FLOW CYTOMETRY
PRINCIPLES AND PRACTICE

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Aims and Objectives

- Principles of flow cytometry
  - Preparation
  - Steps involved
  - Interpretation of flow cytometry plots
  - Use of panels
- Cases

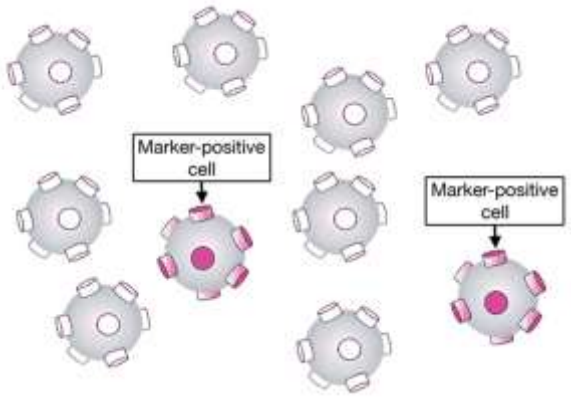
Introduction

- Identifies cell types present in cell suspension
- Fluorescent-labelled antibodies specific for lineage or maturation markers
- ‘marker’ molecules: usually on cell surface
- Possible to permeabilize cells to allow antibody penetration to detect intracellular antigens e.g. TdT and Myeloperoxidase

Preparation of cells

- Blood and bone marrow - red cells lysed (e.g. ammonium chloride).
- White cells recovered by washing and centrifugation
- Aliquots of the cell suspension are incubated with different combinations of fluorescently-conjugated antibodies
- CSF, ascitic fluid, pleural fluid or a cell suspension made by ‘mashing’ solid tumour samples can be analyzed in a similar way.

Step 1: Add a fluorescently-labelled antibody specific for the cell marker



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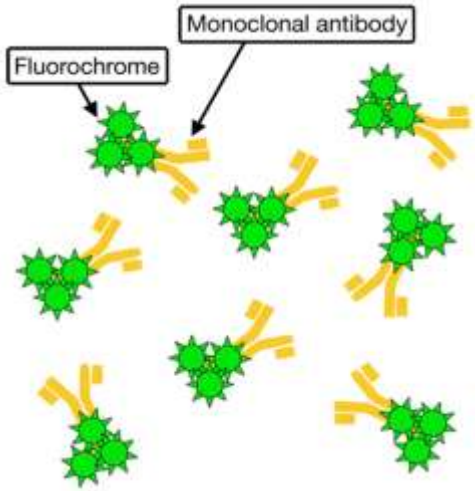
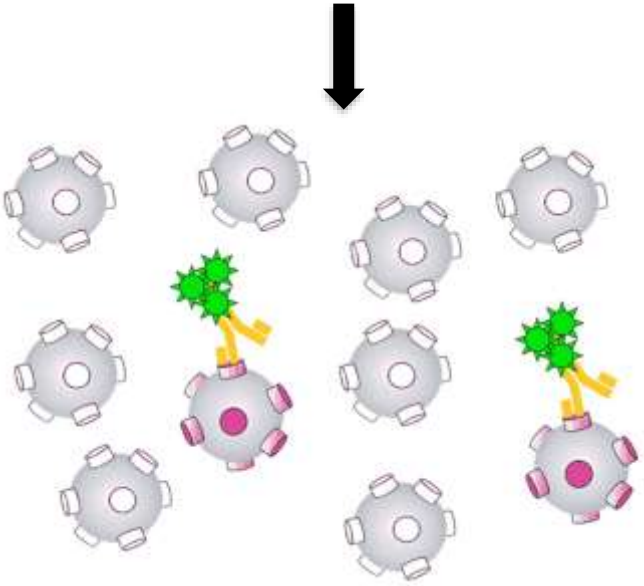
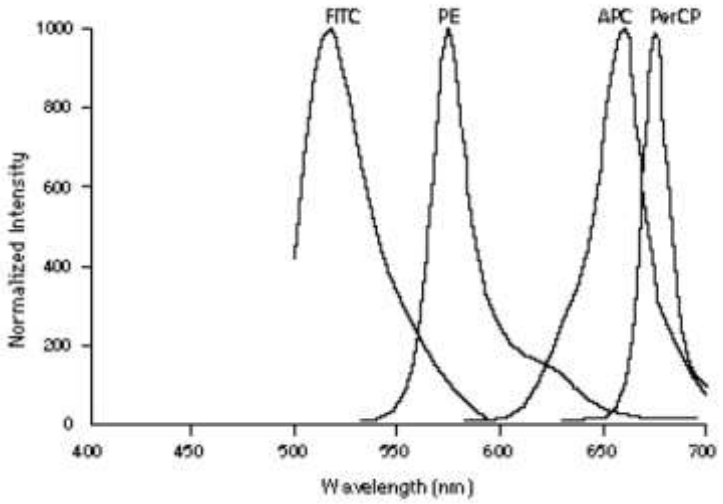




