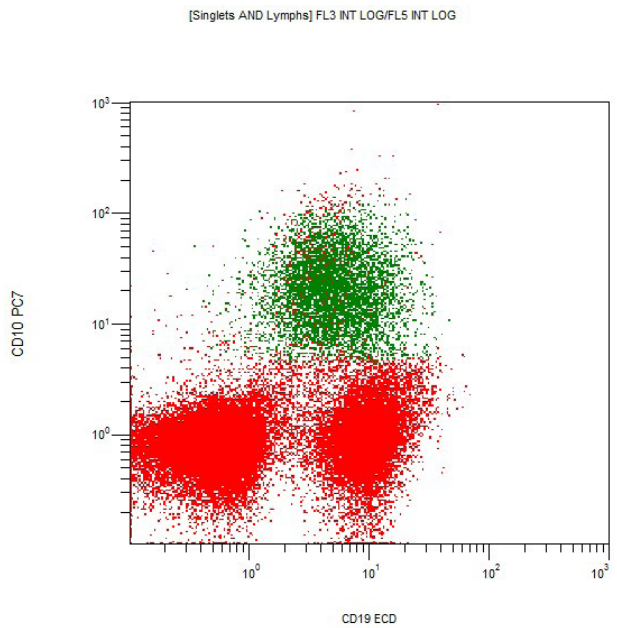
This CD34/CD5 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blue events are consistent with debris.

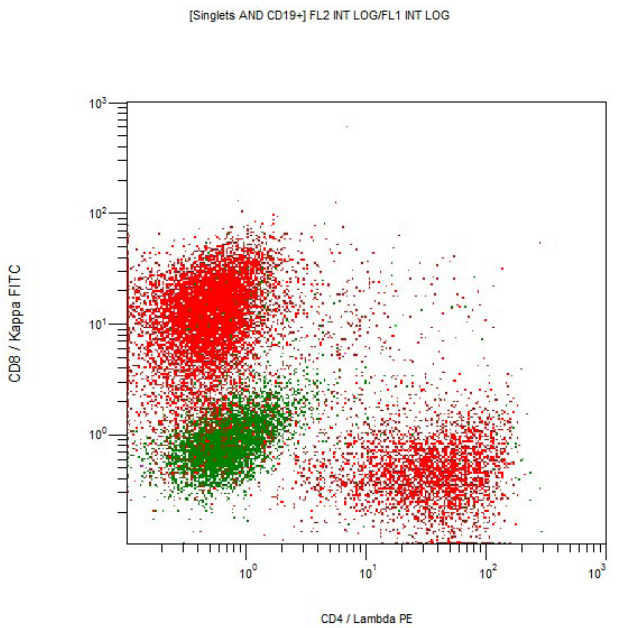
Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells properties that express CD10, CD19, CD20, and CD45 and are negative for CD5 and CD34. These cells fail to display either Kappa or Lambda immunoglobulin light chains.

Taken together, the findings in this case are consistent with a CD10 positive B cell lymphoproliferative disorder. The differential diagnosis includes follicular lymphoma, diffuse large B cell lymphoma, and Burkitt lymphoma. Note that correlation with clinical and laboratory data is recommended. Histologic findings in this case (data not shown) confirm a diagnosis of follicular lymphoma.

Note that it is not uncommon for both normal and aberrant B lymphocytes to be present in the same sample, sometimes complicating assessment of light chain restriction. In order to evaluate them independently, it is helpful to identify parameters that distinguish the two and construct gates that can then be applied to the Kappa/Lambda display. In this case, the CD19/CD10 display clearly distinguishes the normal B lymphocytes from the aberrant population. By coloring the population of interest in green, we can see that the CD10 negative B lymphocytes are clearly polyclonal, whereas the CD10 positive B lymphocytes are negative for surface immunoglobulin light chains.





NEOPLASTIC PROCESS OF T CELL ORIGIN

T cell and NK cell neoplasms also include acute lymphoblastic and mature lymphoid neoplasms. They are relatively uncommon, but many of them are among the most aggressive of all lymphoid neoplasms. Some, however, have a more prolonged clinical course. Immunophenotypically, these neoplasms often show aberrant expression or loss of T cell markers that aid in the differential diagnosis. Additionally, they may be associated with a viral infection. Epstein-Barr virus (EBV) is most often associated with NK cell leukemias and extranodal NK/T cell lymphomas. Human T cell leukemia virus (HTLV-1) is etiologically linked to adult T cell leukemia/lymphoma. Besides morphologic, immunophenotypic, and genetic characteristics, clinical features play an important part in the definition of these diseases.

T LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

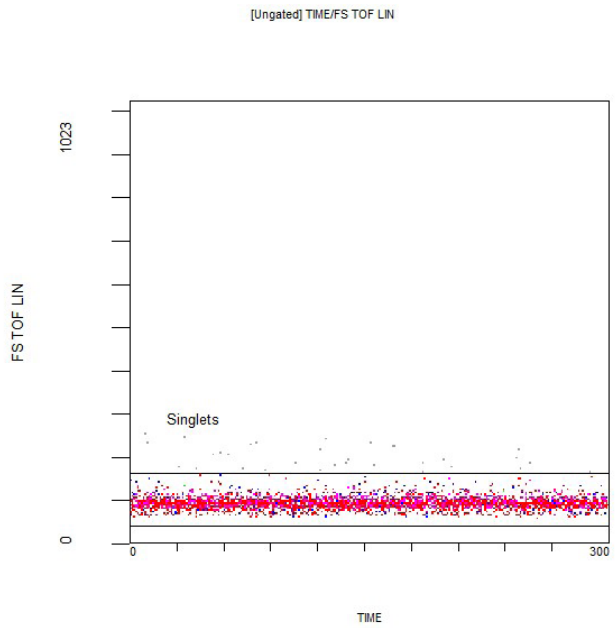
Case #9: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

Clinical Vignette

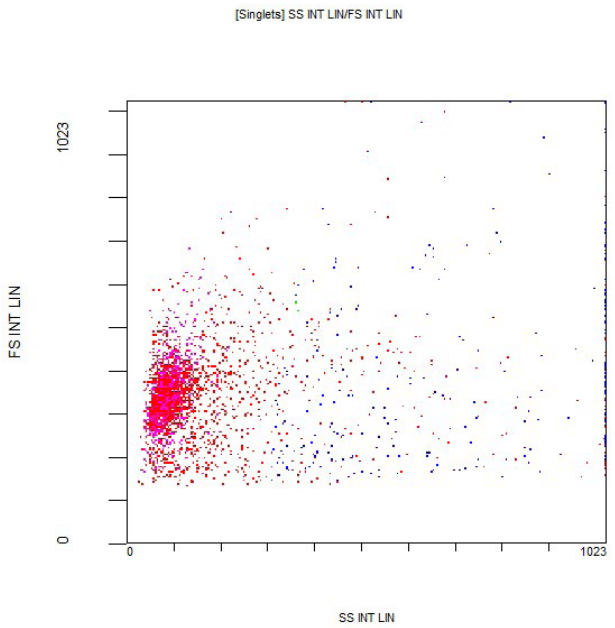
This 18-year-old female with presents with pancytopenia and a mediastinal mass. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

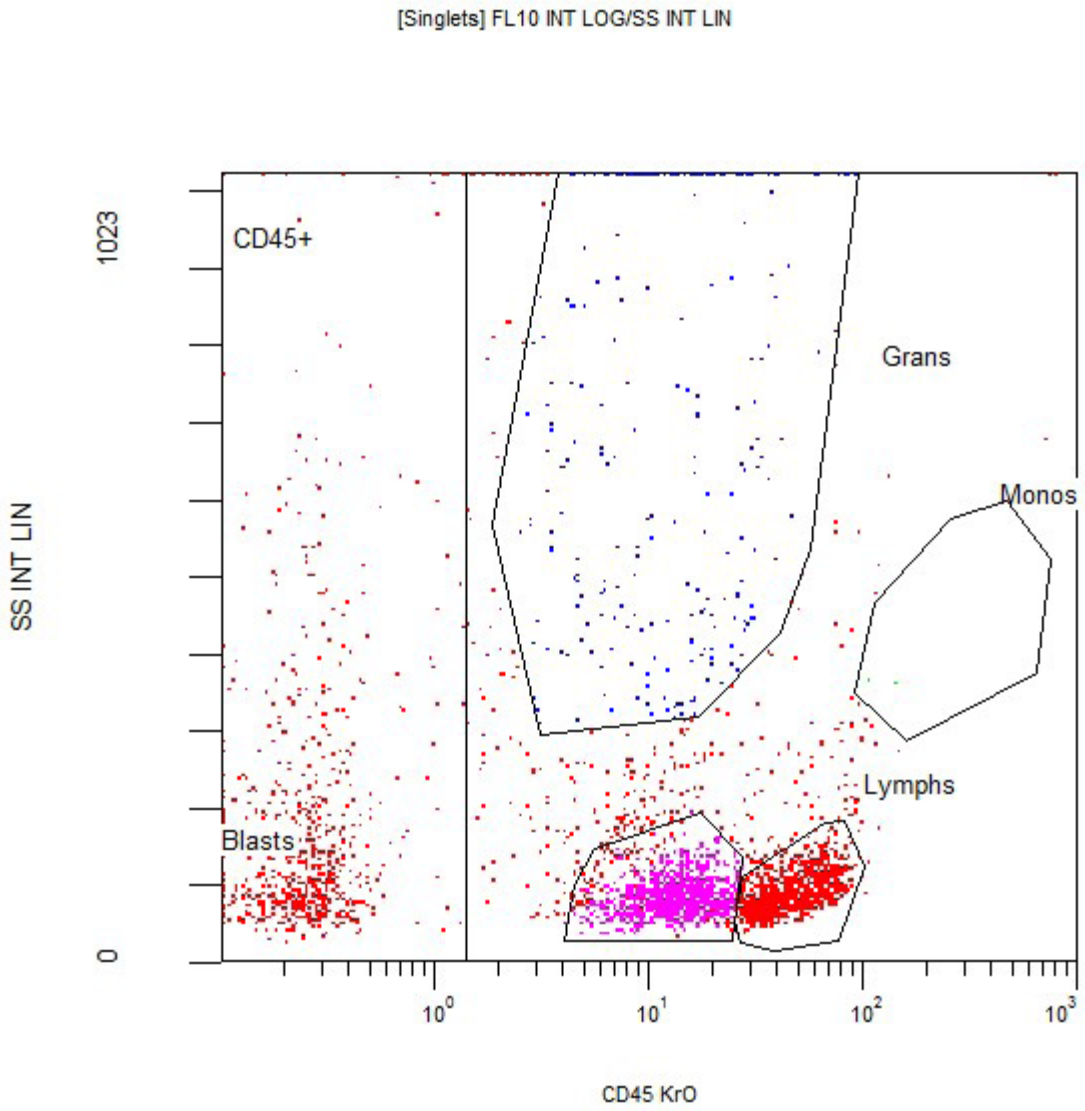
Access Case #9 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

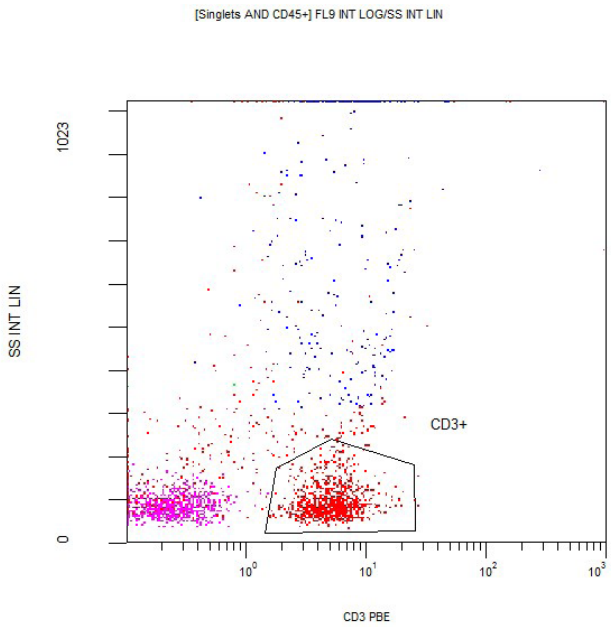


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of apparent lymphocytes. No other distinct cells populations are present.



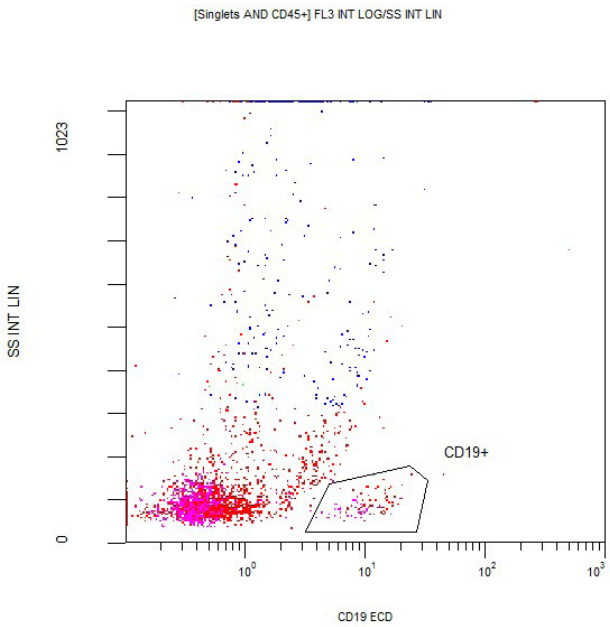
This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). Additionally, the "Blast" gate (pink) has been used to define tightly clustered population of mononuclear cells in this case. Note that the label for the Blast gate is not immediately adjacent to it---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.

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. In this case the monocyte gate is not being used and has been pushed to the side. The granulocyte gate is in its usual position, but the events within it do not in fact appear to be granulocytes.

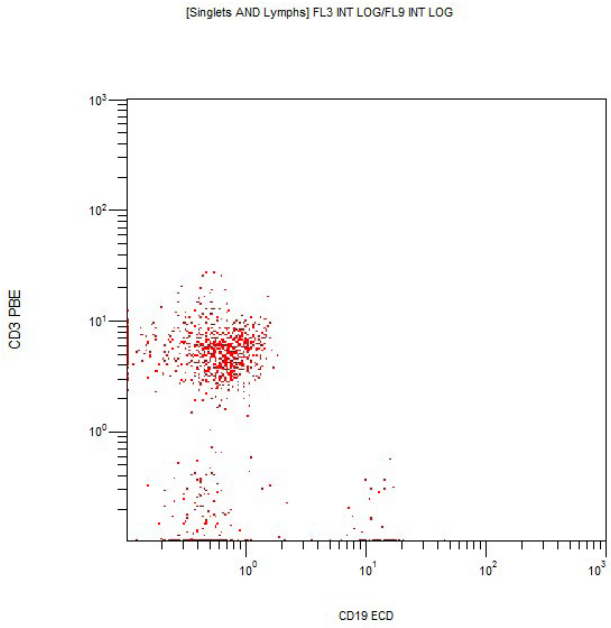


This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.

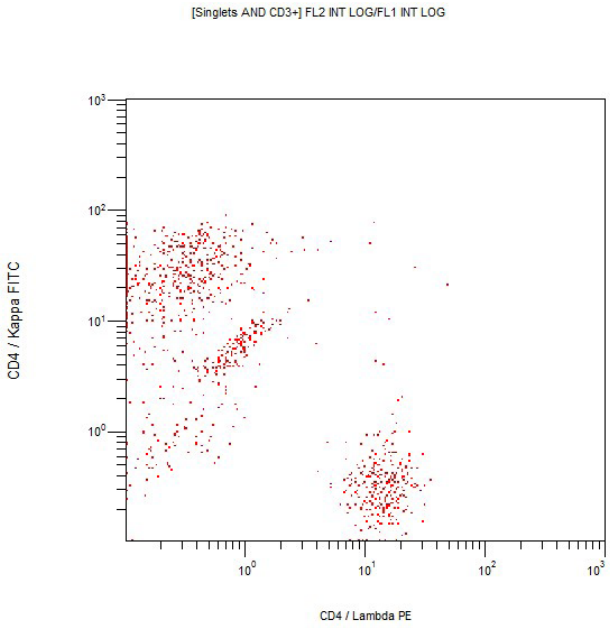
Note that the blasts (pink) are negative for CD3. For this particular case it's important to note that this assay tests only for surface CD3. Cells that express only cytoplasmic CD3 will not be identified as such.



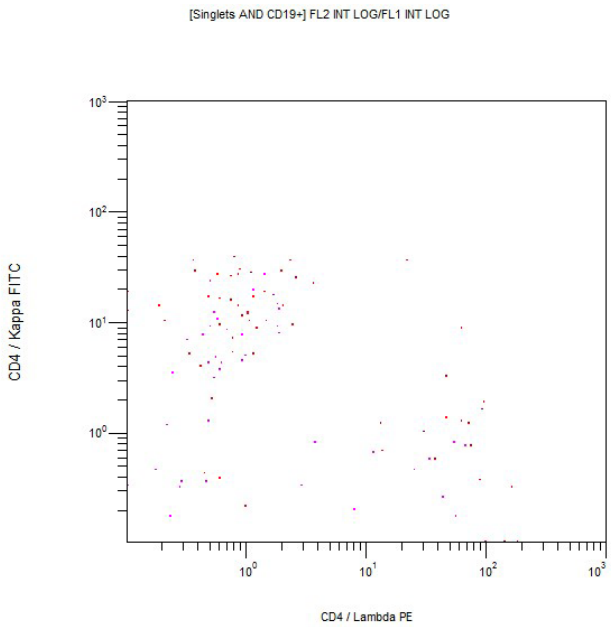
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that some of events in the CD19+ gate are pink. These are B lymphocytes that were included in the Blast gate rather than the Lymphocyte gate. Normal B lymphocytes express slightly lower density CD45 than normal T lymphocytes, so this overlap is expected. Normal B cell progenitors ("hematogones") may be also be present and might also be either pink or red depending on the gating.



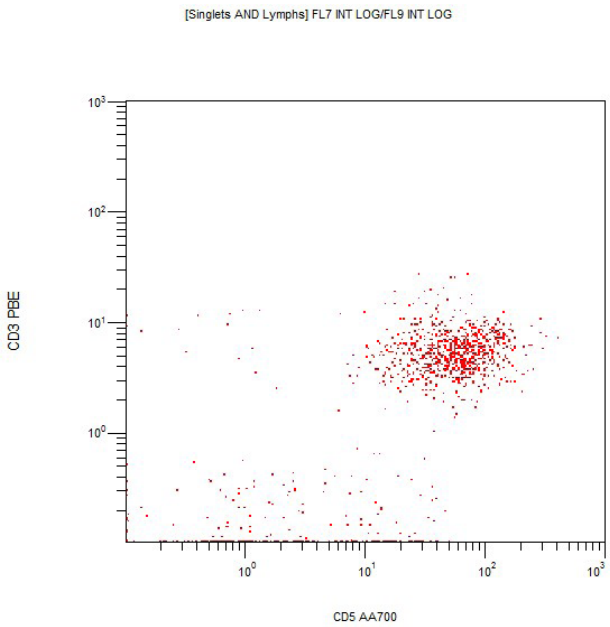
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present.



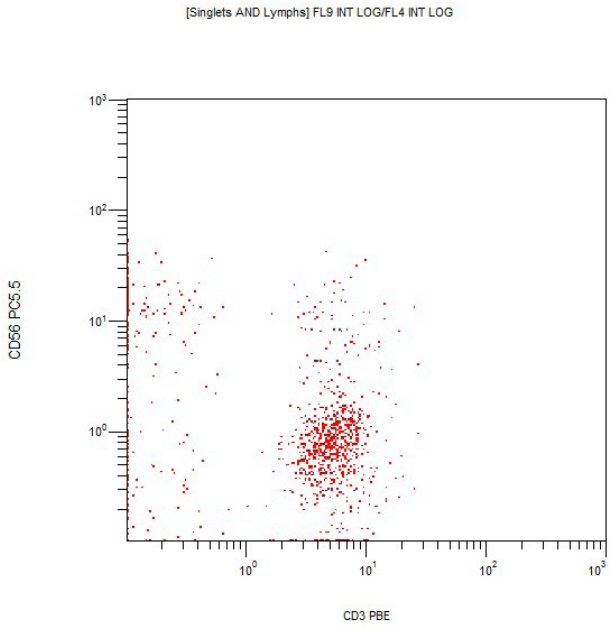
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



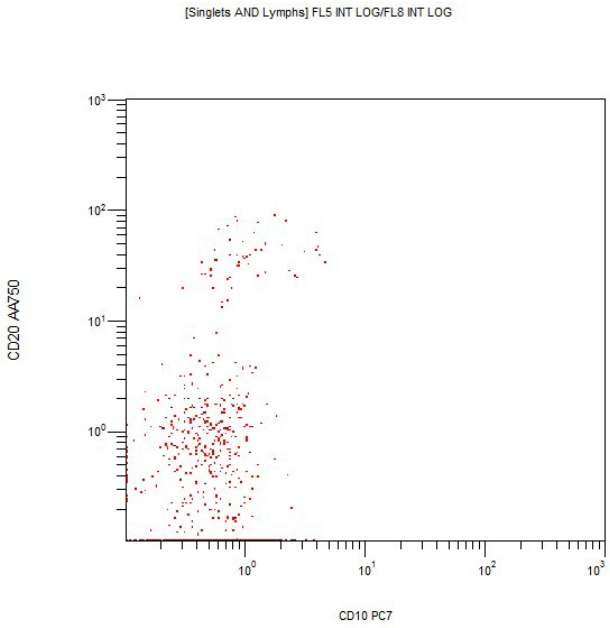
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. The mixture of pink and red events is due to the original overlap between the lymphocyte and blast gates.



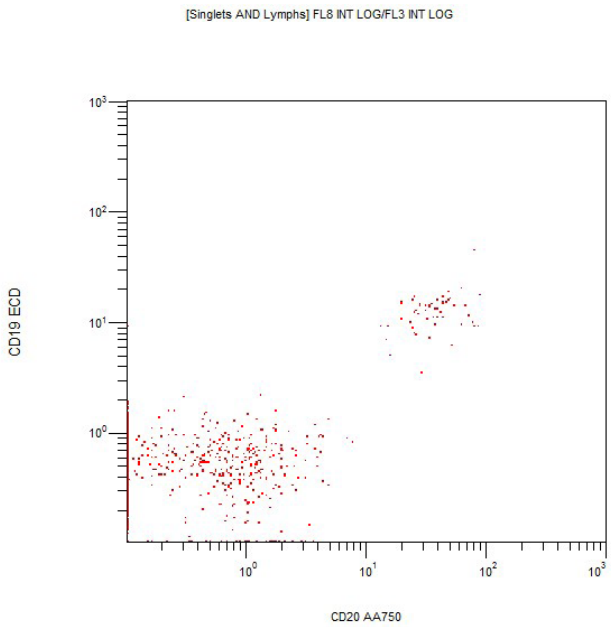
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable T lymphocytes comprise the majority of cells.