Permeabilisation:

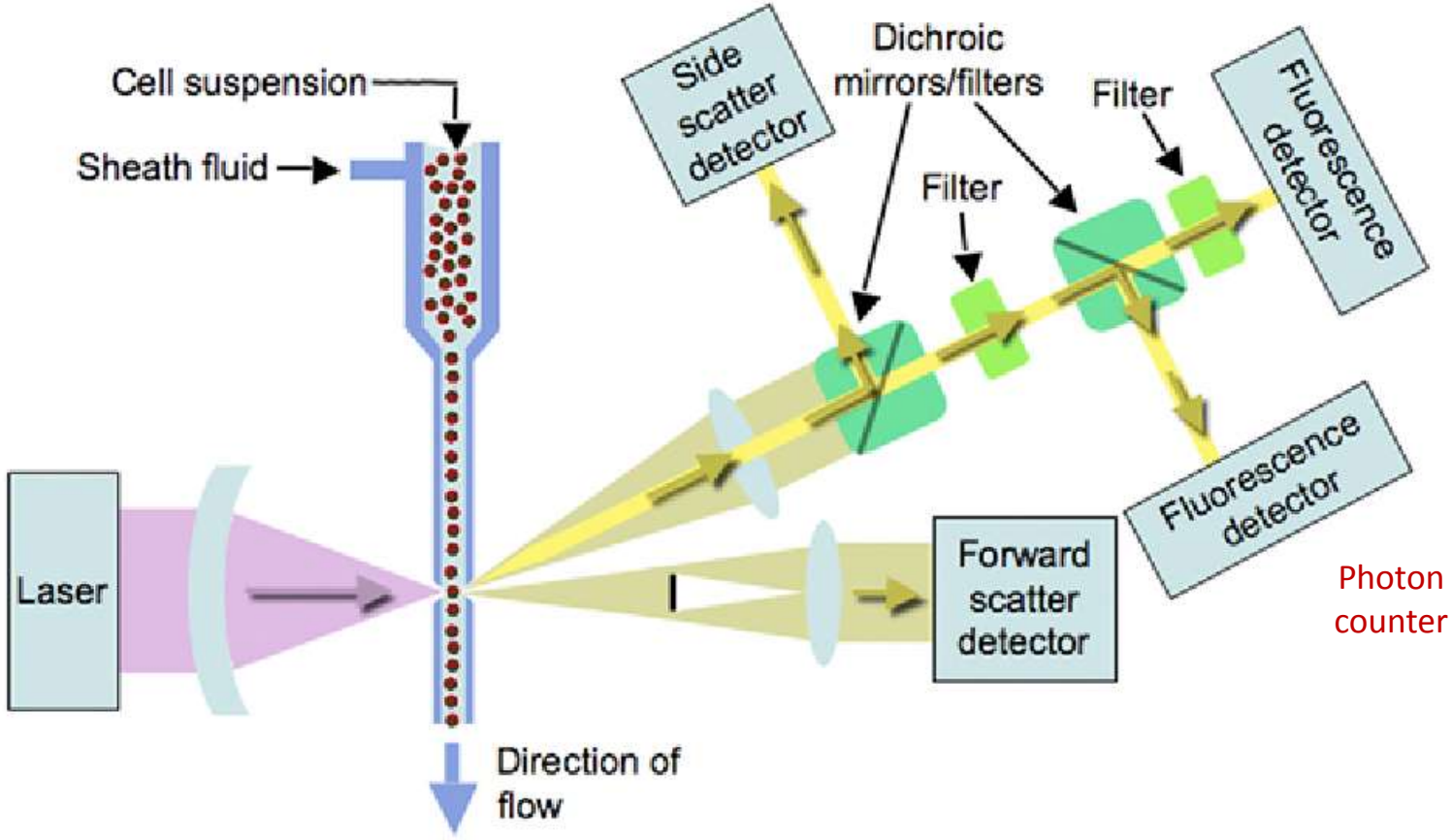
Fix in suspension and commercial permeabilisation reagents: incubation

Different Fluorochromes



Different emission spectra for different fluorochromes means that multiple cell surface markers may be interrogated.

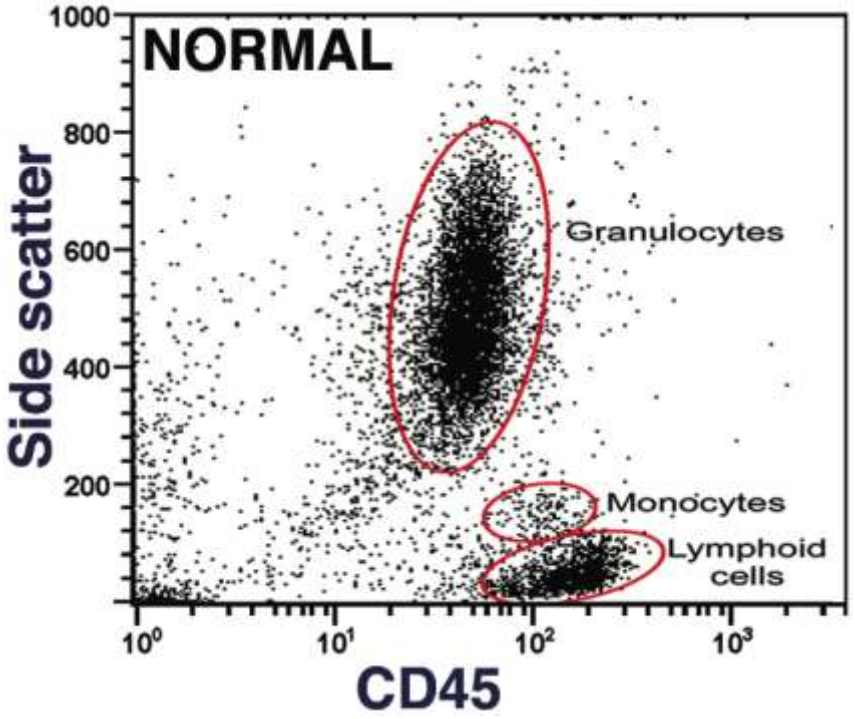
Step 2: Separating out subpopulations of cells for identification



Three parameters are recorded

- Forward scatter - this reflects cell size
- Side scatter - reflects granularity
- Fluorescence signal – no. of fluorochromes/cell

Using CD45/SCC to ‘gate’ different sorts of cells in blood



- CD45: common leucocyte antigen

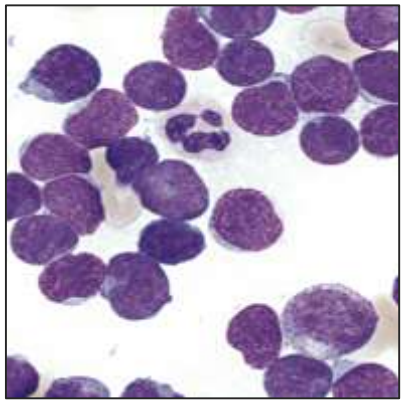
- protein tyrosine phosphatase that is specifically expressed in haemopoietic cells.