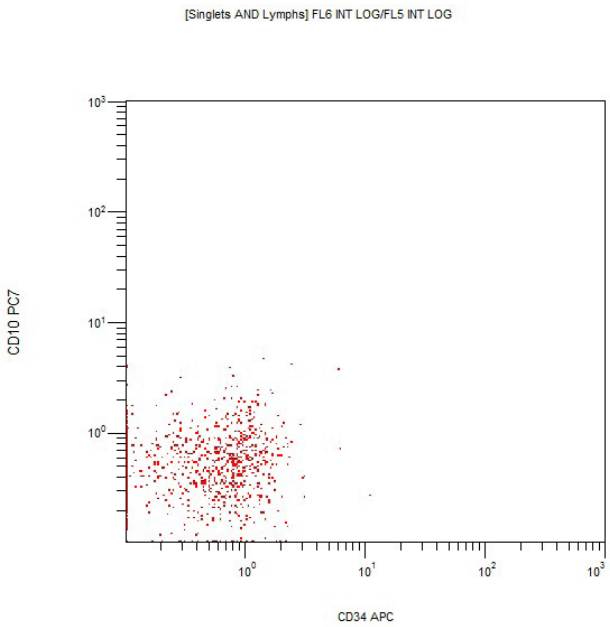
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. B lymphocytes are CD3 and CD56 dual negative.



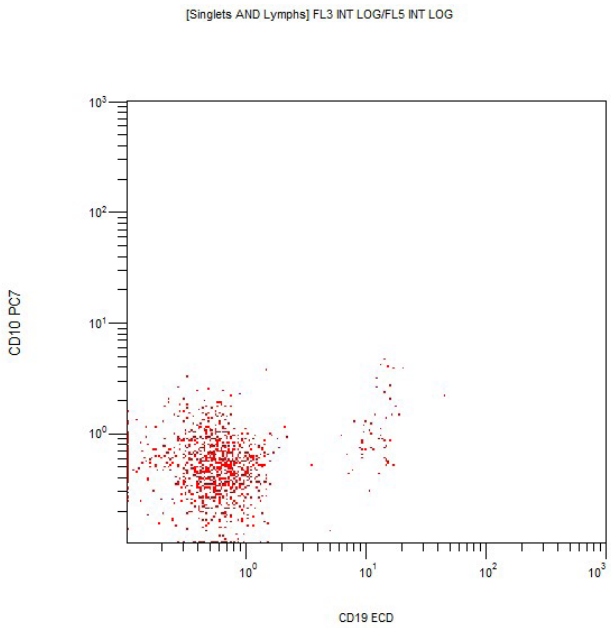
This CD10/CD20 dot plot is gated on Lymphocytes. Very few B lymphocytes are present.



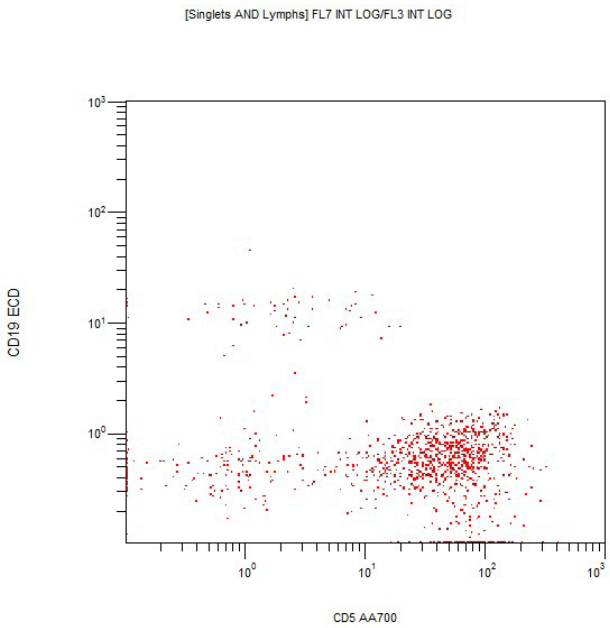
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display expected normal co-expression of CD19 and CD20.



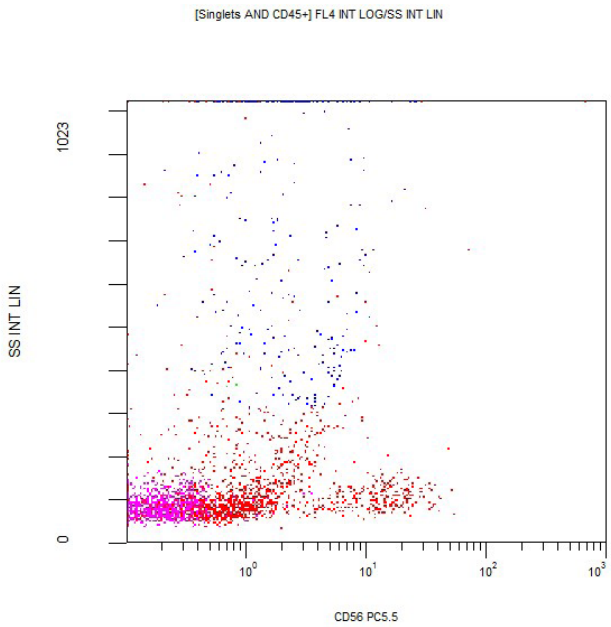
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, consistent with expected presence of mature T and B lymphocytes and NK cells.



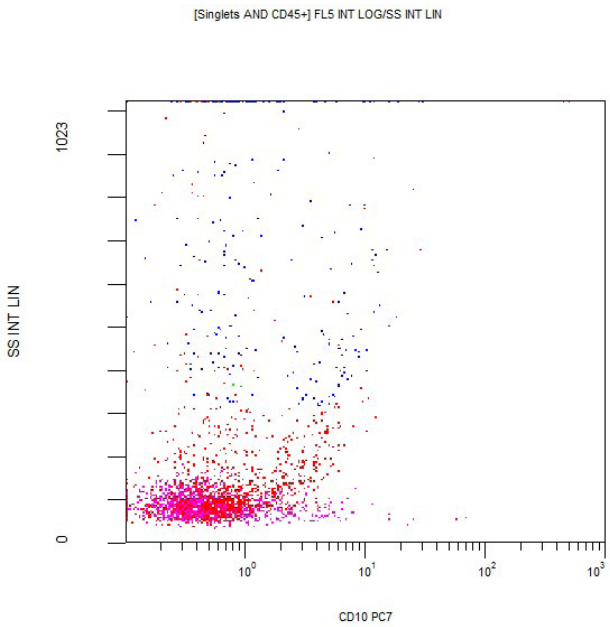
This CD19/CD10 dot plot is gated on Lymphocytes. B lymphocytes comprise the minority of lymphocytes.



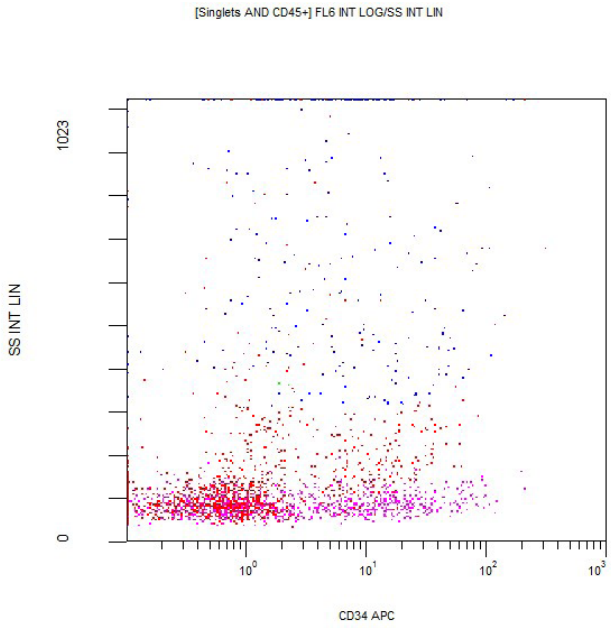
This CD5/CD19 dot plot is gated on Lymphocytes. B lymphocytes comprise the minority of lymphocytes.



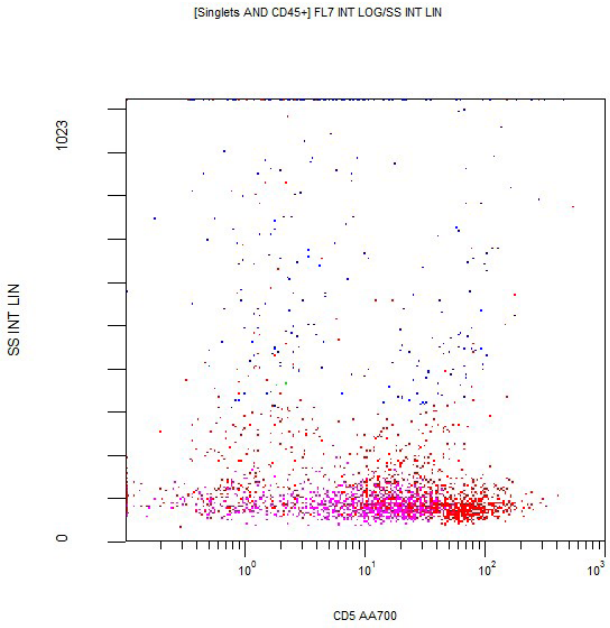
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells (red). The blasts (pink) do not express CD56.



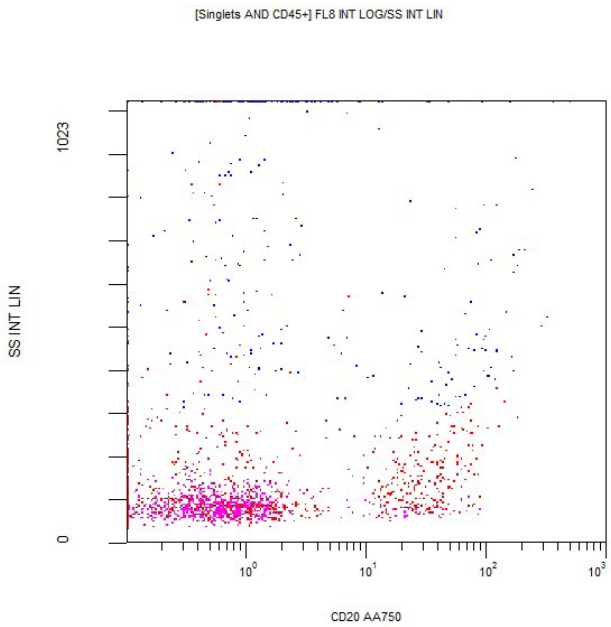
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A few of the blasts (pink) appear to express low density CD10, but the majority of the blasts are negative. At least some of the CD34 positive pink events may represent hematogones. The blue events are negative for CD10 and, as demonstrated elsewhere in this analysis, are not consistent with granulocytes.



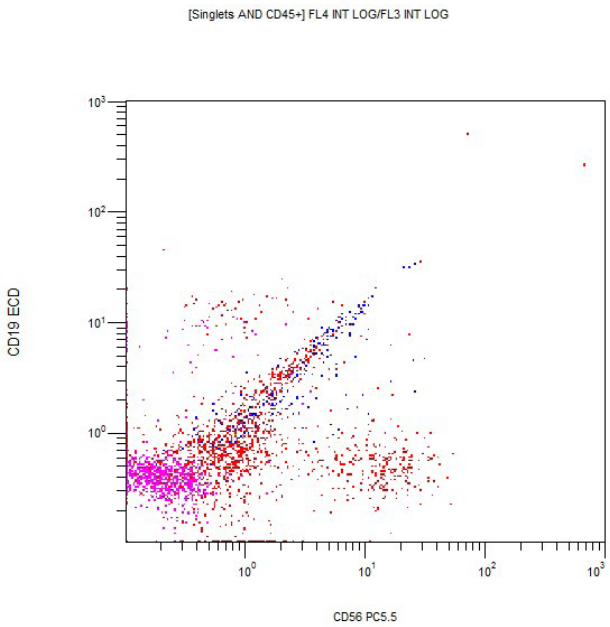
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). CD34 expression is noted by a portion of the blasts (pink).



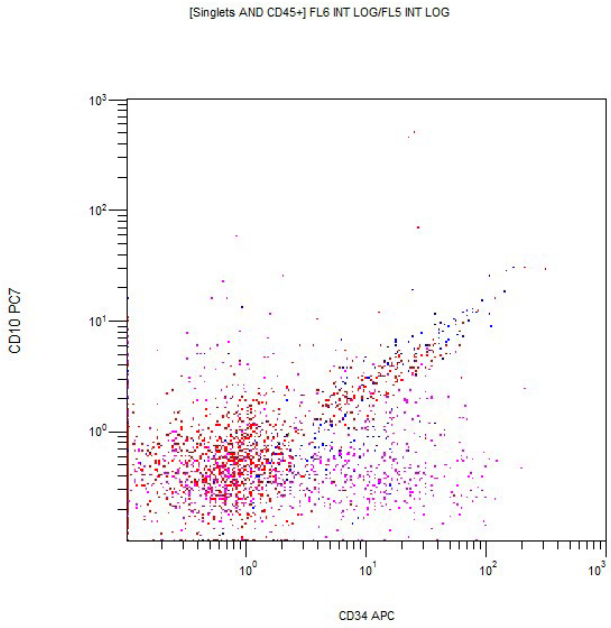
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The majority of the lymphocytes (red) express CD5, consistent with T lymphocytes. The blasts (pink) are also generally positive for CD5 but at lower density relative to the T lymphocytes.



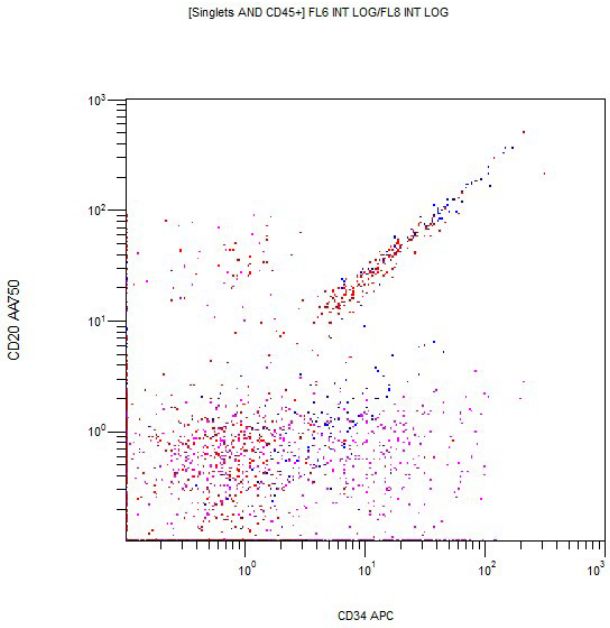
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The majority of the blasts (pink) do not express CD20, and those that do may represent hematogones.



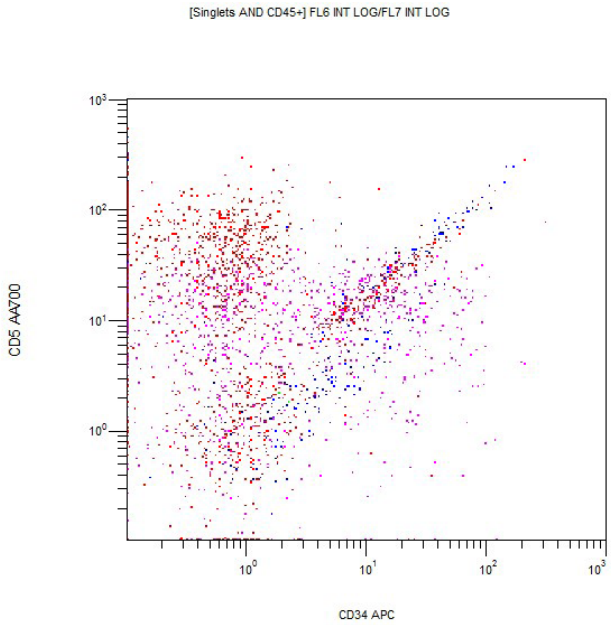
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The majority of the blasts are CD56 and CD19 dual negative, with the small subset of CD19 positive pink events being consistent with B lymphocytes and/or hematogones. NK cells (red, CD56 positive, CD19 negative) are also present. The blue and red 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 the blasts (pink) express CD34. The blue and red events noted on the diagonal are consistent with high background fluorescence.



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). As noted elsewhere the blasts (pink) express CD34. They do not express CD20. The blue and red events noted on the diagonal are consistent with high background fluorescence.



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). As noted elsewhere, both the T lymphocytes (red) and the blasts (pink) are positive for CD5, with a portion of the blasts co-expressing CD34. The blue and red events noted on the diagonal are consistent with high background fluorescence.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a small but phenotypically distinct population of cells that express CD34, partial CD5, and low density CD45. These cells fail to express surface CD3, CD19, CD20, and CD10. In order to assess expression of CD4 and CD8 by these cells, additional dot plots may be analyzed.

Taken together, the results are suspicious for acute leukemia. Diagnostic considerations include acute myeloid leukemia as well as T acute lymphoblastic leukemia/lymphoblastic lymphoma (T ALL), with T ALL being favored by the CD45 antigen density and light scatter properties. Additional flow cytometric immunophenotyping of this sample (data not shown) confirms an immunophenotype consistent with T ALL. Correlation with clinical and laboratory data is also recommended---note that the presence of a mediastinal mass in this patient also supports a diagnosis of T ALL.

T LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

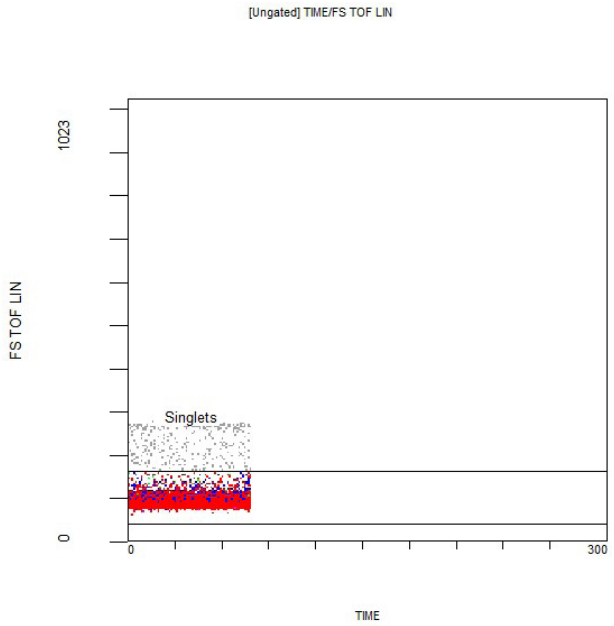
Case #10: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

Clinical Vignette

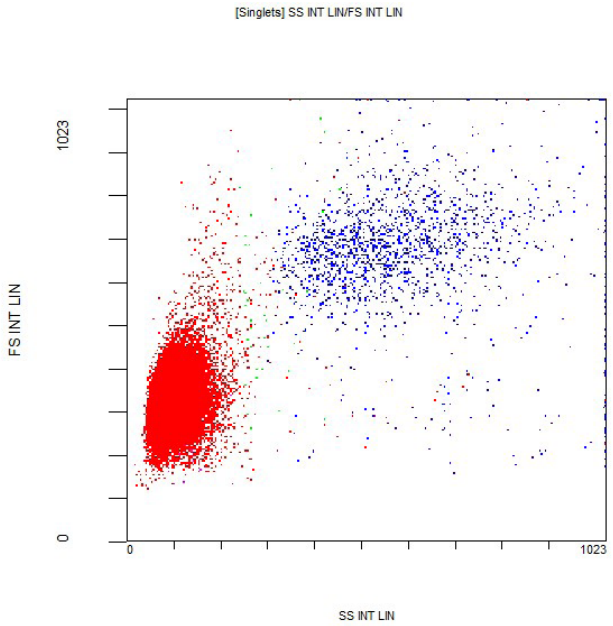
This 28-year-old male with presents with anemia and lymphadenopathy. Microscopic examination of a peripheral blood smear demonstrates a predominance of small mononuclear cells. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

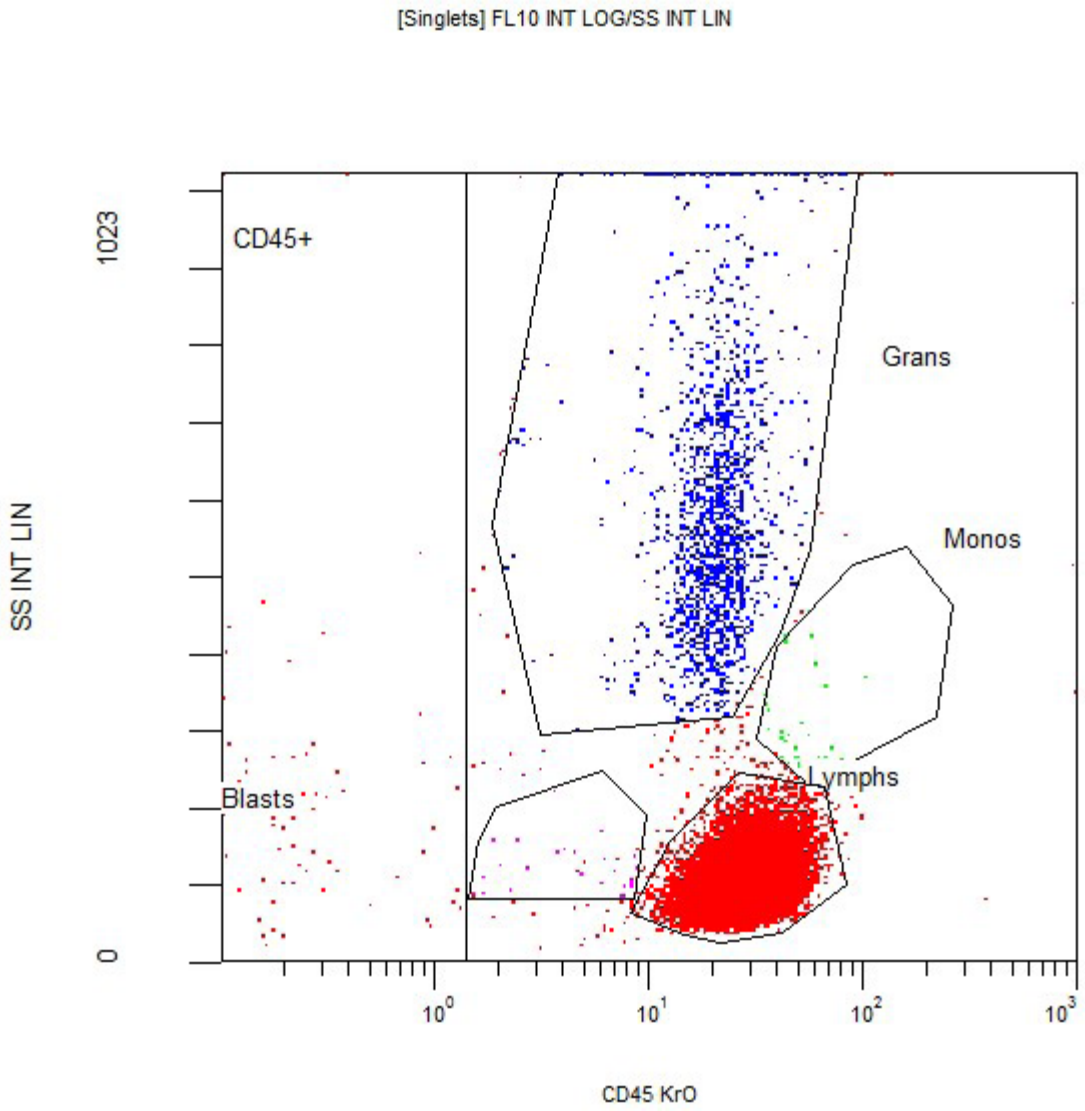
Access Case #10 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

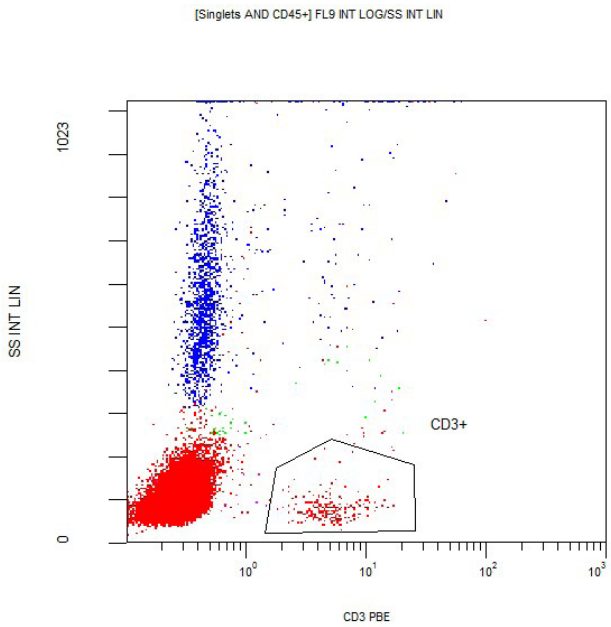


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of small mononuclear cells. Apparent granulocytes are also noted.