- Its variable positivity allows separation of blood cells.

- It is negative on plasma cells, RBCs and some ALL

Choosing a panel of antibodies

Inspect morphology + clinical picture, and choose a panel:

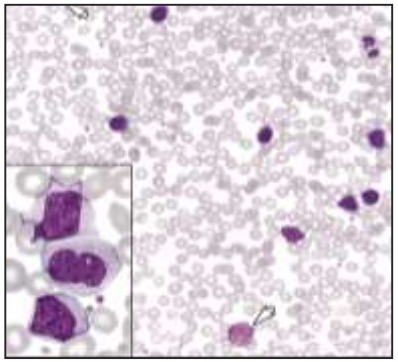


Acute leukaemia panel

Lineage
CD79a – B cell receptor component
CD3 – T cell receptor component
MPO – myeloperoxidase

CD117 – c-kit (most AML, few ALL)
CD34 – expressed on stem cells
CD19 – pan-B cell marker
CD10 – expressed on ALL
CD7 – T cell marker
HLADR
CD33 – myeloid marker
TdT – all ALL, 25% AML
Lysozyme – monocytic marker

Chronic lymphoproliferative disease panel



Lineage
CD3 – T cell receptor component
CD19 – pan-B cell marker
CD79b – B cell receptor component
Clonality of B cells ?
Kappa/lambda light chains

CD20 –B cells; not early blasts/plasma cells
CD23 – B-CLL
FMC7 – conformational variant of CD20
CD5 – T cell marker, also expressed in B-CLL
CD45 – pan-leucocyte marker
CD10 – T/B ALL and germinal centre marker
If T cell population: extended T cell panel (e.g. CD2, CD7, TCR, CD4, CD8, CD16, CD56, CD57…)

Analysis of Acute Leukaemia: Identify Lineage:

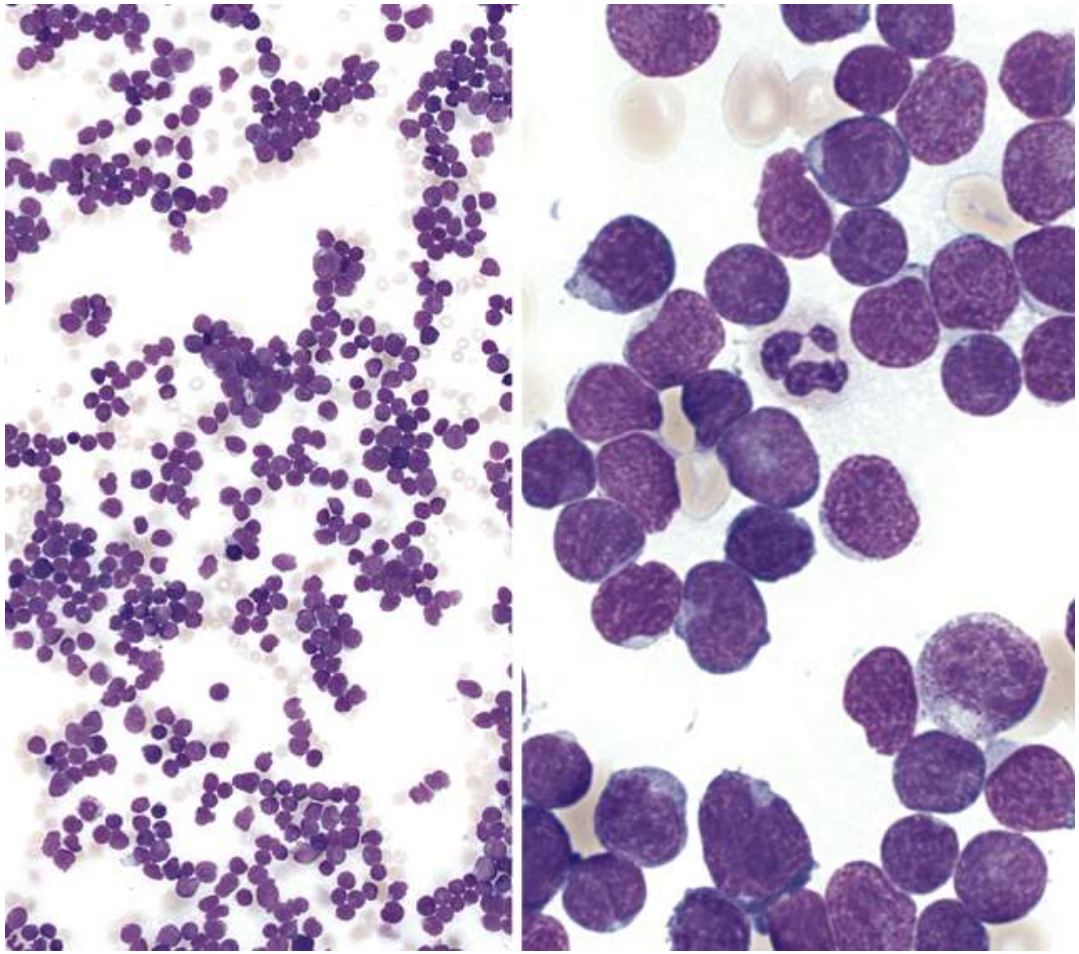
Does the case express:-

1. MPO?
   myeloid

2. CD79a?
   B cell

3. Cyt CD3 OR sCD3 ?
   T cell

4. TdT ?
   Primitive; often ALL



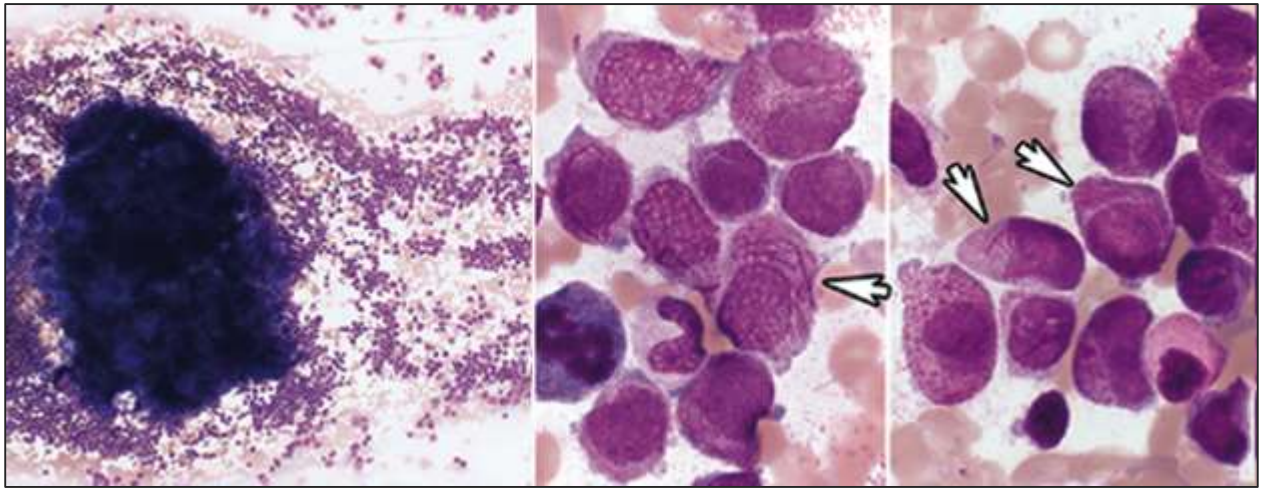
Case 1

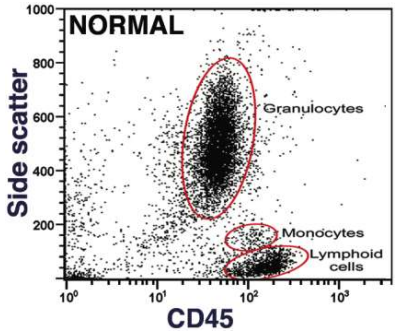
40 year old Female