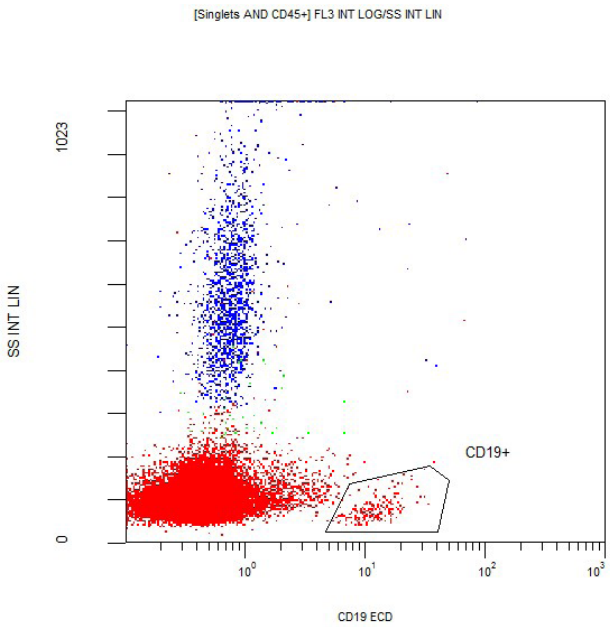
This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). The "Blast" gate (pink) contains only a few cells. Note that the label for the Blast gate is not immediately adjacent to it---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.

Note that the minimal overlap among the various populations in this particular case renders it easy to distinguish among them by adjusting the gates to conform to the naturally occurring separations among the populations. Remember, however, that populations that overlap in CD45 antigen density and light scatter cannot be distinguished.



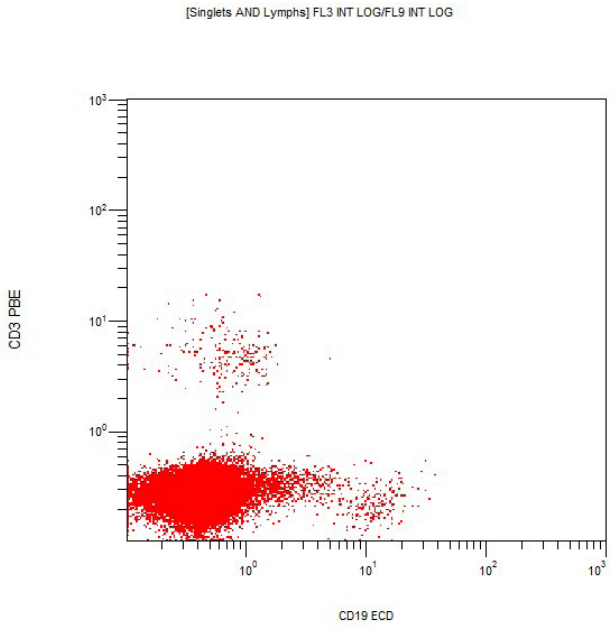
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.

Note that the majority of the red events are negative for (surface) CD3.

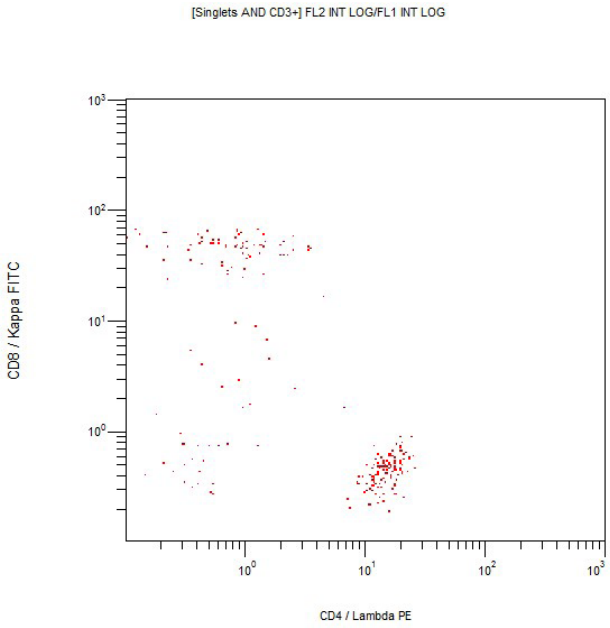


This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate should be adjusted to include apparent B lymphocytes only.

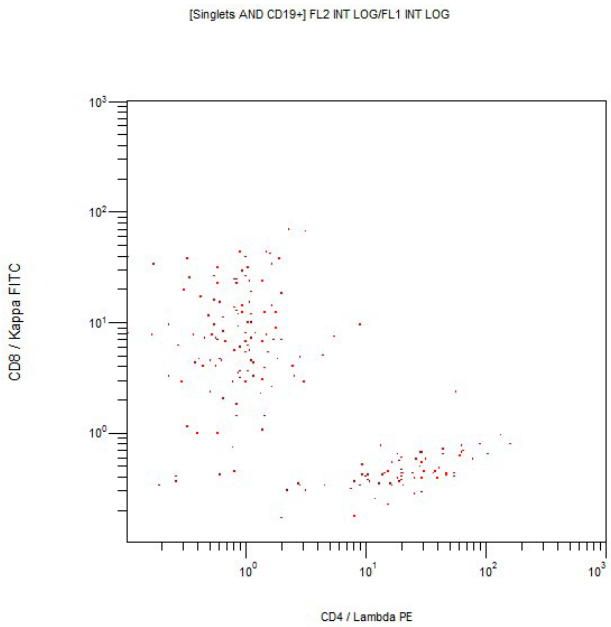
Note that the majority of the red events are negative for CD19.



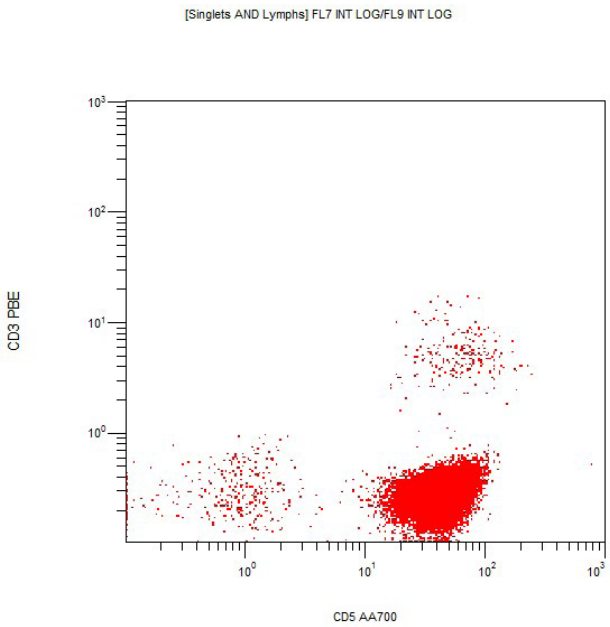
This CD19/CD3 dot plot gated on Lymphocytes confirms the presence of small numbers of B and T lymphocytes in this sample. The CD19 negative, CD3 negative cells comprise the majority of the cells in this gate.



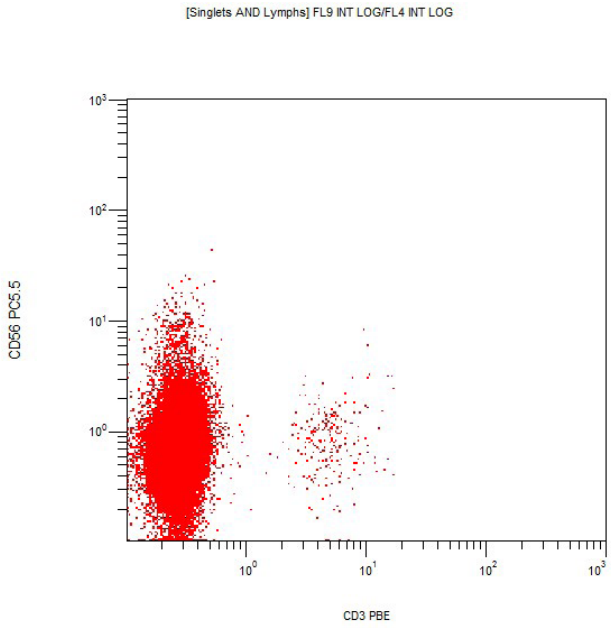
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



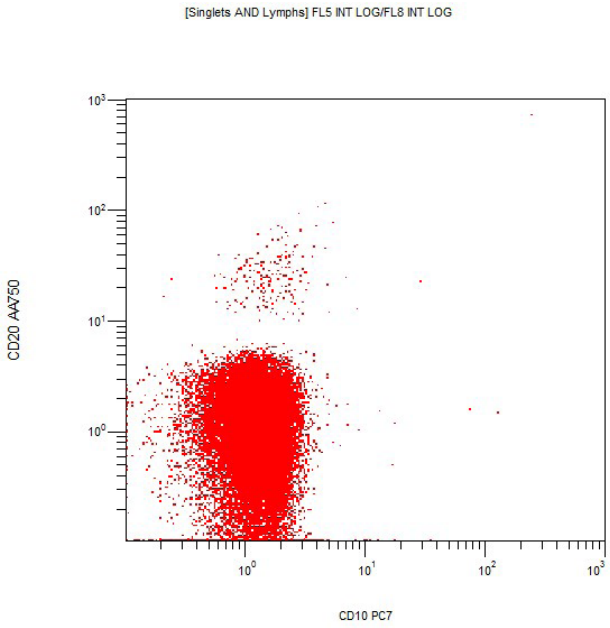
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations.



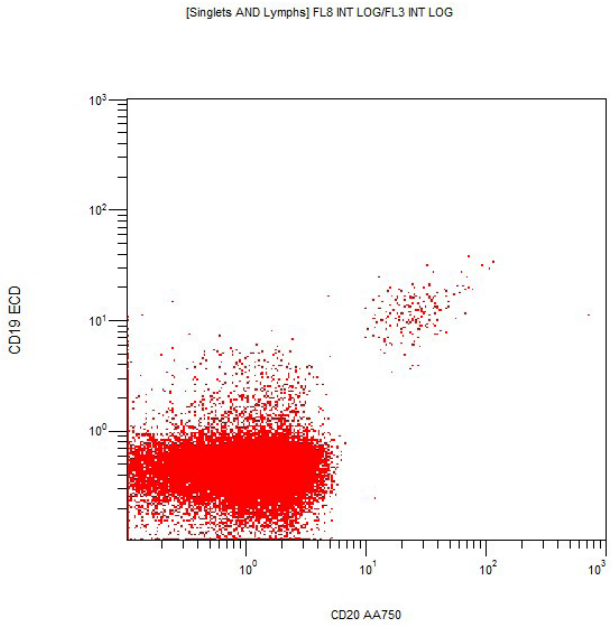
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable CD3 and CD5 dual positive T lymphocytes are present, but majority of cells are CD5 positive, CD3 negative. Note that the CD5 antigen density on the aberrant population is nearly as high as that of the normal T lymphocytes.



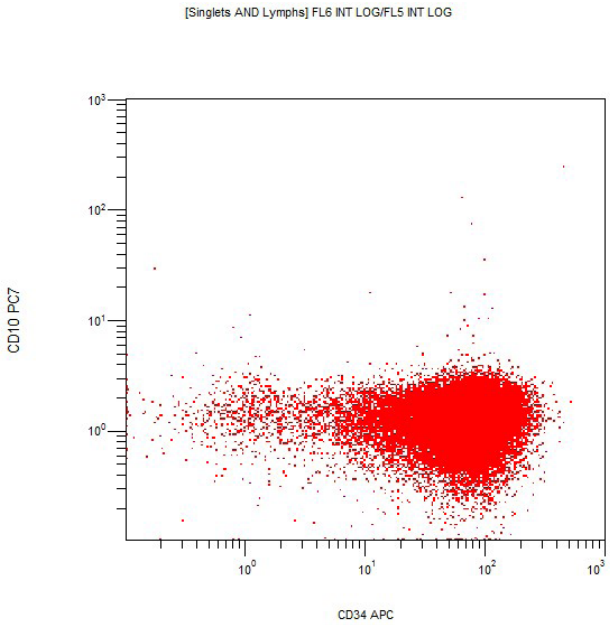
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. The majority of the events are CD3 and CD56 dual negative.



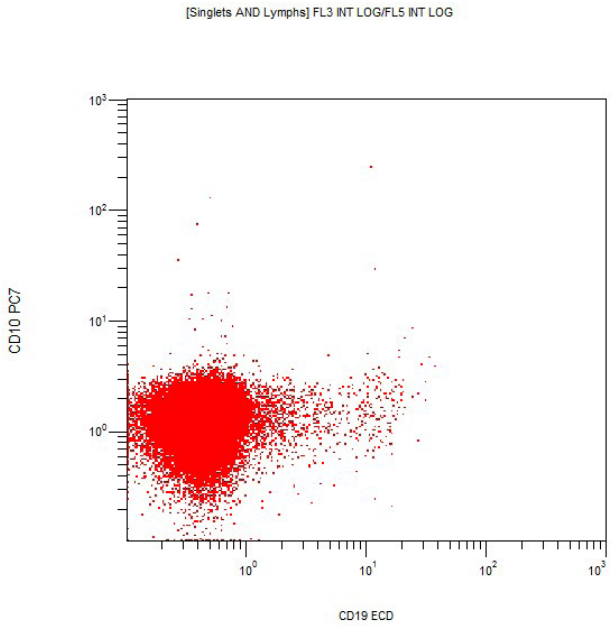
This CD10/CD20 dot plot is gated on Lymphocytes. A small number of B lymphocytes is present. The aberrant population is negative for both CD10 and CD20.



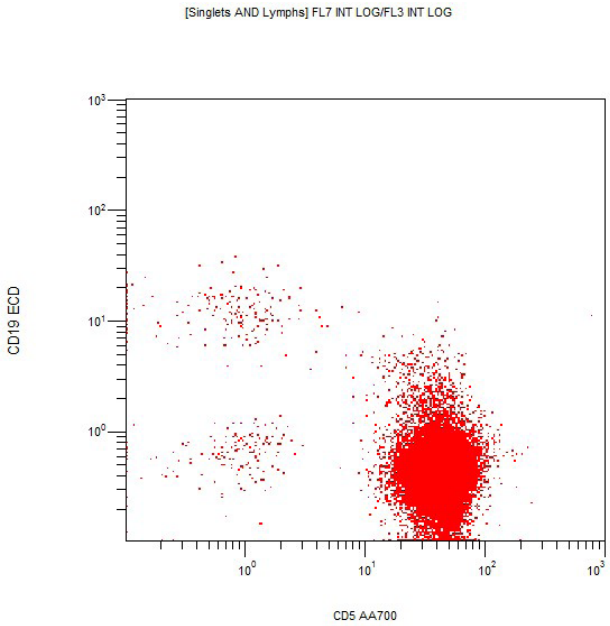
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display expected normal co-expression of CD19 and CD20. The aberrant population is negative for both CD19 and CD20.



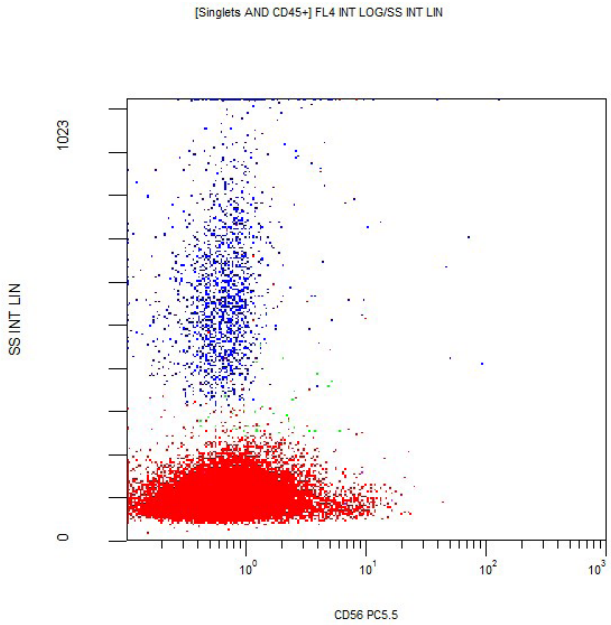
This CD34/CD10 dot plot is gated on Lymphocytes. The majority of events, however, express high density CD34, indicating that they are not, in fact, lymphocytes but instead represent blasts. Although blasts are generally colored pink in ClearLLab LS analysis, the overlap in CD45 antigen density and light scatter properties between normal lymphocytes and the aberrant population in this case results in their being included in the Lymphocytes gate.



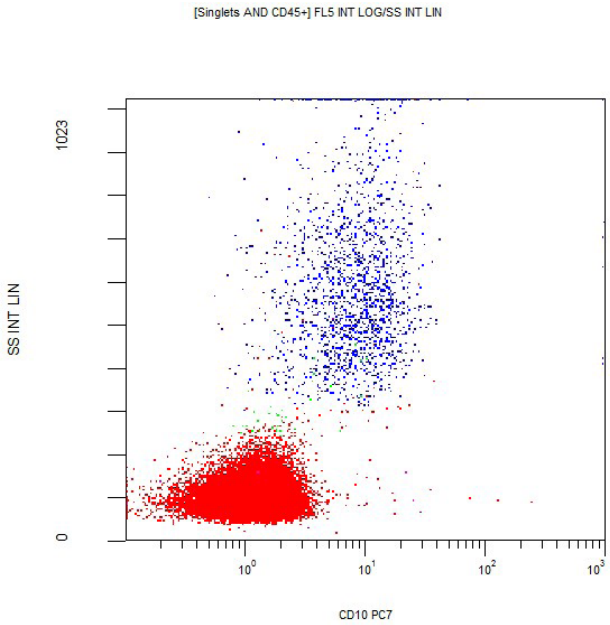
This CD19/CD10 dot plot is gated on Lymphocytes. A small number of B lymphocytes is present in this sample. The aberrant population is negative for both CD19 and CD10.



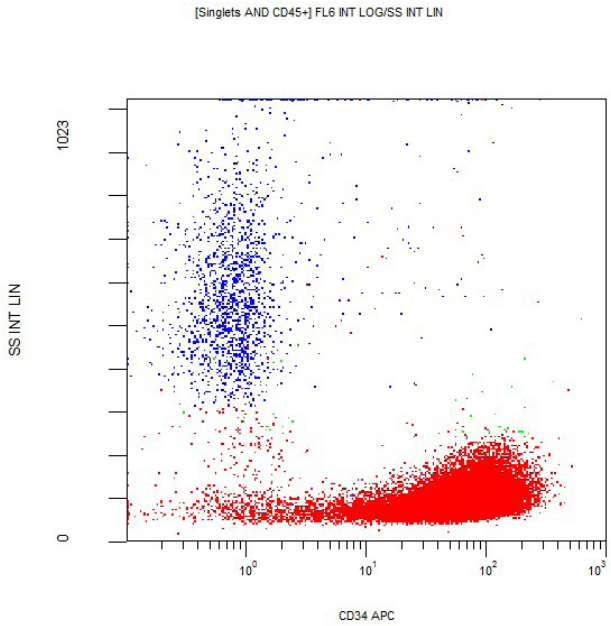
This CD5/CD19 dot plot is gated on Lymphocytes. Normal B lymphocytes are present. The presence of normal T lymphocytes is obscured by the overlapping aberrant population which displays similarly high density CD5 expression.



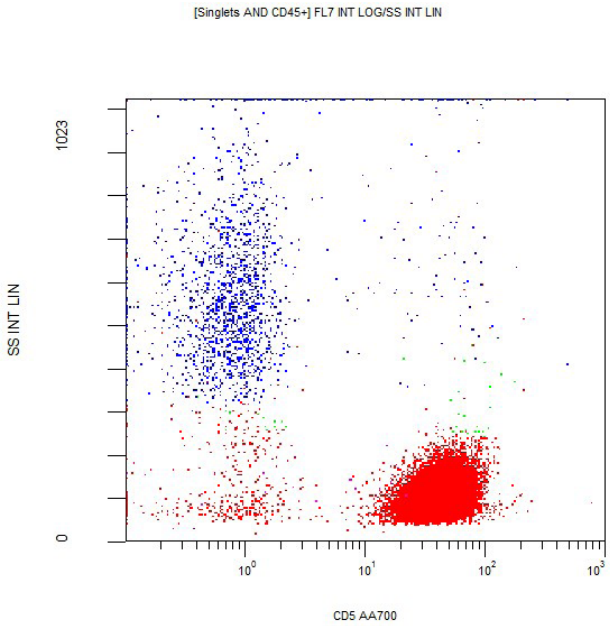
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells. The blasts (normally colored pink but in this case colored red) do not express CD56.



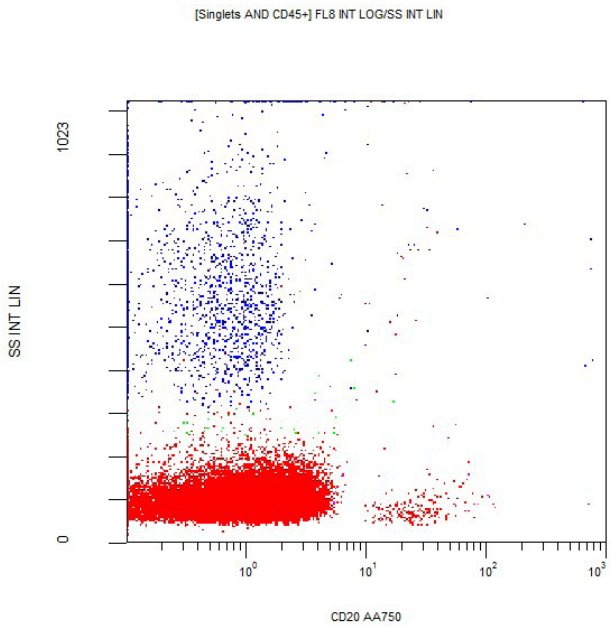
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are positive for CD10. Blasts in this case are colored red, and they are negative for CD10.