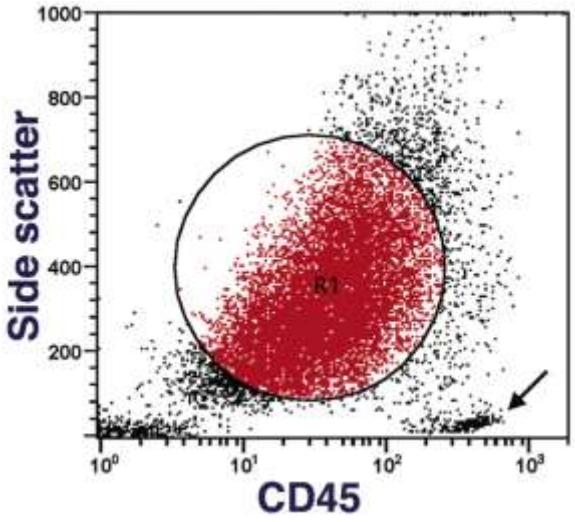
Mucous bleeding
and easy bruising

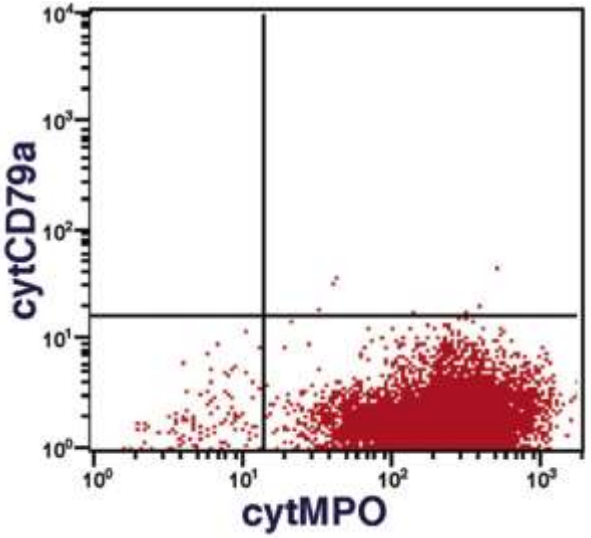
Hb 9
Plt 12
WCC 15

Deranged clotting

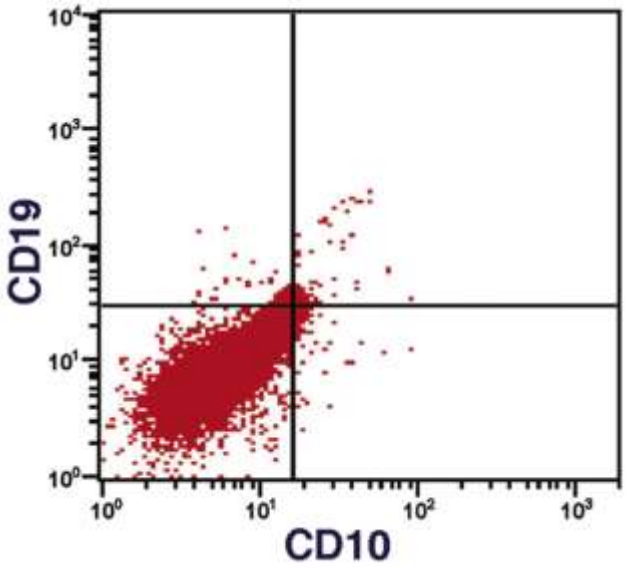


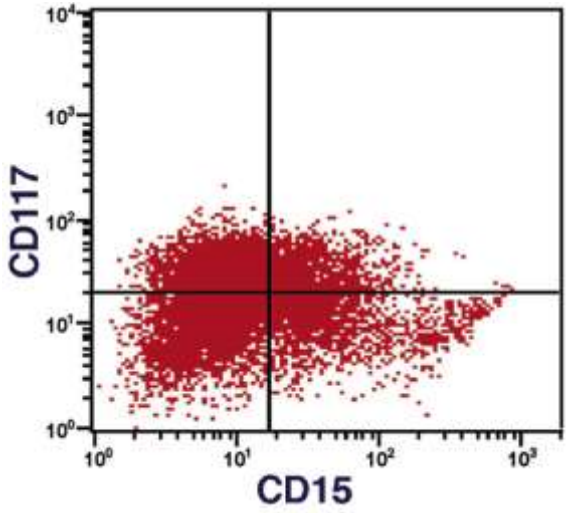


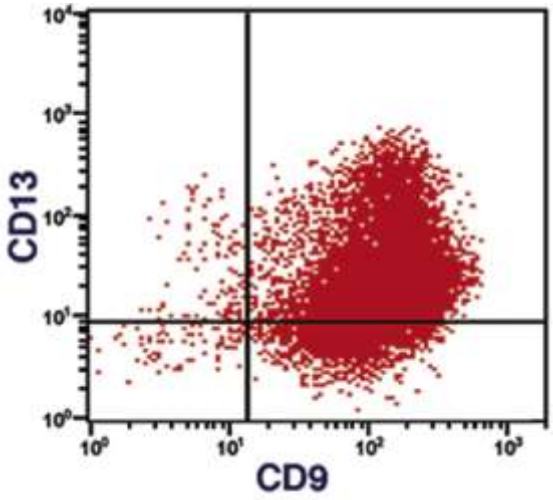