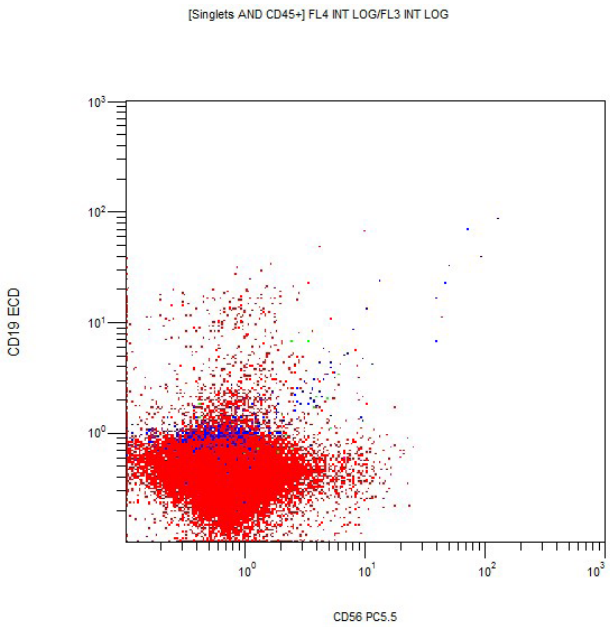
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (normally colored pink but colored red in this case) express CD34.



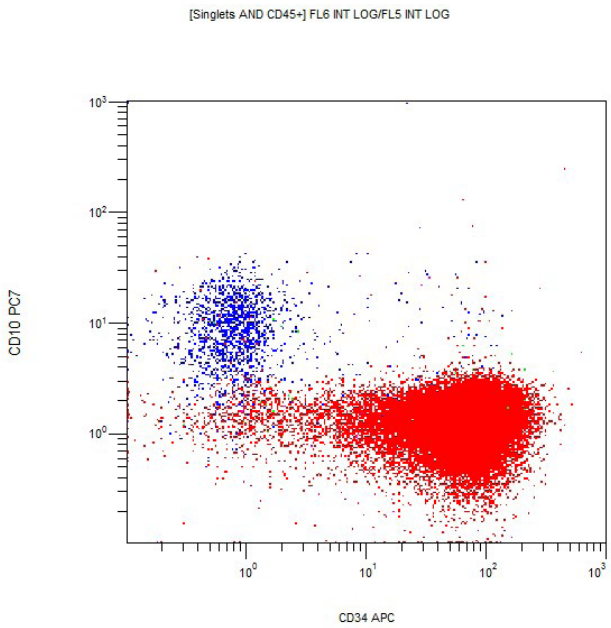
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (normally colored pink but colored red in this case) express high density CD5.



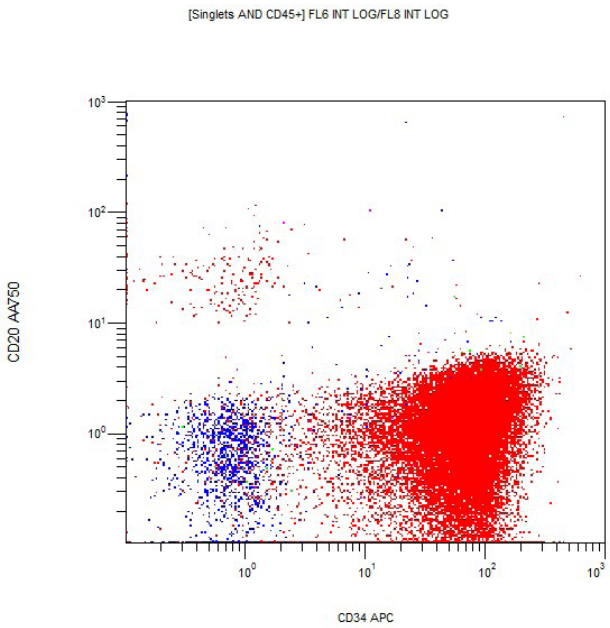
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (normally colored pink but colored red in this case) do not express CD20. A small population of normal B lymphocytes is present in this sample.



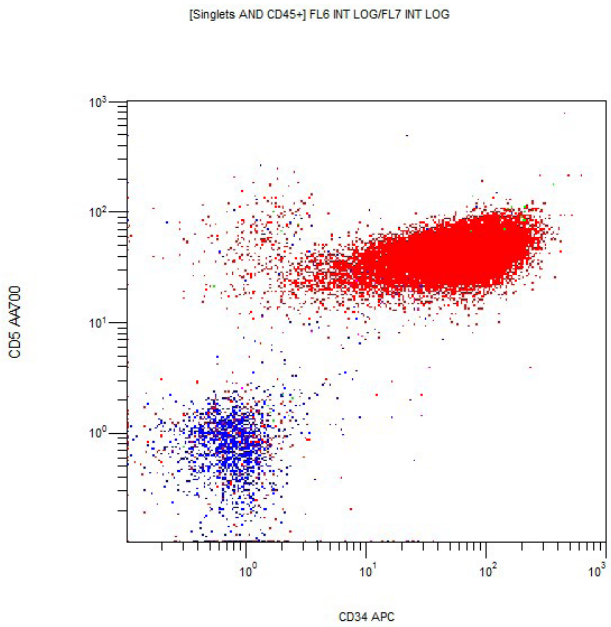
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (normally colored pink but colored red in this case) do not express either CD56 or CD19.



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. The blasts (normally colored pink but colored red in this case) express high density CD34.



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). As noted elsewhere the blasts (normally colored pink but colored red in this case) express CD34.



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). In this display it is possible to separate the small number of CD5 positive, CD34 negative T lymphocytes from the large number of CD5 and CD34 dual positive blasts (normally colored pink but colored red in this case).

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with low light scatter properties that express high density CD34, high density CD5, and CD45. Note that the CD45 expression is much higher density than is typically found together with CD34 expression, resulting in overlap between the aberrant population and normal lymphocytes. These cells fail to express surface CD3, CD19, CD20, CD56, and CD10. In order to assess expression of CD4 and CD8 by these cells, additional dot plots may be analyzed.

Taken together, the results are consistent with acute leukemia. Diagnostic considerations include acute myeloid leukemia as well as T acute lymphoblastic leukemia/lymphoblastic lymphoma (T ALL), with T ALL being favored by the CD45 and CD5 antigen density and light scatter properties. Additional flow cytometric immunophenotyping of this sample (data not shown) confirms an immunophenotype consistent with T ALL. Correlation with clinical and laboratory data is also recommended.

T LARGE GRANULAR LYMPHOMA

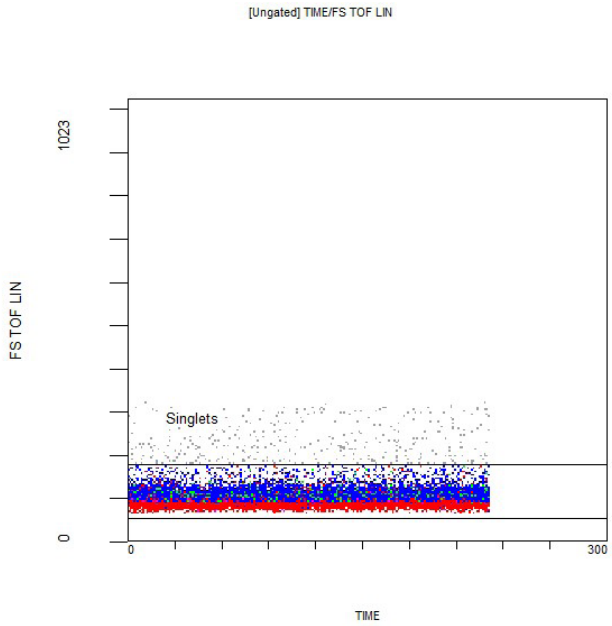
Case #11: T Cell Large Granulocytic Lymphocytic Leukemia

Clinical Vignette

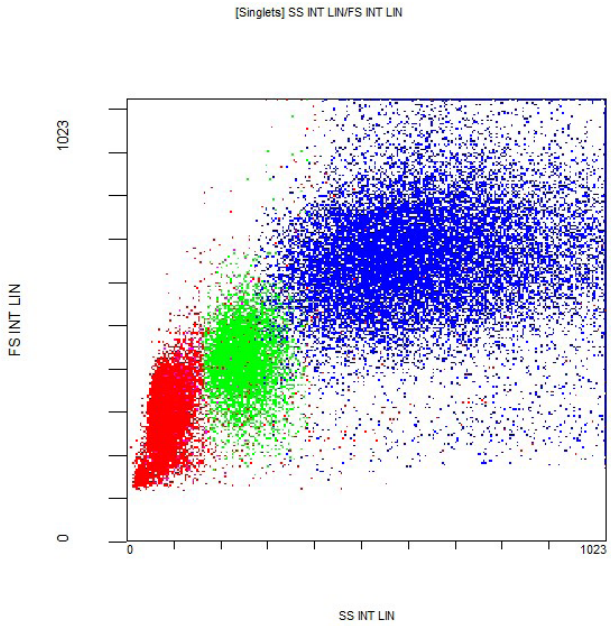
This 51-year-old female presents with lymphocytosis. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

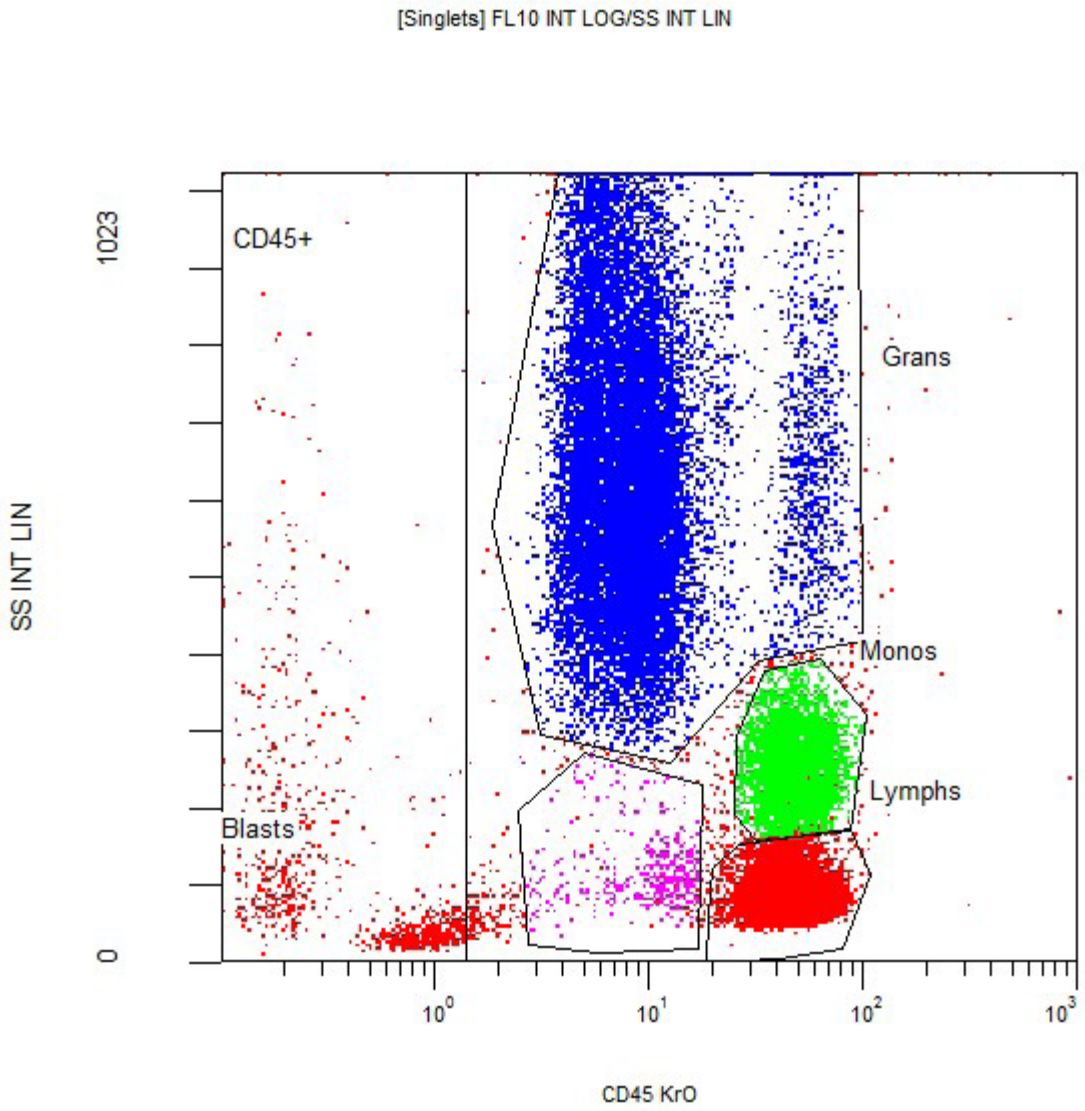
Access Case #11 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

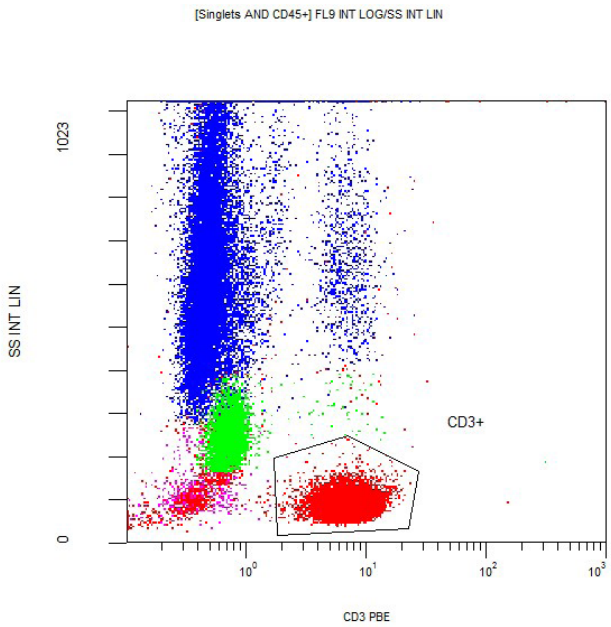


This Side Scatter/Forward Scatter dot plot demonstrates lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink).

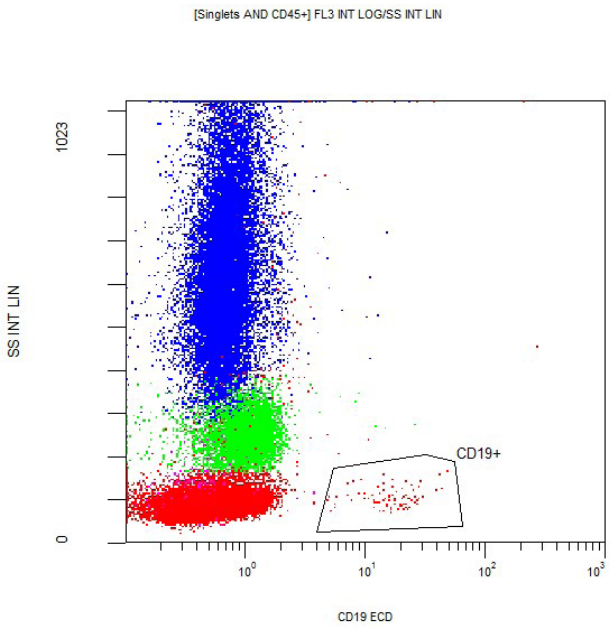


This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). The "Blast" gate contains a small cluster of events. Note that 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.

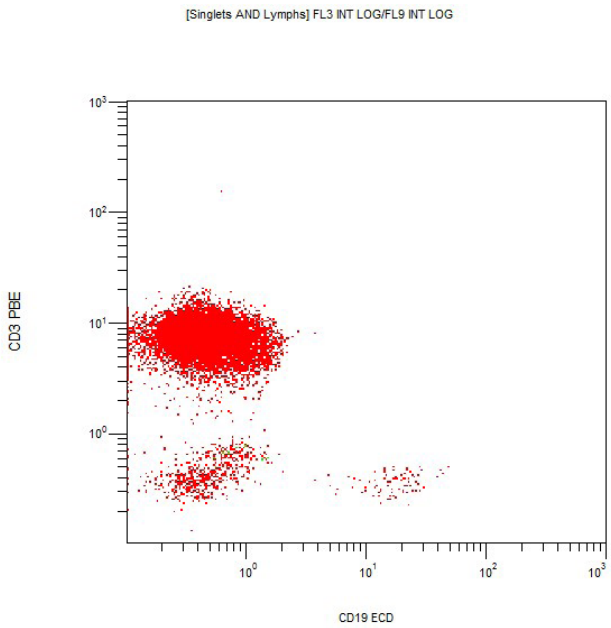
Note the mild degree of overlap in this case between apparent monocytes and lymphocytes. The gates should be adjusted such that they conform to the naturally occurring separations among the populations, but where no separation is observed an estimate based on experience may be used.



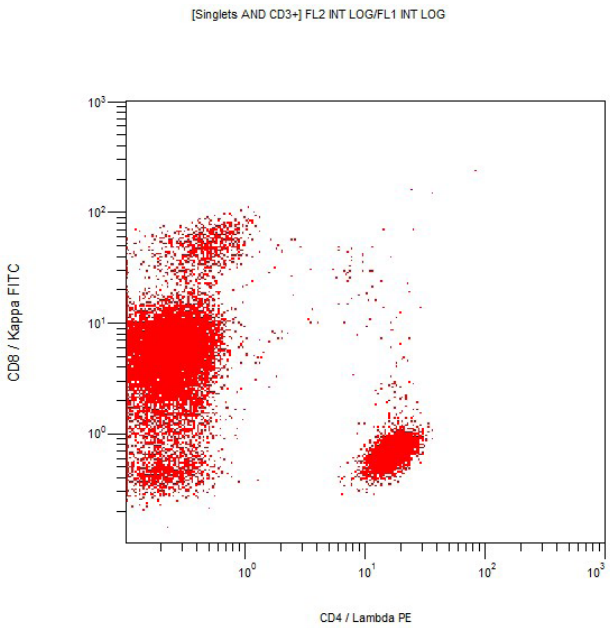
This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.



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.

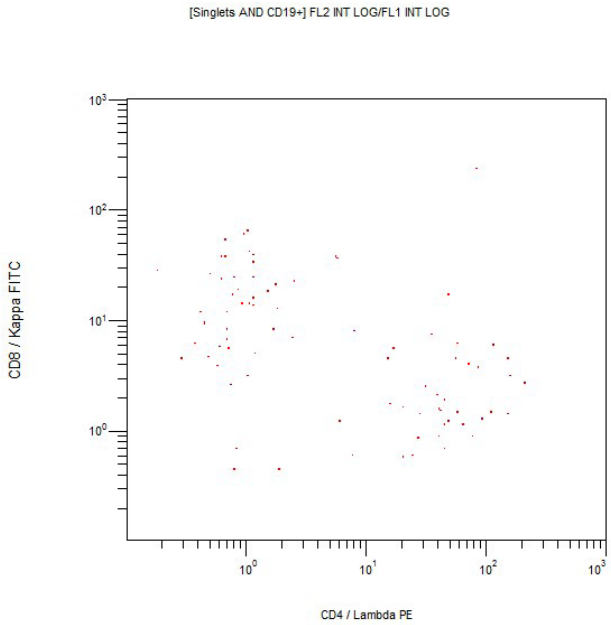


This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present.

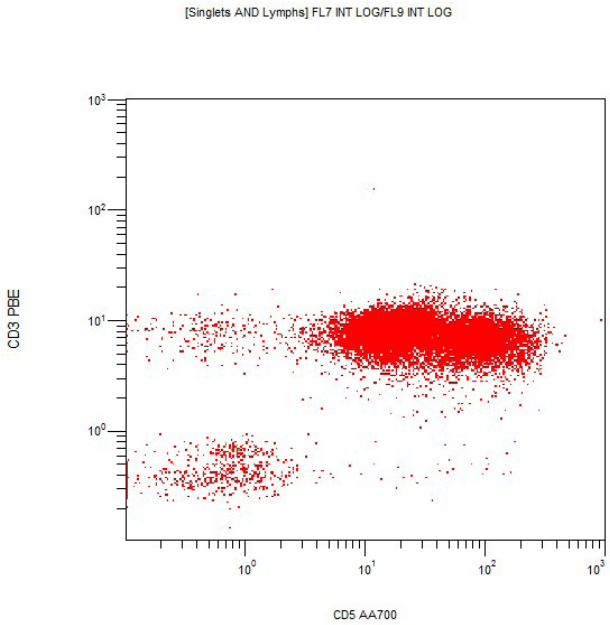


Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.

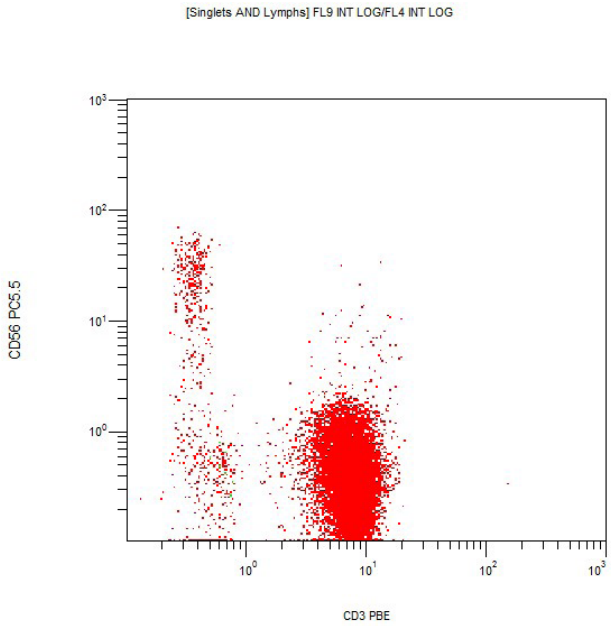
In addition to the expected populations, a predominant CD8 positive, CD4 negative population is present. Note that the CD8 density for this aberrant population is lower than that of the normal CD8 positive T lymphocytes.



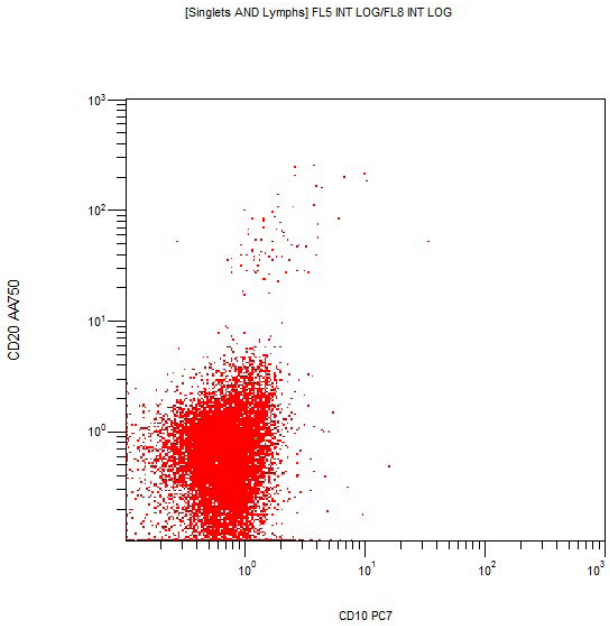
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations.



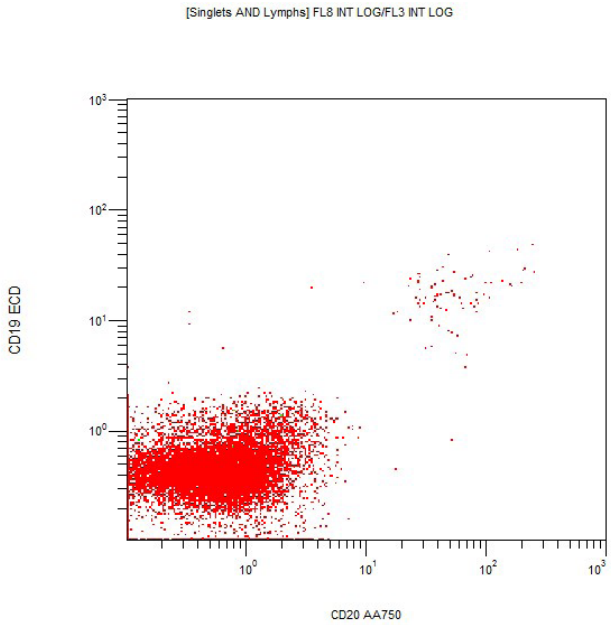
This CD5/CD3 dot plot is gated on Lymphocytes. Two populations of CD3 positive, CD5 positive T lymphocytes can be seen, with the higher density CD5 corresponding to normal cells and the lower density CD5 corresponding to the aberrant population.



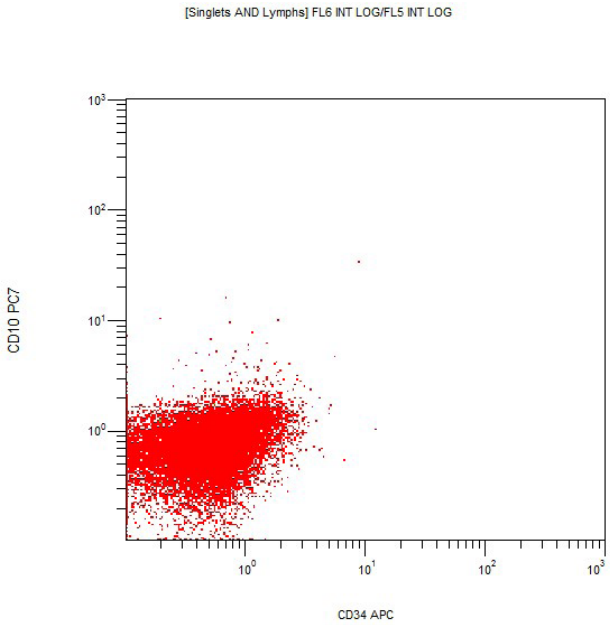
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. The normal and aberrant T lymphocyte populations are CD3 positive and indistinguishable based on CD3 antigen density.



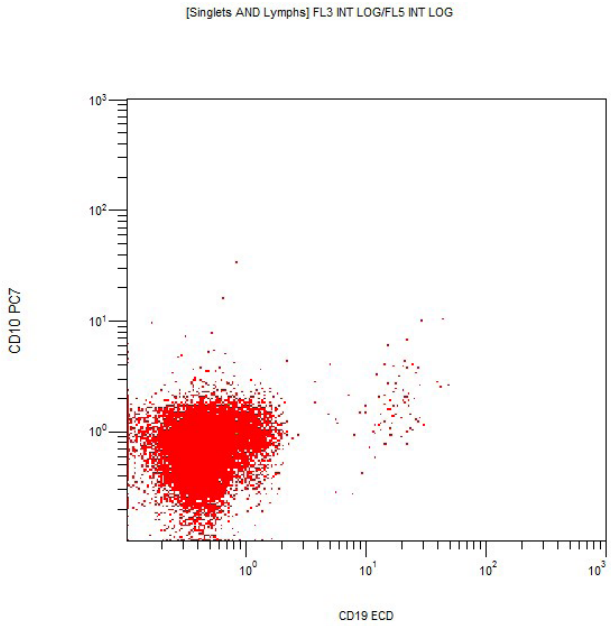
This CD10/CD20 dot plot is gated on Lymphocytes. A small population of unremarkable B lymphocytes is present.



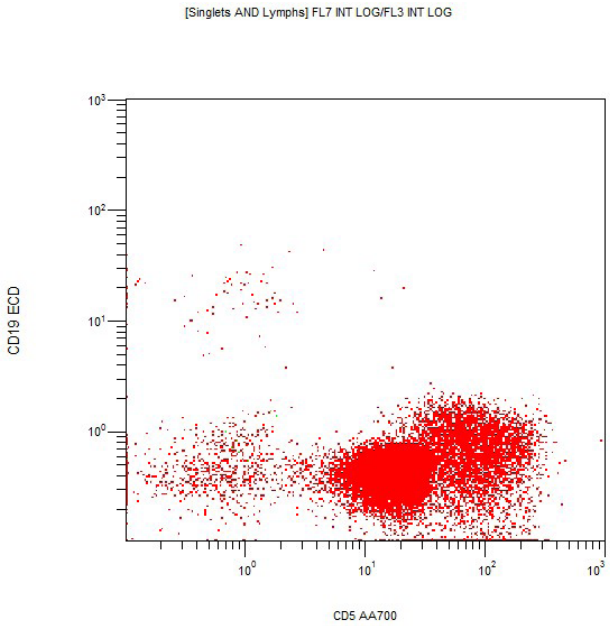
This CD20/CD19 dot plot is gated on Lymphocytes. A small population of unremarkable B lymphocytes is present.



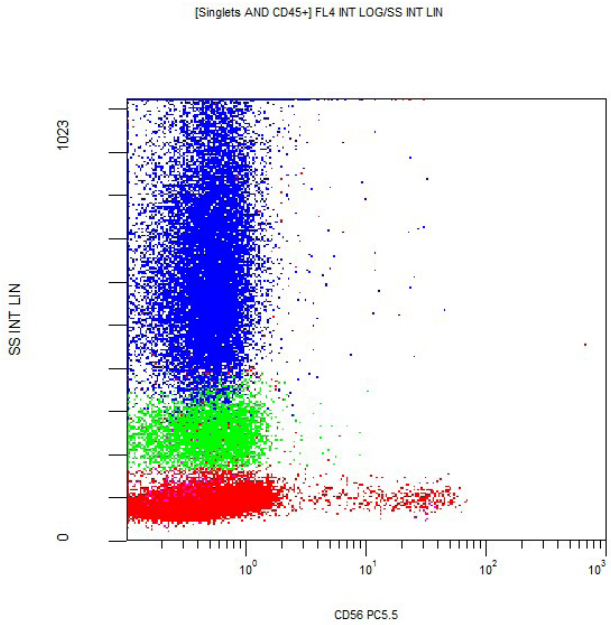
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, as expected for both normal mature peripheral blood lymphocytes as well as mature lymphoproliferative disorders.



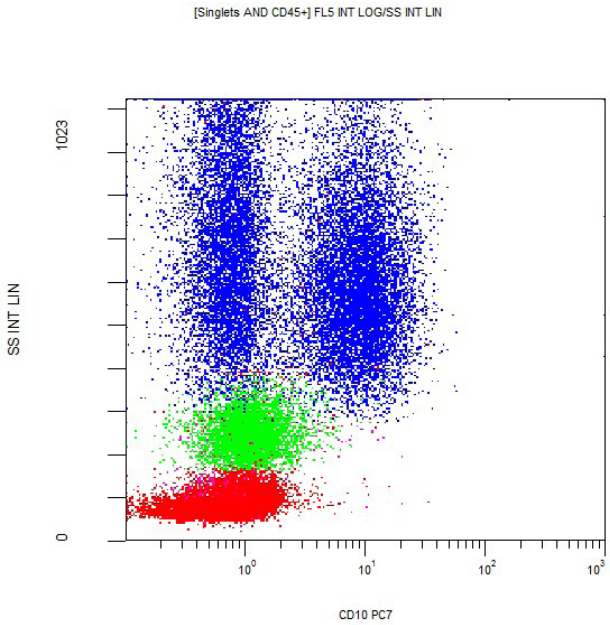
This CD19/CD10 dot plot is gated on Lymphocytes. A small population of unremarkable B lymphocytes is present.



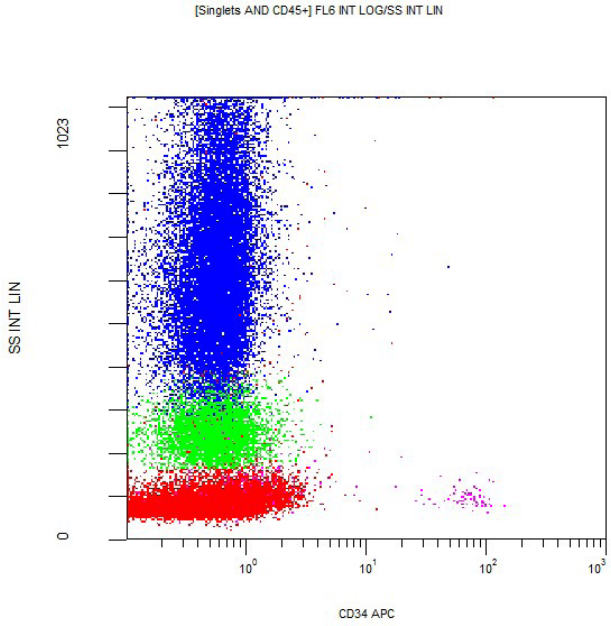
This CD5/CD19 dot plot is gated on Lymphocytes. Two populations of CD5 positive T lymphocytes can be seen, with the higher density CD5 corresponding to normal cells and the lower density CD5 corresponding to the aberrant population.



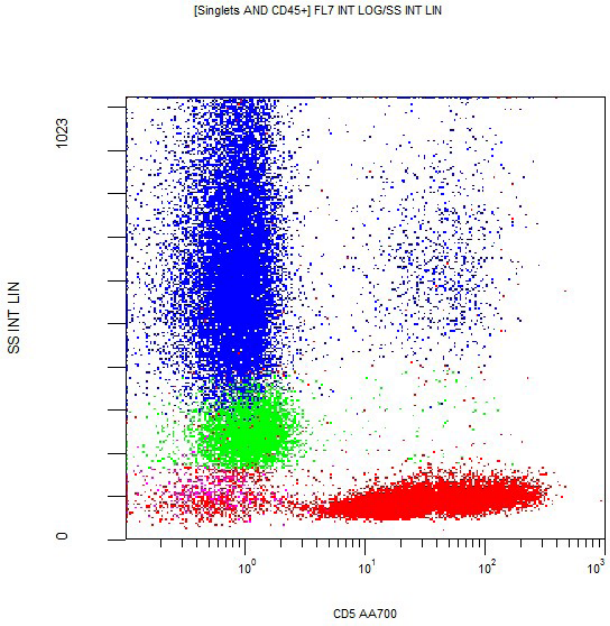
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells.



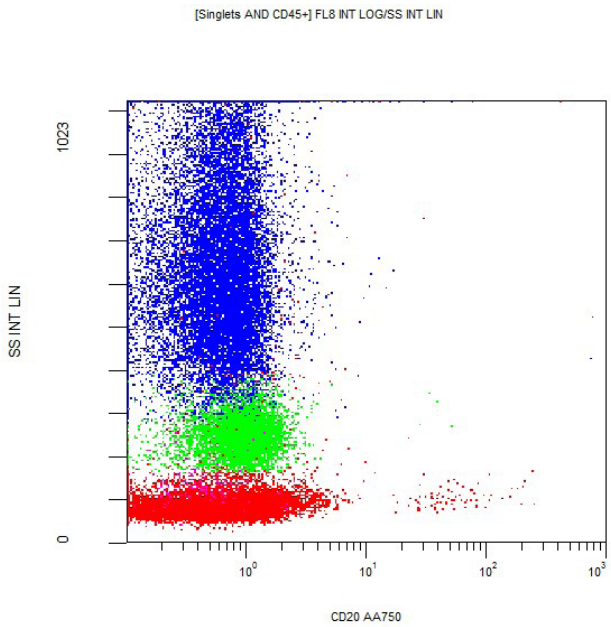
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Granulocytes (blue) are positive for CD10.



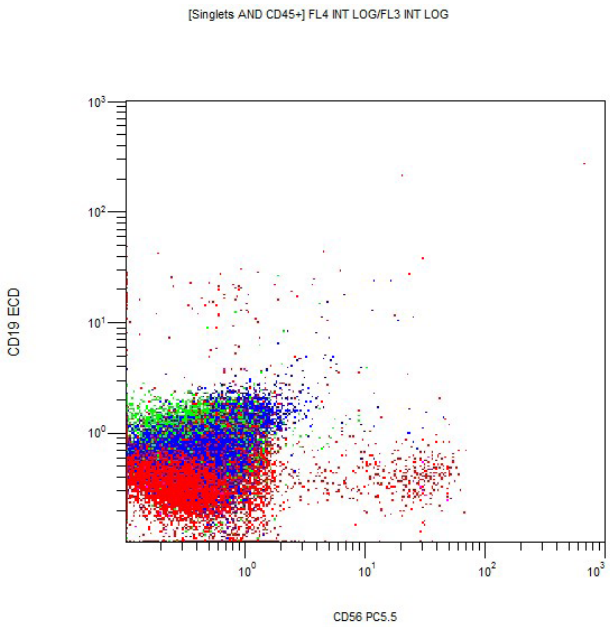
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A small population of CD34 positive blasts (pink) is noted. Although this sample is peripheral blood, this population is not necessarily aberrant---peripheral blood stem cells are a normal finding and may be increased under a number of conditions.



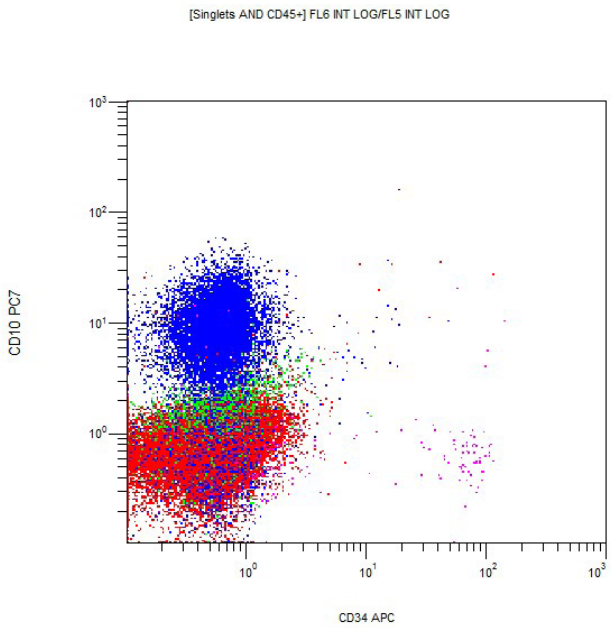
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The CD5 positive events depicted here include both normal T lymphocytes and the aberrant T lymphocyte population. Two populations can be discerned, with the higher density CD5 population being normal T lymphocytes and the lower density CD5 population being the aberrant T lymphocyte population.



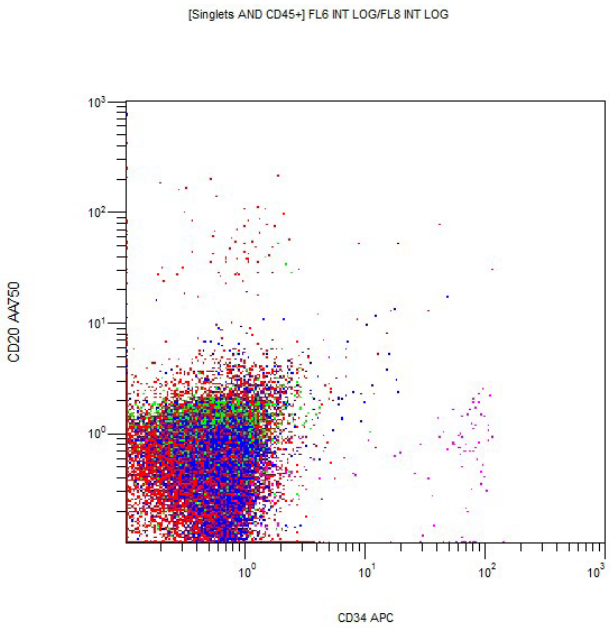
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A small population of unremarkable B lymphocytes is present.



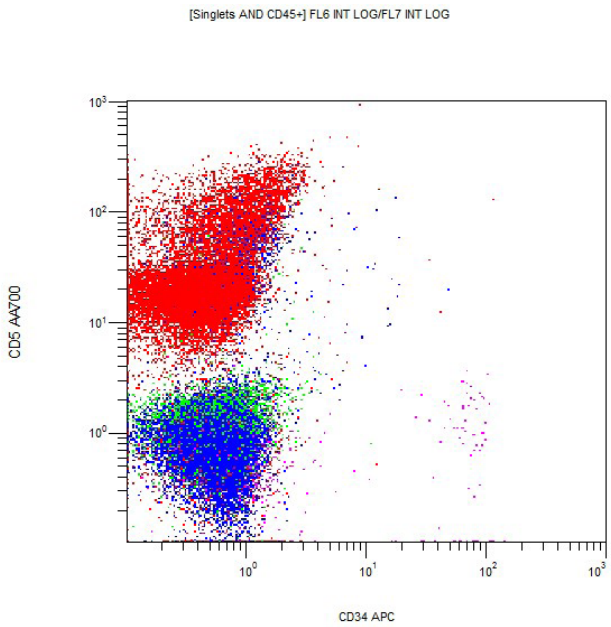
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Both B lymphocytes and NK cells are present.



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. The blasts (pink) are CD34 positive, consistent with circulating peripheral blood stem cells.



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. Small numbers of normal CD20 positive B lymphocytes and normal CD34 positive peripheral blood stem cells are present.



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). The higher density CD5 T lymphocytes and lower density CD5 aberrant T lymphocytes are apparent here, as are a small number of CD34 positive peripheral blood stem cells.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with low light scatter properties that express CD3, low density CD5, low density CD8, and CD45. These cells fail to express CD56.

Taken together, the findings in this case are most consistent with a T cell lymphoproliferative disorder. Note that correlation with clinical and laboratory data is recommended. The final diagnosis, taking all data into account (not shown), is T cell large granular lymphocytic leukemia.

NEOPLASTIC PROCESS OF MYELOID ORIGIN

Myeloid malignancies are clonal diseases of hematopoietic stem or progenitor cells. These malignancies can be present in the bone marrow and peripheral blood. Some myeloid disorders, such as the myeloid leukemias, have long been considered malignant while other myeloid disorders have been considered non-malignant or pre-leukemia blood disorders which may become malignant over time. Based on the morphology, cytochemistry, immunophenotype, genetics, and clinical features of myeloid disorders, the World Health Organization (WHO) categorizes myeloid malignancies into five primary types: (1) acute myeloid leukemia; (2) myelodysplastic syndromes (MDS); (3) myeloproliferative neoplasms (MPN); (4) myelodysplastic and myeloproliferative (MDS/MPN) neoplasms; and (5) myeloid neoplasms associated with eosinophilia and abnormalities of growth factor receptors derived from platelets or fibroblasts.

ACUTE MYELOID LEUKEMIA, NOT OTHERWISE SPECIFIED

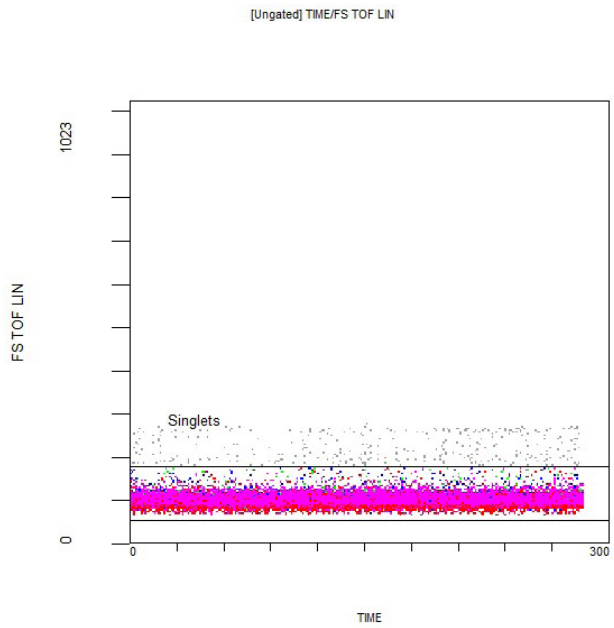
Case #12: Acute Myeloid Leukemia Not Otherwise Specified

Clinical Vignette

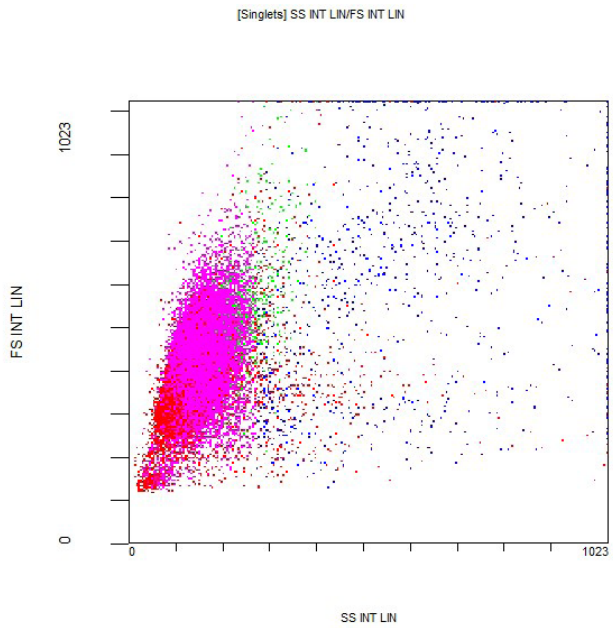
This 46-year-old female presents with anemia, thrombocytopenia, and neutropenia. Circulating atypical mononuclear cells are noted on microscopic examination. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

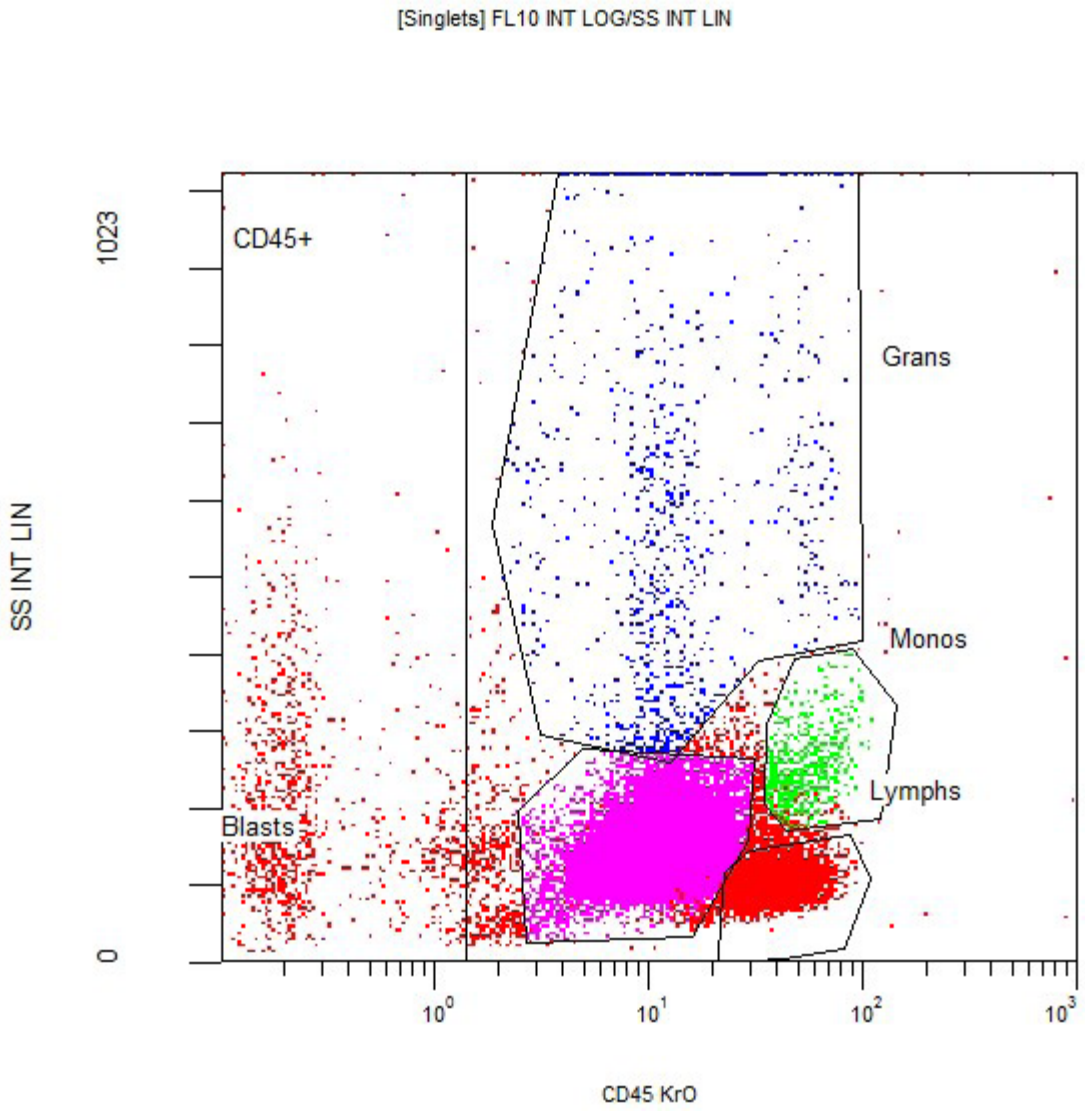
Access Case #12 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

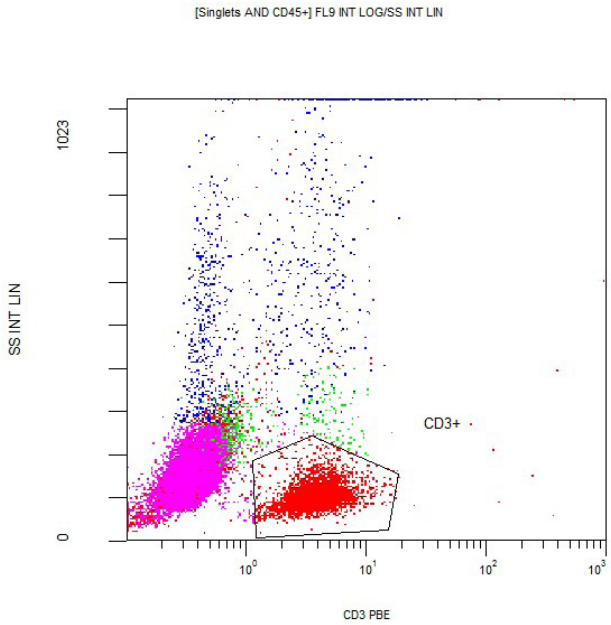


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of mononuclear cells and a relative paucity of apparent granulocytes.



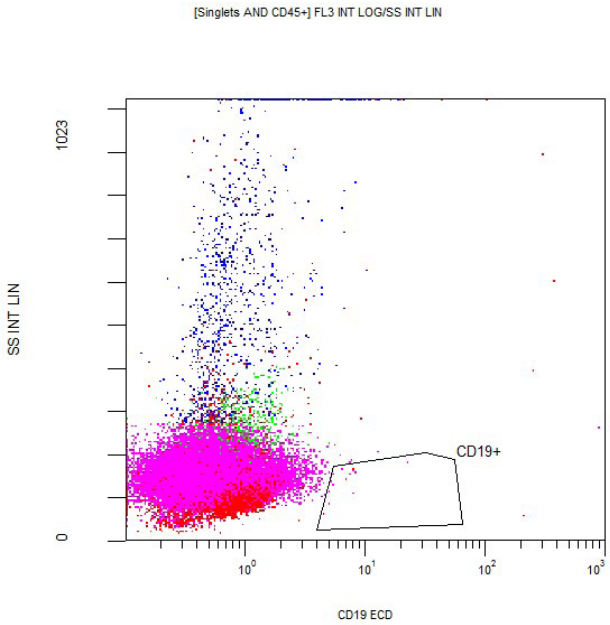
This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). Additionally, the "Blast" gate (pink) has been used to define a predominant population of mononuclear cells in this case. Note that the label for the Blast gate is not immediately adjacent to it---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.

Note that the overlap among the various populations in this particular case renders it somewhat difficult to distinguish among them. 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.

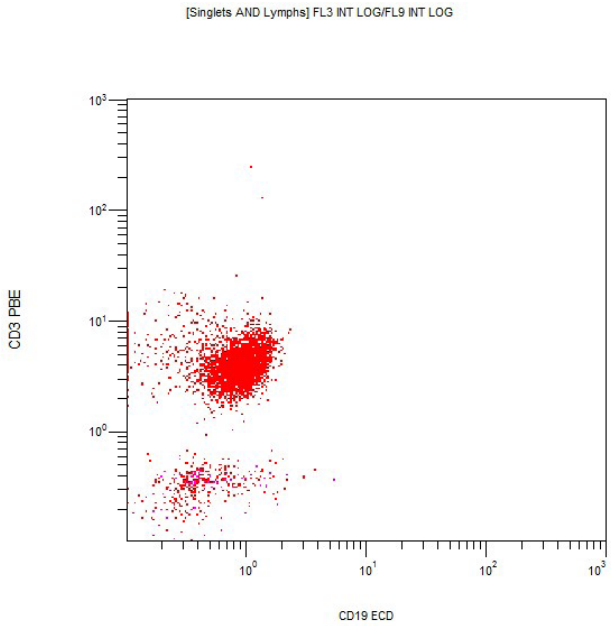


This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.

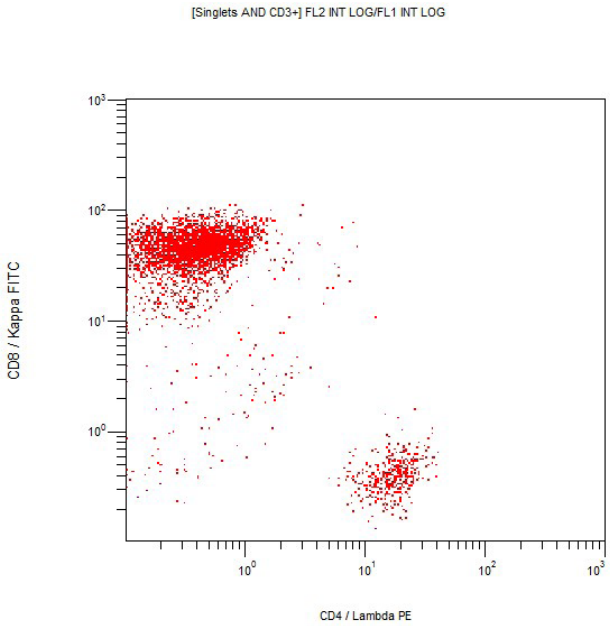
Note that the blasts (pink) are negative for CD3.



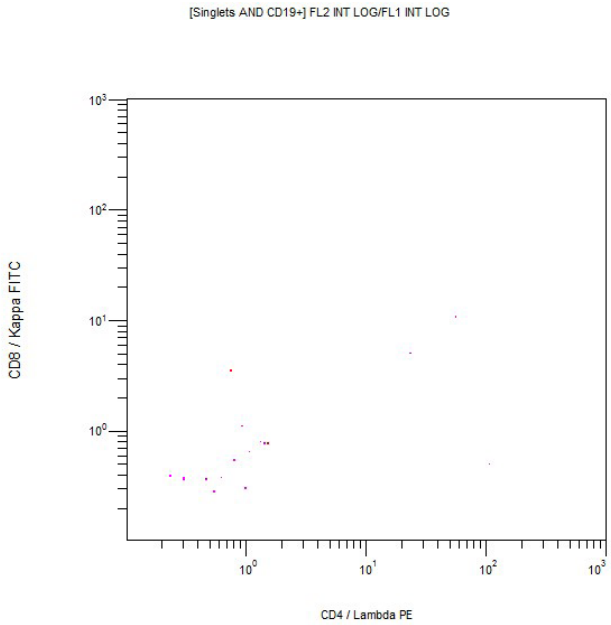
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot. Note that this gate would normally be adjusted to include apparent B lymphocytes only, but the paucity of B lymphocytes in this sample means that the gate is essentially empty.



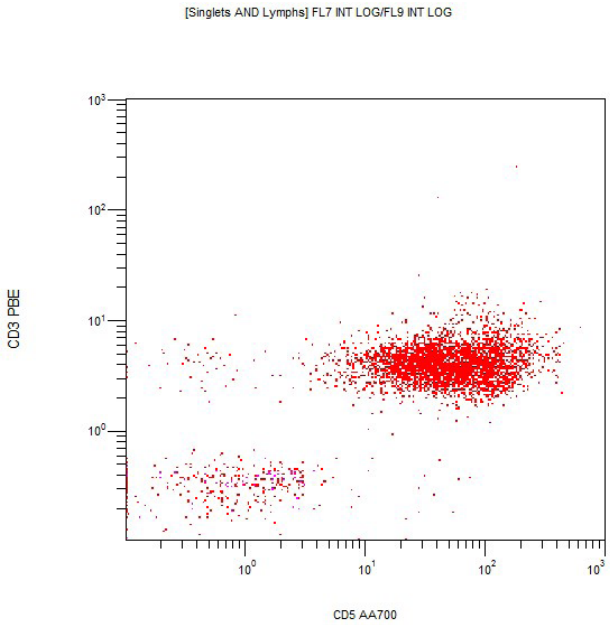
This CD19/CD3 dot plot gated on Lymphocytes confirms the absence of B lymphocytes in this sample. The CD19 negative, CD3 negative cells include NK cells.



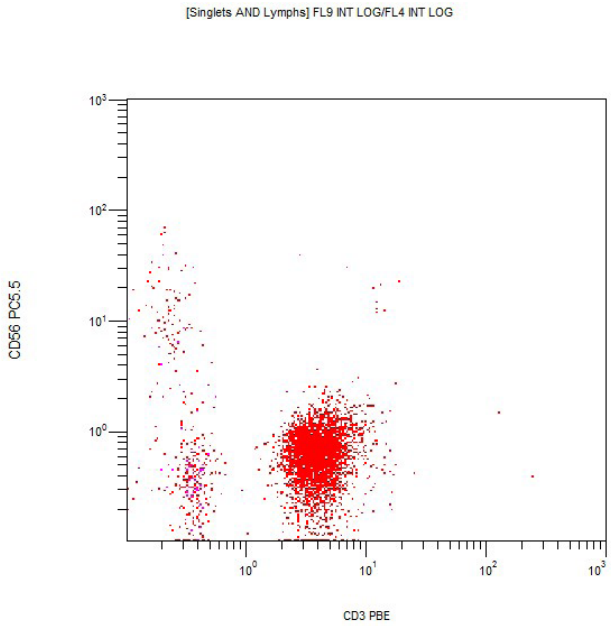
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



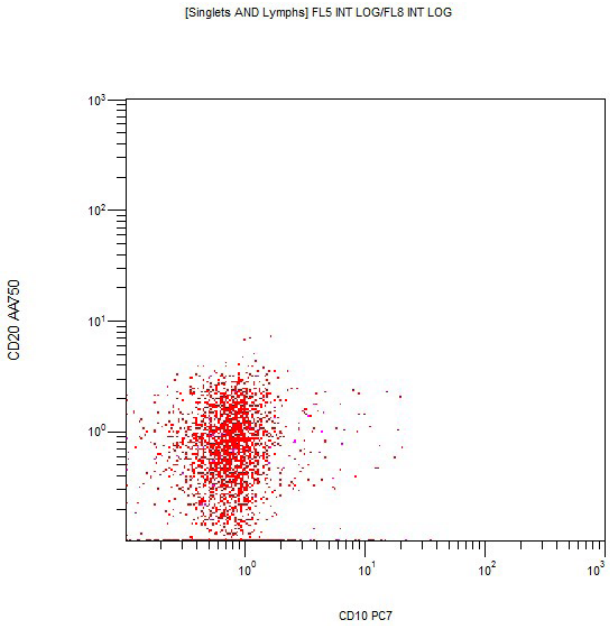
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations. As this sample contains essentially no B lymphocytes, this dot plot is not informative in this case.



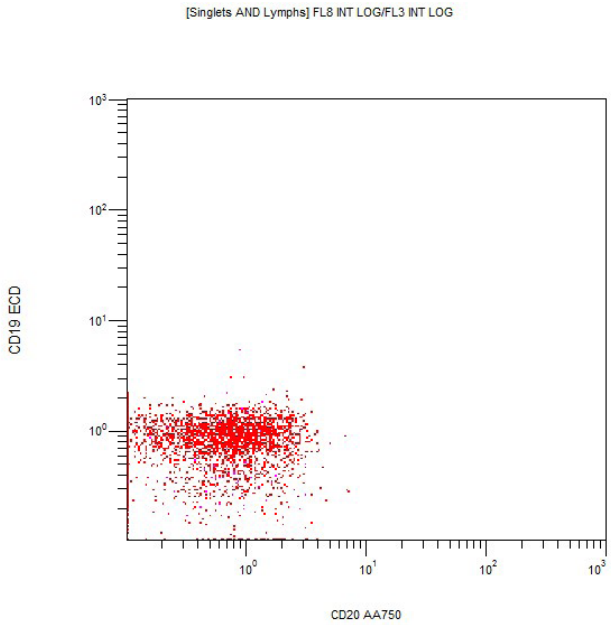
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable T lymphocytes comprise the majority of cells. Apparent NK cells (CD3 and CD5 dual negative) are also present.



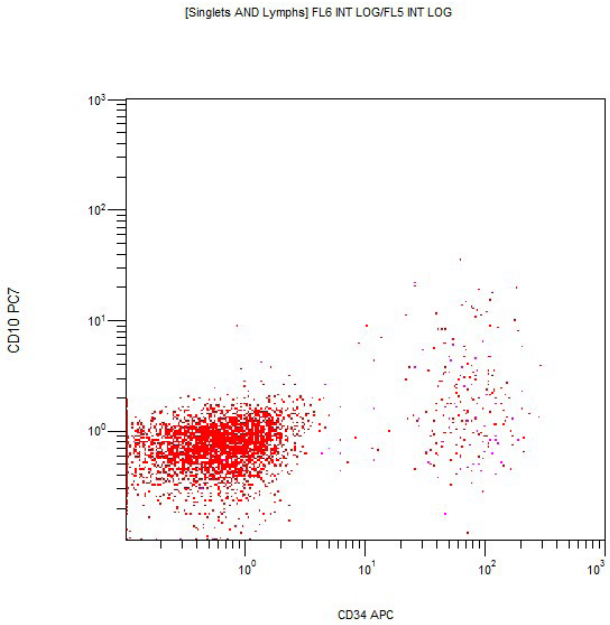
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present.



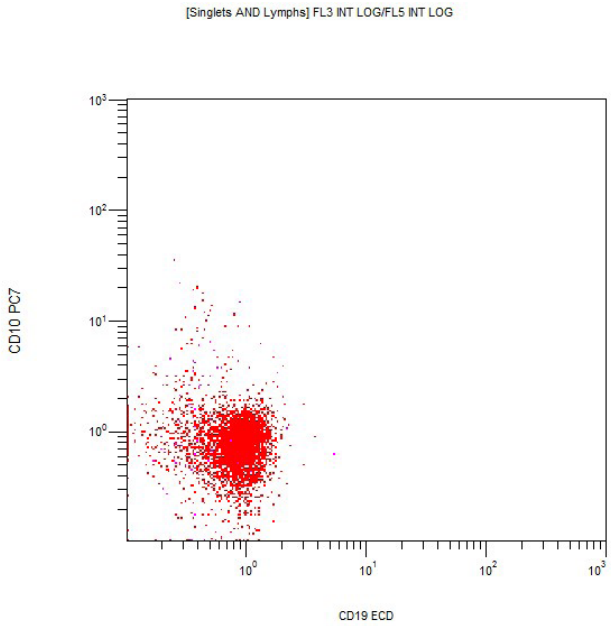
This CD10/CD20 dot plot is gated on Lymphocytes. No B lymphocytes that co-express CD10 are present.



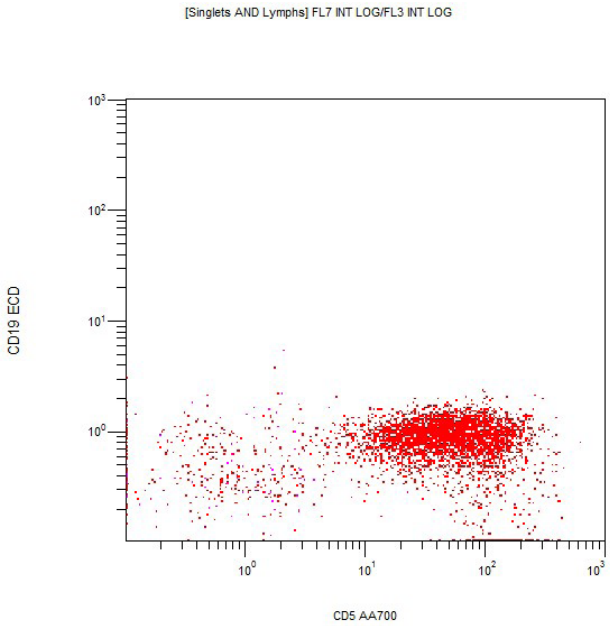
This CD20/CD19 dot plot is gated on Lymphocytes. No B lymphocytes are present.



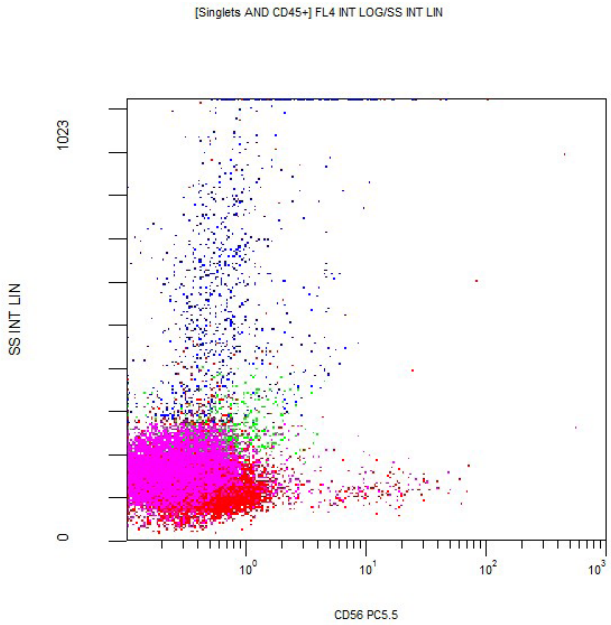
This CD34/CD10 dot plot is gated on Lymphocytes. The majority of these cells are negative for both markers, consistent with expected presence of mature T and B lymphocytes and NK cells. A small population of cells expressing CD34 is noted: these cells represent overlap between the Lymphocyte and Blast gates. Note the mixture of red and pink events.



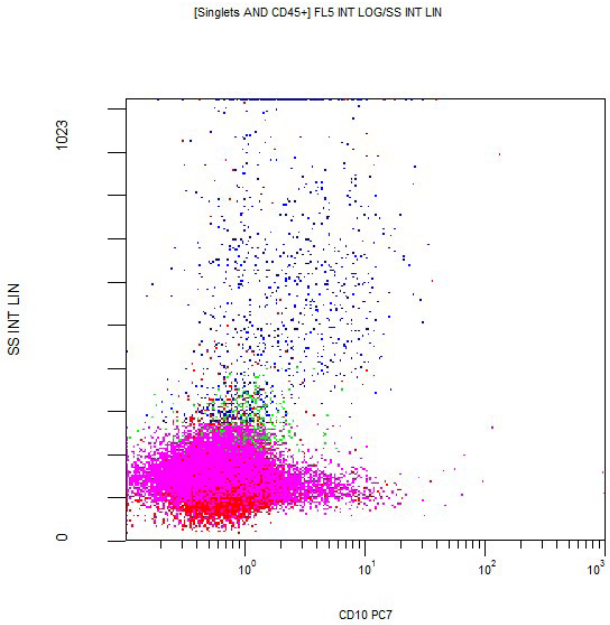
This CD19/CD10 dot plot is gated on Lymphocytes. Essentially no B lymphocytes are present in this sample.



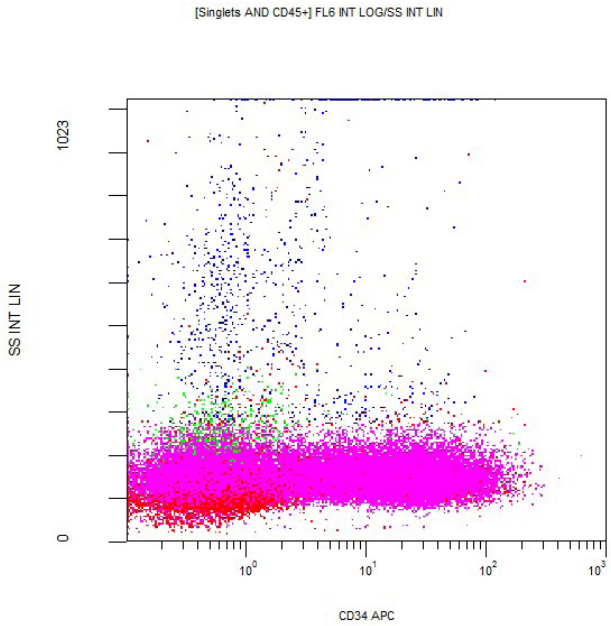
This CD5/CD19 dot plot is gated on Lymphocytes. No co-expression of CD5 and CD19 is identified.



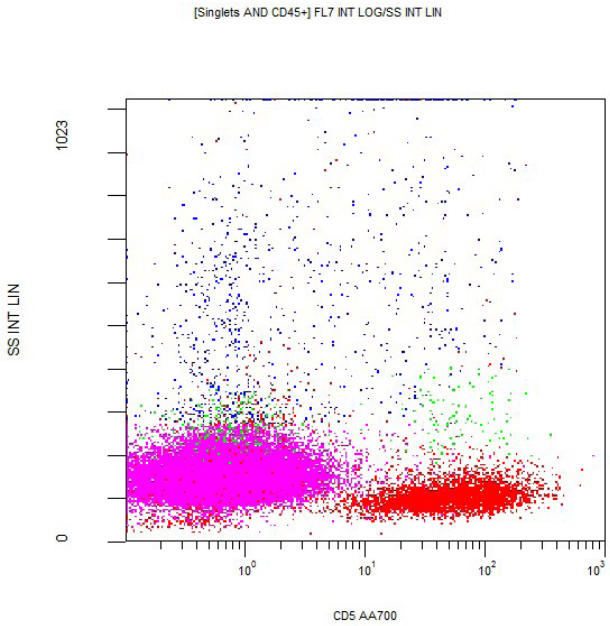
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells. The blasts (pink) do not express CD56.



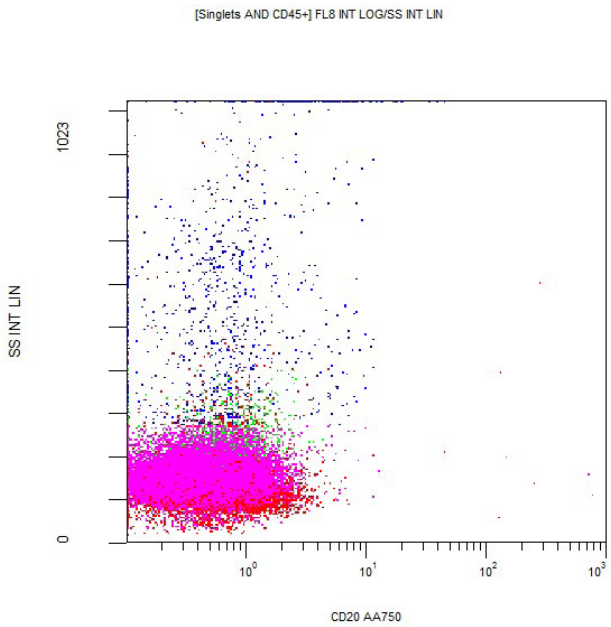
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A subset of the blasts (pink) appear to express low density CD10, but the majority of the blasts are negative. Some CD10 expression by granulocytes (blue) is noted, but the expression is not as high density as usual.



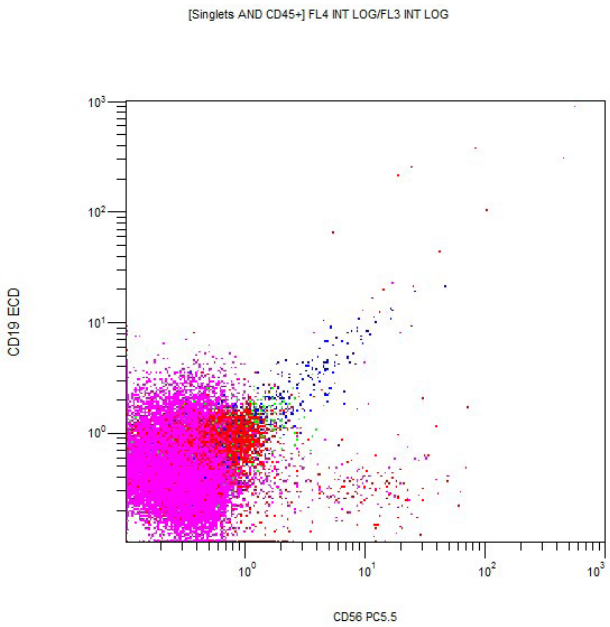
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). Many of the blasts (pink) express CD34.



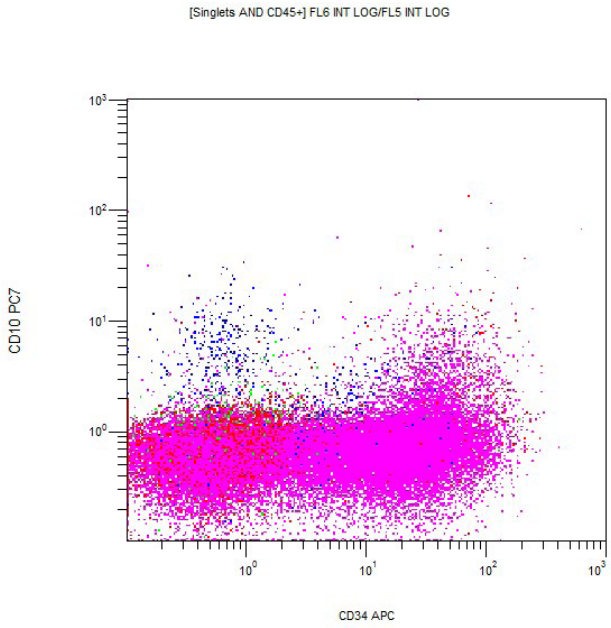
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) do not express CD5. The majority of the lymphocytes (red) do express CD5, consistent with T lymphocytes.



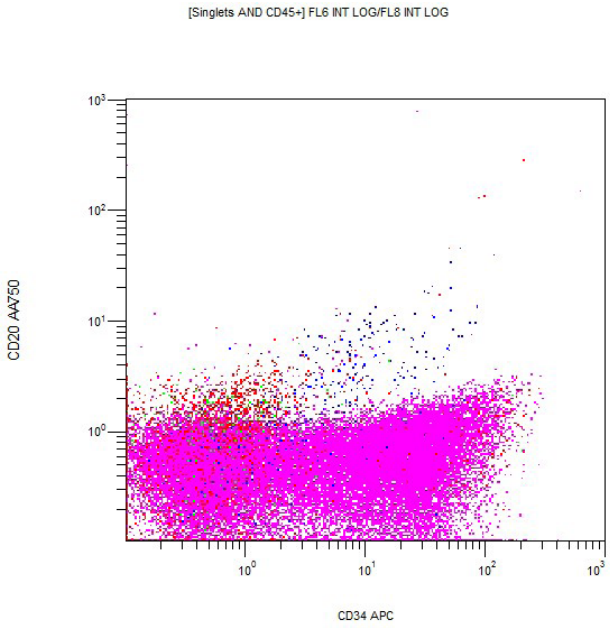
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) do not express CD20. As noted elsewhere in this analysis, very few B lymphocytes are present in this sample.



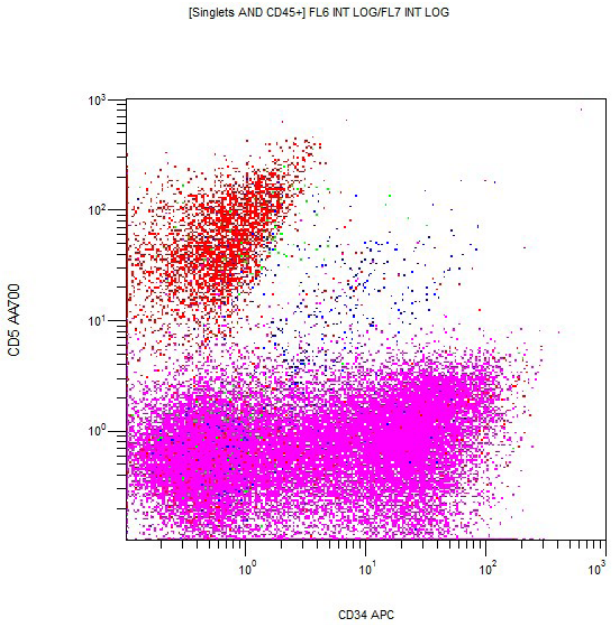
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) do not express either CD56 or CD19. As noted elsewhere in this analysis, very few B lymphocytes are present in this sample. A small population of NK cells is 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 the blasts (pink) express CD34. A small but distinct population of CD10 positive/CD34 negative events corresponds to granulocytes (blue). The blue events that appear to co-express CD34 and CD10 are consistent with high background fluorescence.



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). As noted elsewhere the blasts (pink) express CD34. The blue events that appear to co-express CD34 and CD20 are consistent with high background fluorescence.



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). As noted elsewhere the blasts (pink) express CD34; the blasts do not express CD5. CD5 positive T lymphocytes (red) are also present. The blue events that appear to co-express CD34 and CD5 are consistent with high background fluorescence.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with increased light scatter properties that express CD34 and low density CD45. These cells fail to express surface CD3, CD5, CD19, CD20, and CD56. Possible partial CD10 expression is noted. In order to assess expression of CD4 and CD8 by these cells, additional dot plots may be analyzed.

Taken together, the results are consistent with acute leukemia. Diagnostic considerations include acute myeloid leukemia (AML) as well as T acute lymphoblastic leukemia. Note that the light scatter properties and CD45 antigen density are both more consistent with AML. Additional flow cytometric immunophenotyping of this sample (data not shown) confirms an immunophenotype consistent with acute myeloid leukemia. Correlation with clinical and laboratory data is also recommended.

ACUTE MYELOID LEUKEMIA, NOT OTHERWISE SPECIFIED

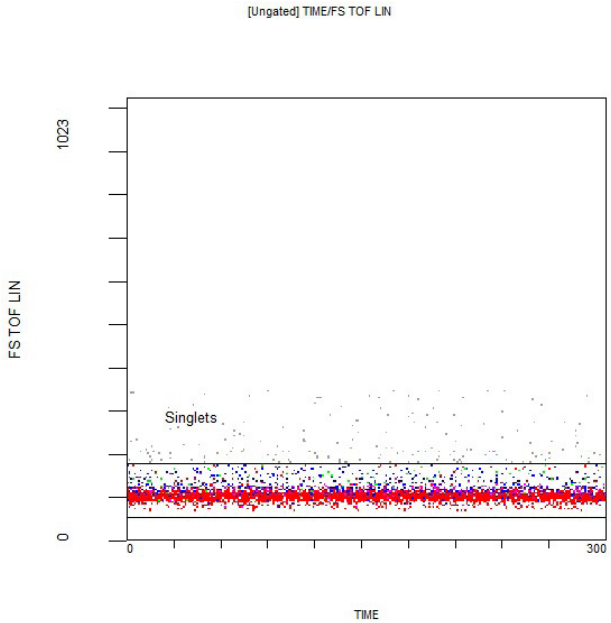
Case #13: Acute Myeloid Leukemia Not Otherwise Specified

Clinical Vignette

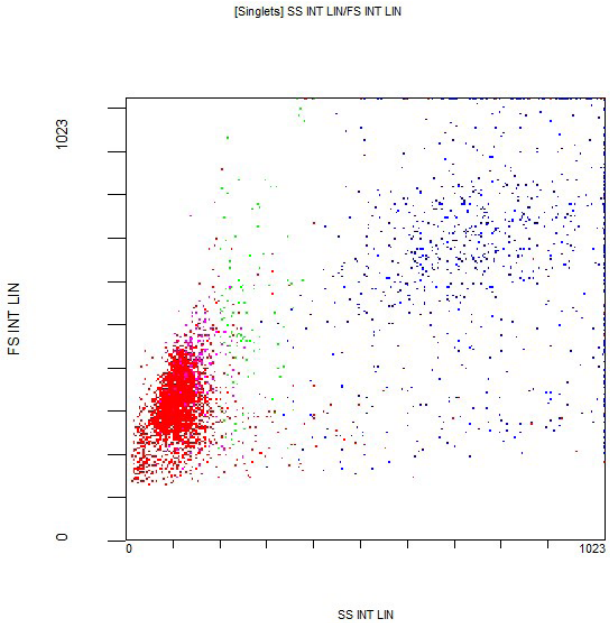
This 57-year-old female with presents with neutropenia. Rare circulating atypical mononuclear cells are noted on microscopic examination. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab LS Lymphoid Screen reagent.

Flow cytometric Immunophenotyping

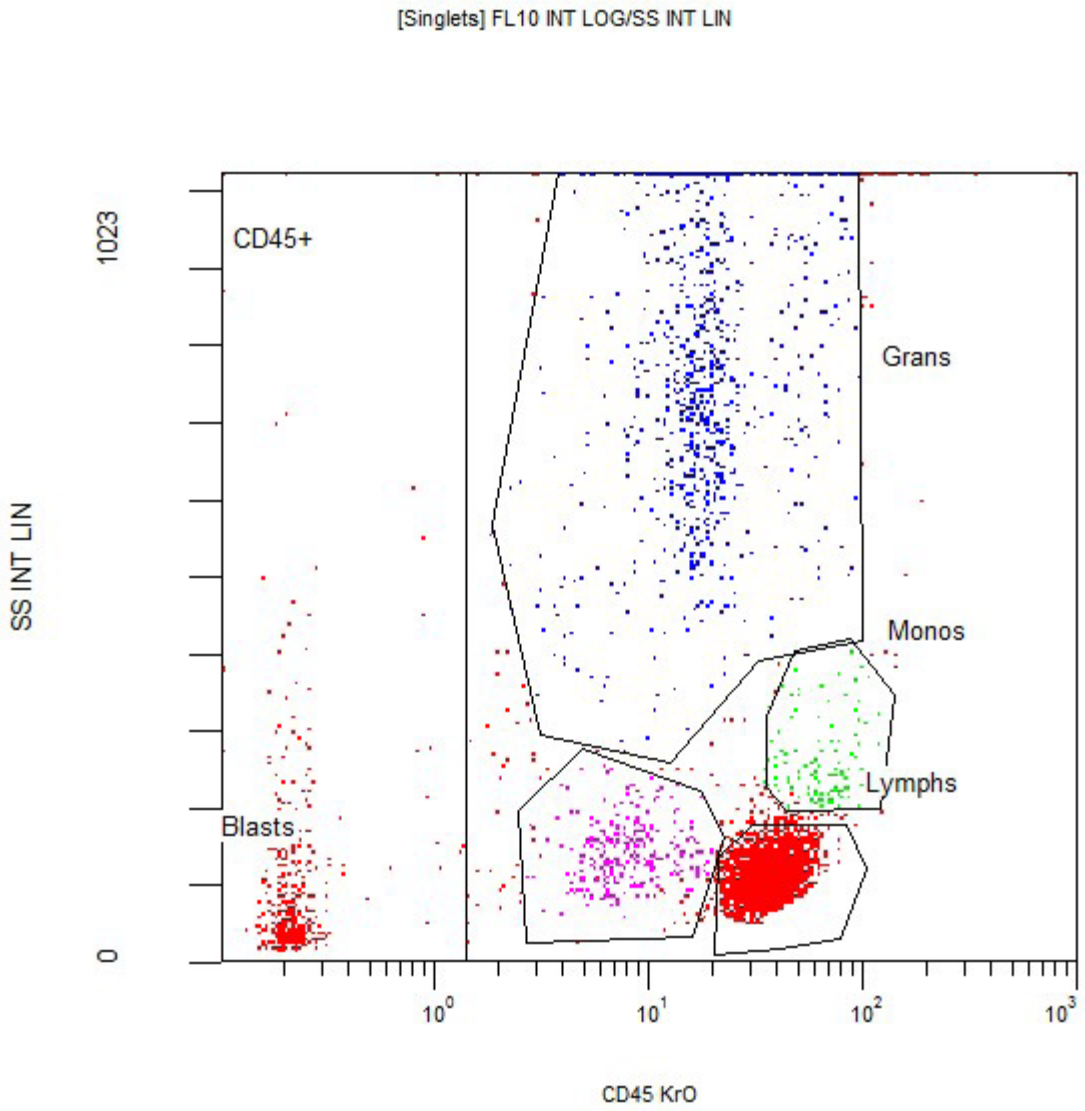
Access Case #13 list mode data



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 employee the Singlets gate. Additionally, were an interruption to be noted here, those events could be gated out as well.

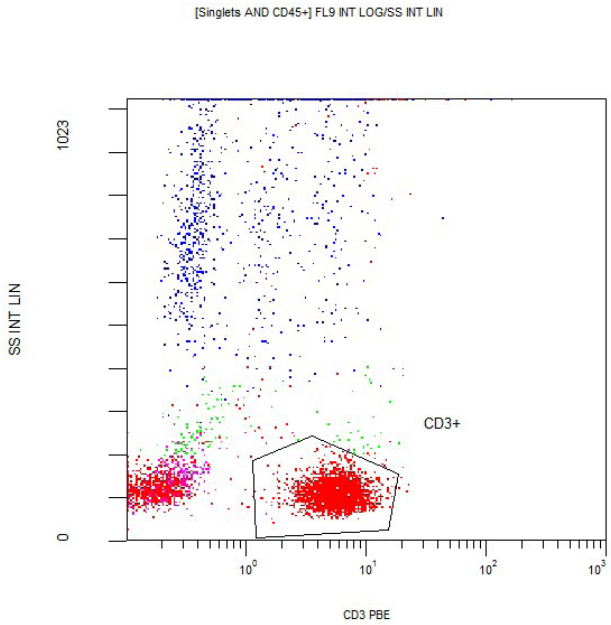


This Side Scatter/Forward Scatter dot plot demonstrates a predominance of apparent lymphocytes and a relative paucity of apparent granulocytes.



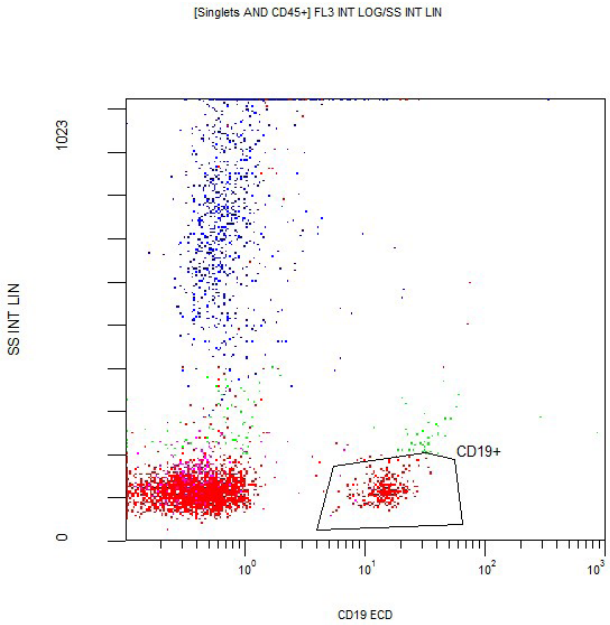
This CD45/Side Scatter dot plot permits distinction of various cell populations according to a combination of CD45 antigen density and side scatter. The usual populations include lymphocytes (red), monocytes (green), and granulocytes (blue). Additionally, the "Blast" gate (pink) has been used to define a small but fairly tightly clustered population of mononuclear cells in this case. Note that the label for the Blast gate is not immediately adjacent to it---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.

Note that the lack of overlap among the various populations in this particular case renders it easy to distinguish among them. 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.

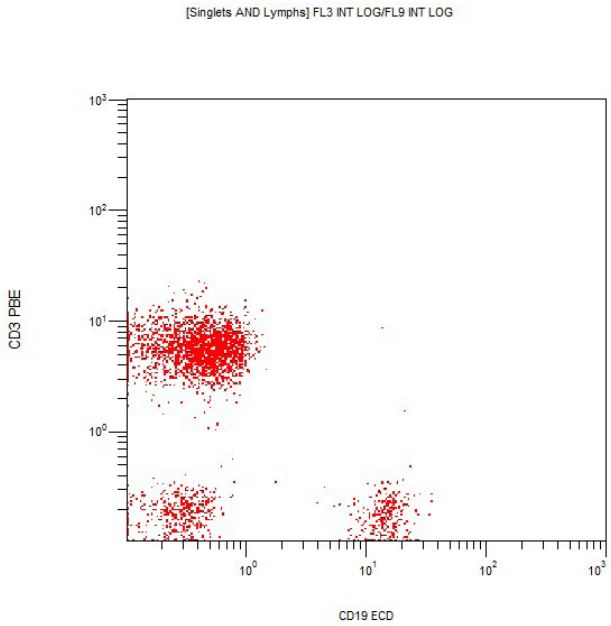


This CD3/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD3+ gate which will be applied eventually to the CD4/CD8 dot plot. Note that this gate should be adjusted to include apparent T lymphocytes only.

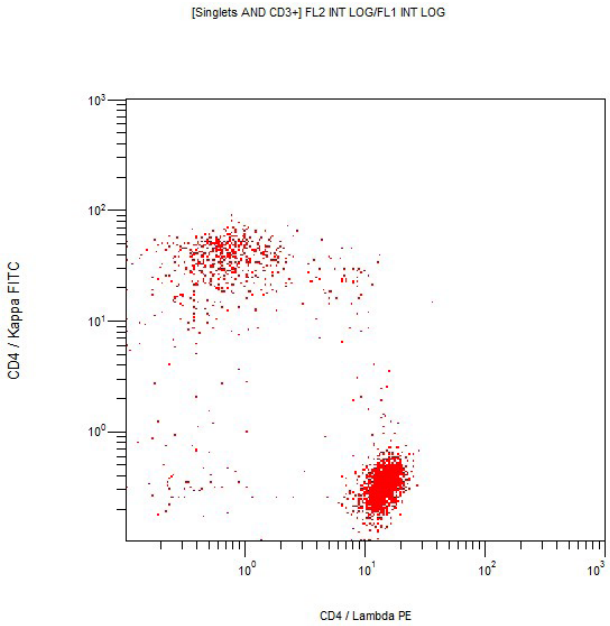
Note that the blasts (pink) are negative for CD3.



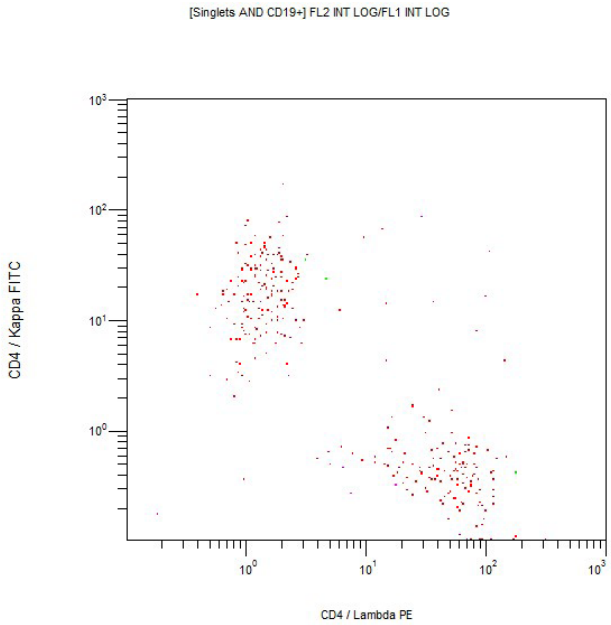
This CD19/Side Scatter dot plot is gated on CD45+ events. This display is used to define the CD19+ gate which will be applied eventually to the Kappa/Lambda dot plot.



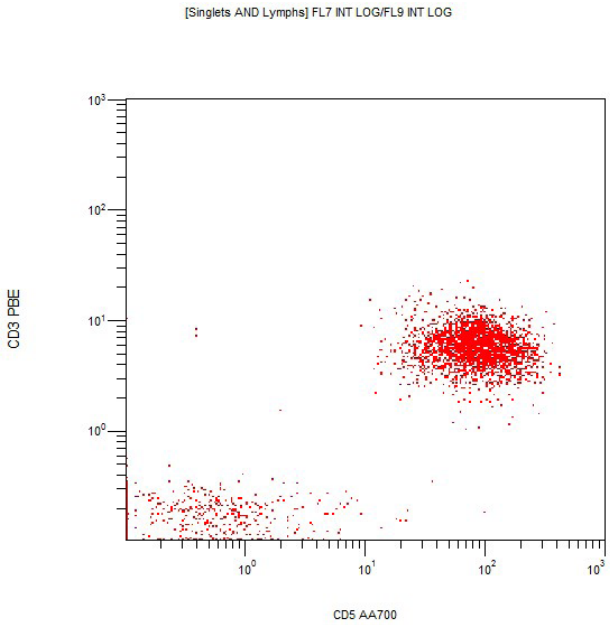
This CD19/CD3 dot plot gated on Lymphocytes is consistent with the remainder of the analysis. T lymphocytes, B lymphocytes, and presumed NK cells (CD3 and CD19 dual negative) are present.



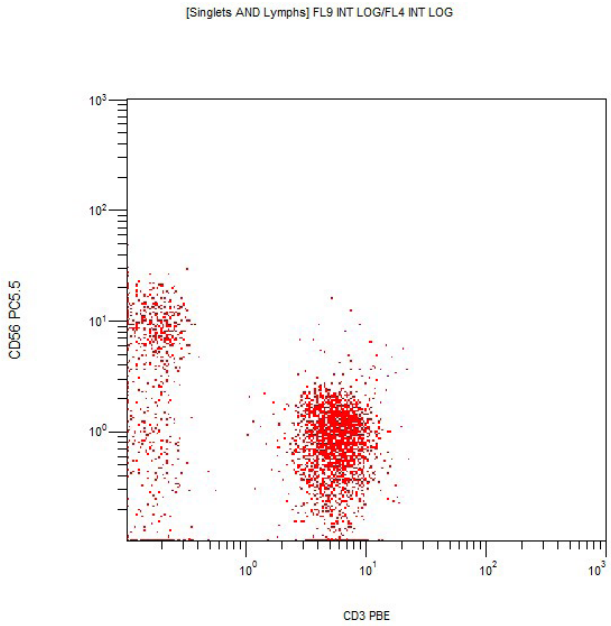
Applying the CD3+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to T lymphocytes only, the user sees only CD4 and CD8 T lymphocytes.



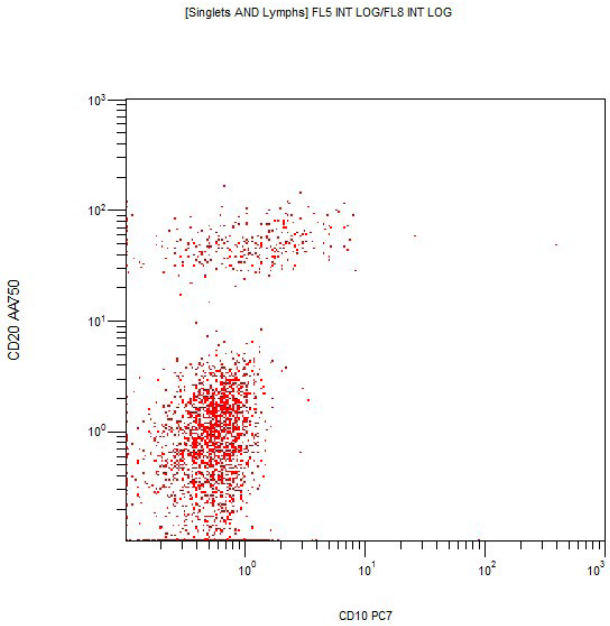
Applying the CD19+ gate to this dot plot permits "unstacking" of the reagents in the FITC and PE channels. By limiting the display to B lymphocytes only, the user sees only the expected Kappa and Lambda populations.



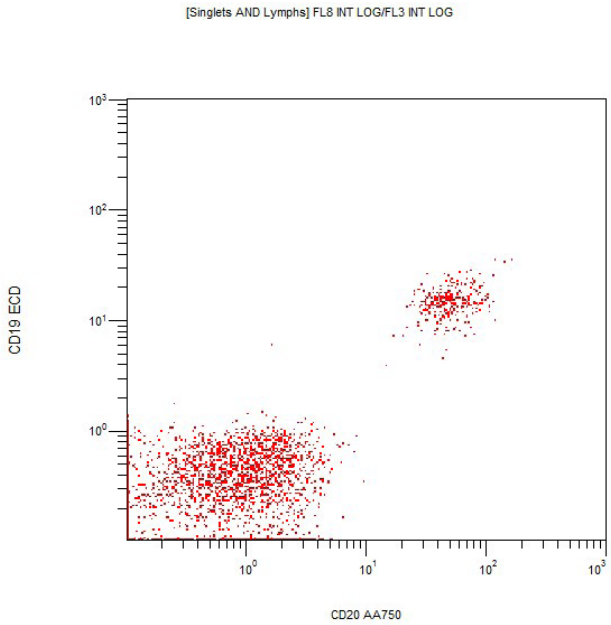
This CD5/CD3 dot plot is gated on Lymphocytes. Unremarkable T lymphocytes comprise the majority of cells. Apparent B lymphocytes and NK cells (CD3 and CD5 dual negative) are also present.



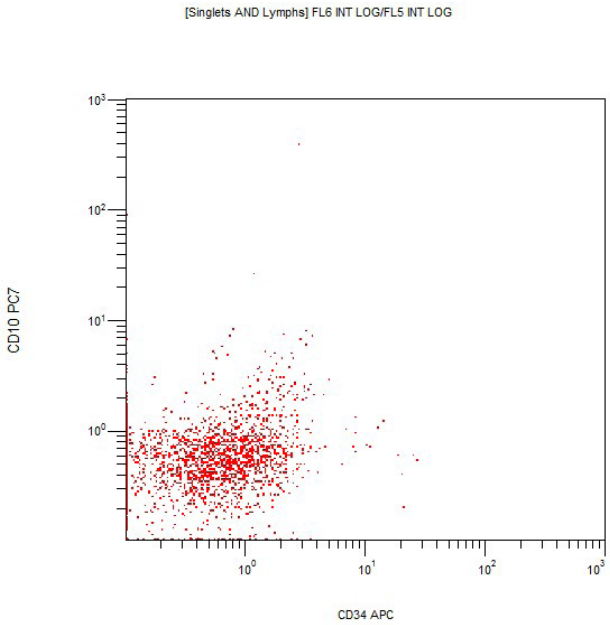
This CD3/CD56 dot plot is gated on Lymphocytes. Both T lymphocytes (CD3 positive, CD56 negative) and NK cells (CD56 positive, CD3 negative) are present. B lymphocytes are CD3 and CD56 dual negative.



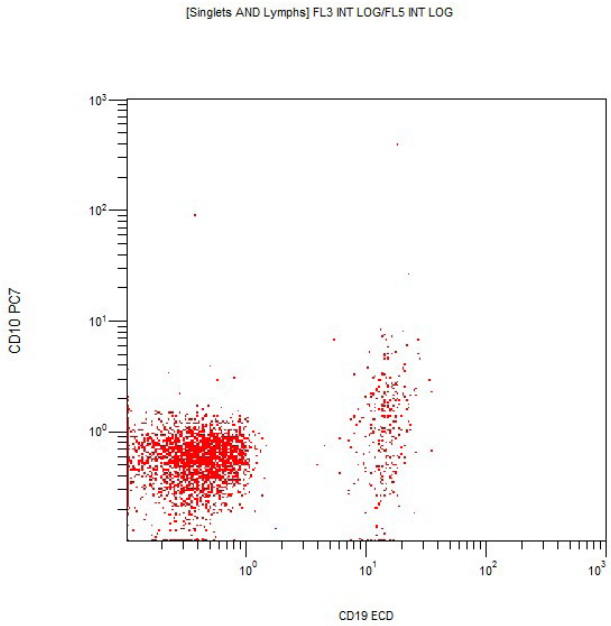
This CD10/CD20 dot plot is gated on Lymphocytes. Possible low density expression of CD10 on B lymphocytes is noted and could be evaluated separately for the presence of light chain restriction within this compartment.



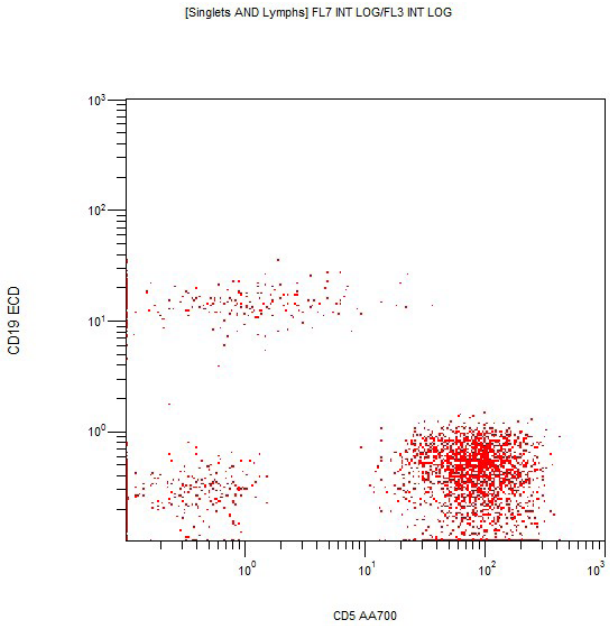
This CD20/CD19 dot plot is gated on Lymphocytes. The B lymphocytes display expected normal co-expression of CD19 and CD20.



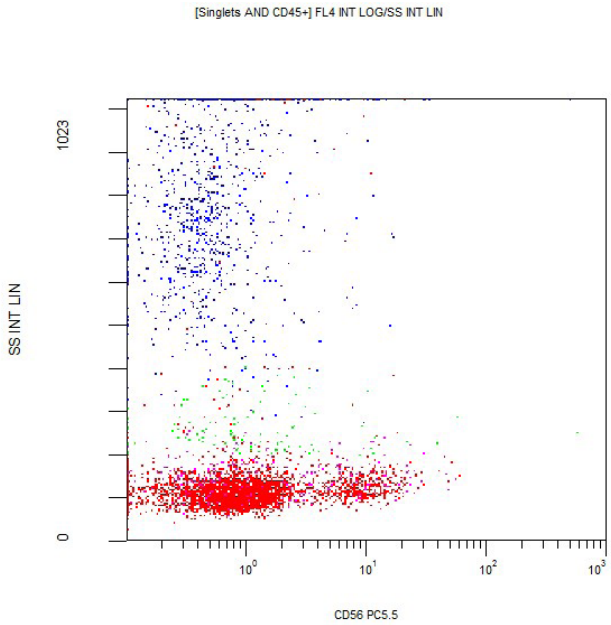
This CD34/CD10 dot plot is gated on Lymphocytes. These cells are essentially negative for both markers, consistent with expected presence of mature T and B lymphocytes and NK cells.



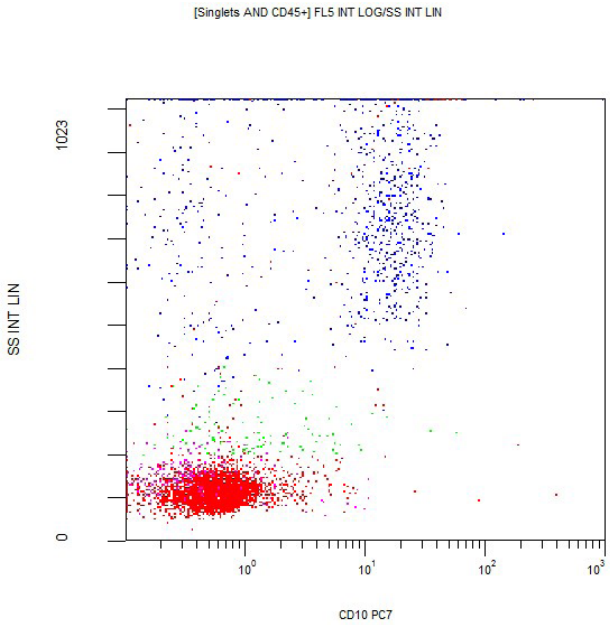
This CD19/CD10 dot plot is gated on Lymphocytes. B lymphocytes comprise the minority of lymphocytes in this peripheral blood sample, as expected.



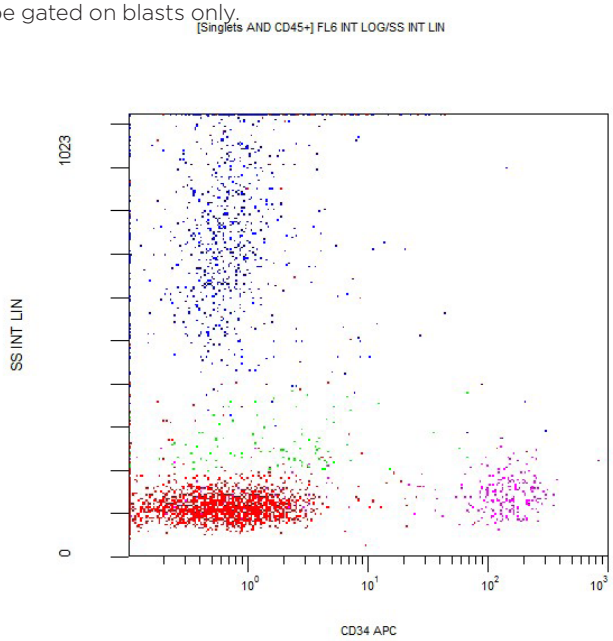
This CD5/CD19 dot plot is gated on Lymphocytes. Possible low density co-expression of CD5 and CD19 is identified on a small subset of B lymphocytes. Additional analysis of this population could be performed in order to establish or rule out immunoglobulin light chain restriction within this compartment.



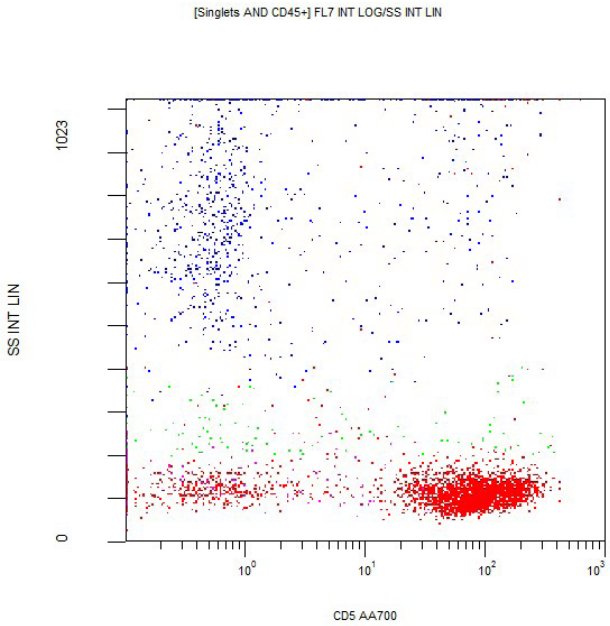
This CD56/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). This dot plot demonstrates a small population with low side scatter light properties that expresses CD56, consistent with apparent NK cells (red). Additionally, notice that at least a portion of the blasts (pink) also express CD56. In order to see these events more clearly, this dot plot could be gated on blasts only.



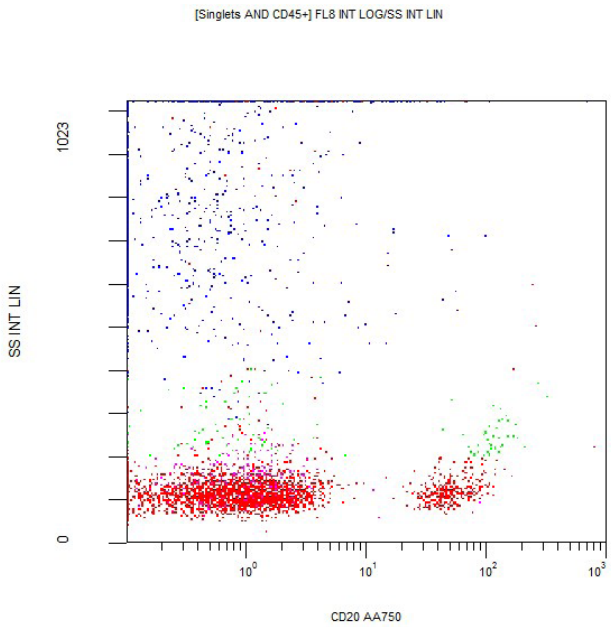
This CD10/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). A few of the blasts (pink) appear to express low density CD10, but the majority of the blasts are negative. Granulocytes (blue) express CD10 as expected.



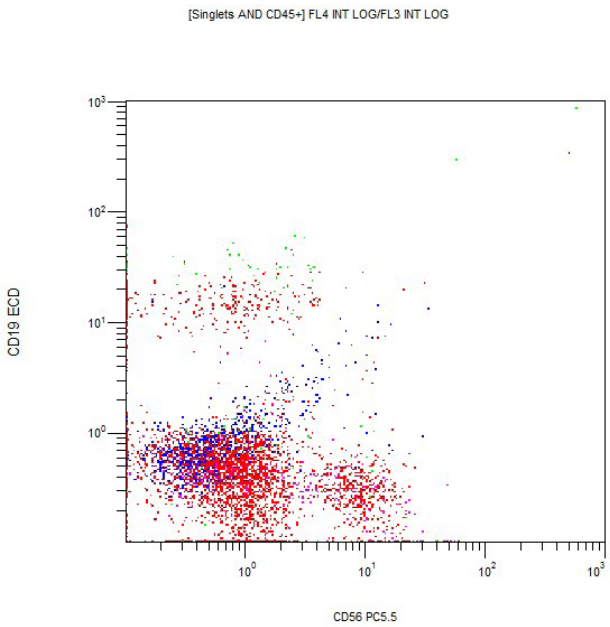
This CD34/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) express high density CD34.



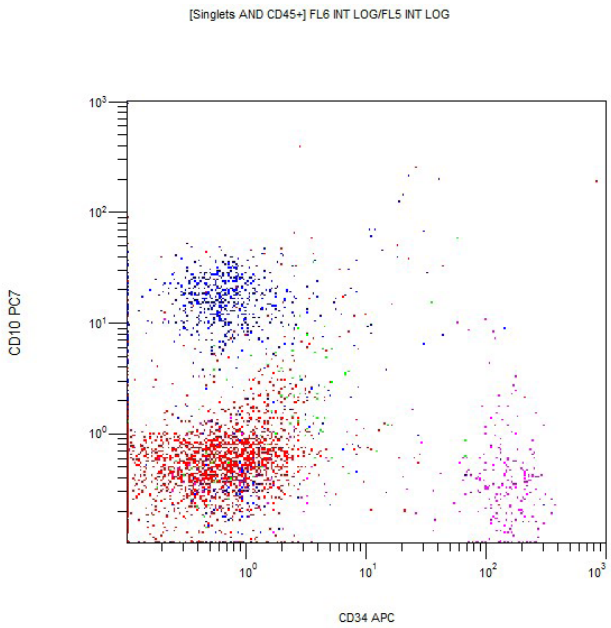
This CD5/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The majority of the lymphocytes (red) express CD5, consistent with T lymphocytes. A subset of the blasts (pink) express low density CD5. In order to see these events more clearly, this dot plot could be gated on blasts only.



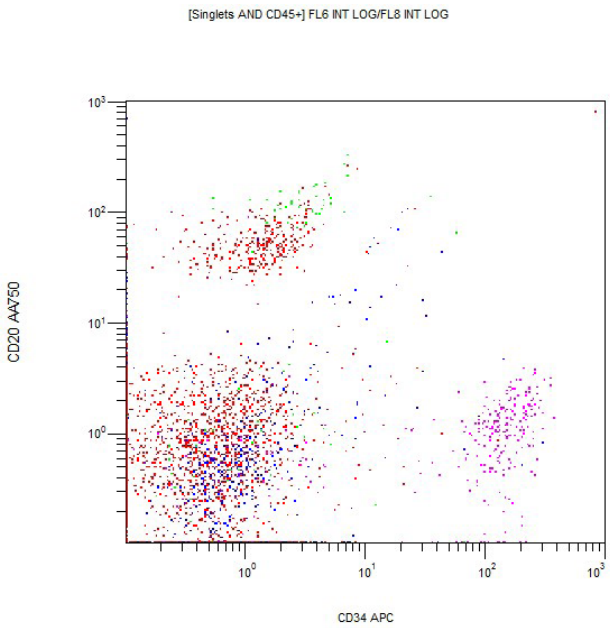
This CD20/Side Scatter dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). The blasts (pink) do not express CD20.



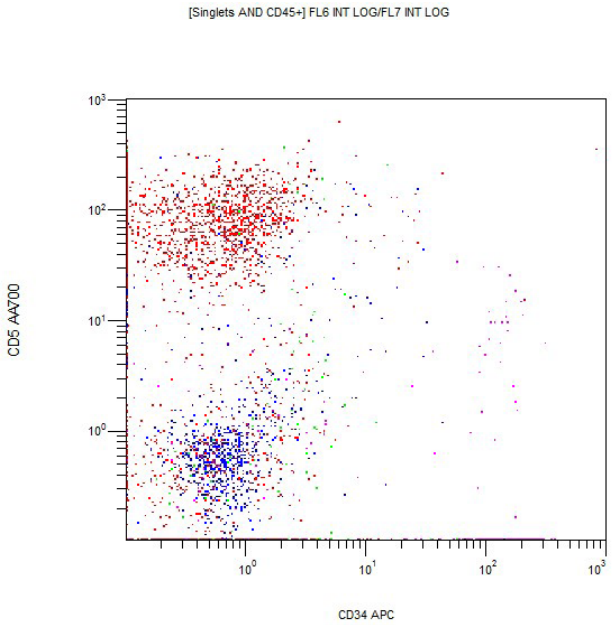
This CD56/CD19 dot plot is gated on CD45+ events. Unlike the dot plots gated on Lymphocytes, it includes the named populations defined on the CD45/Side Scatter dot plot: lymphocytes (red), monocytes (green), granulocytes (blue), and blasts (pink). As noted elsewhere in this analysis, the blasts (pink) express CD56. NK cells (red, CD56 positive, CD19 negative) are also 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 the blasts (pink) express CD34. The do not express CD10. A small but distinct population of CD10 positive/CD34 negative events corresponds to granulocytes (blue).



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). As noted elsewhere the blasts (pink) express CD34. They do not express CD20.



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). As noted elsewhere the blasts (pink) express CD34 and a subset of them also express CD5. The CD5 negative blasts are apparent on the X-axis. CD5 positive T lymphocytes (red) are also present.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a small but phenotypically distinct population of cells that express CD34, CD56, partial CD5, and low density CD45. These cells fail to express surface CD3, CD19, CD20, and CD10. In order to assess expression of CD4 and CD8 by these cells, additional dot plots may be analyzed.

Taken together, the results are suspicious for acute leukemia. Diagnostic considerations include acute myeloid leukemia (AML) as well as T acute lymphoblastic leukemia, with AML being favored by the antigen density and light scatter properties. Additional flow cytometric immunophenotyping of this sample (data not shown) confirms an immunophenotype consistent with AML. Correlation with clinical and laboratory data is also recommended.

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