Case 1


CD19
CD10
10^0
10^1
10^2
10^3
10^4


CD117
CD15
10^0
10^1
10^2
10^3
10^4


CD13
CD9
10^0
10^1
10^2
10^3
10^4

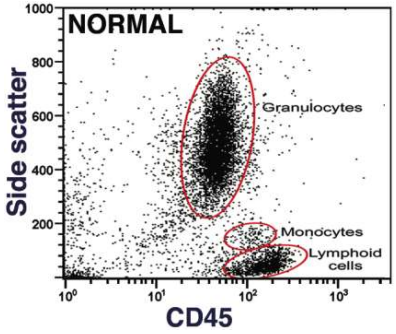
Case 1 Acute promyelocytic Leukaemia (APML)

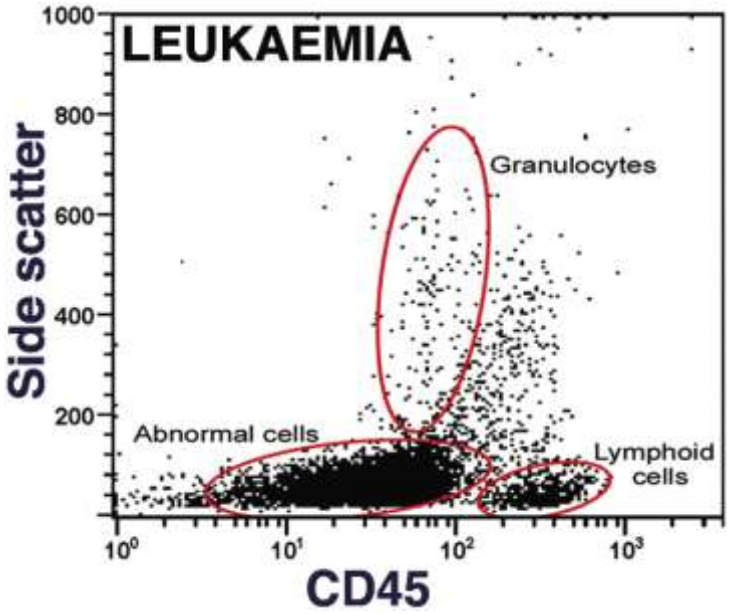
Characteristic markers:

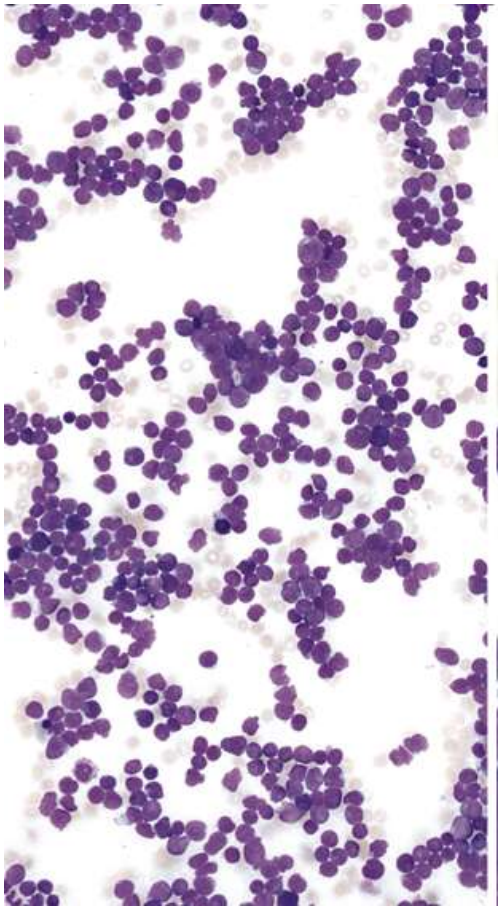
CD117 usually positive may be weak
CD33 positive
MPO strongly positive
HLADR negative
CD 9 positive

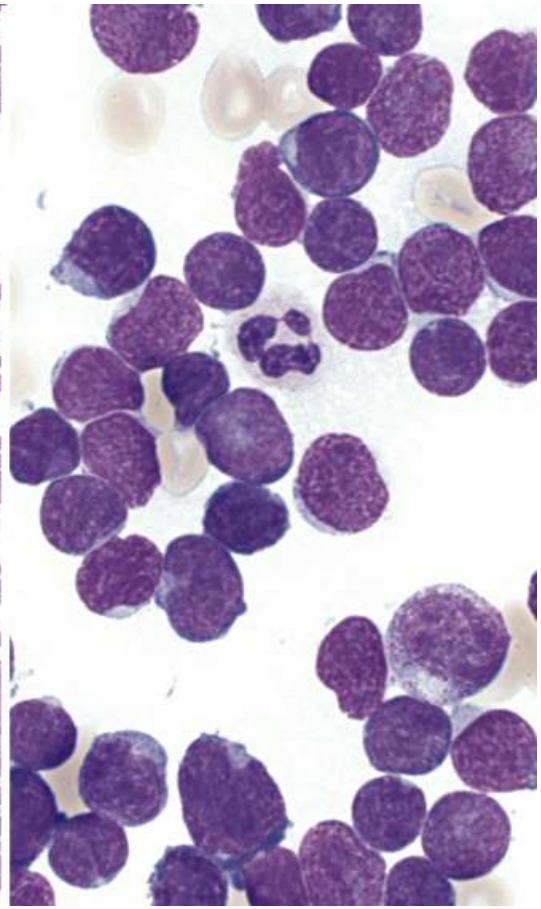
Case 2

12 year old boy
Lethargy and night sweats
Hb 7 WCC 70 Plts 50

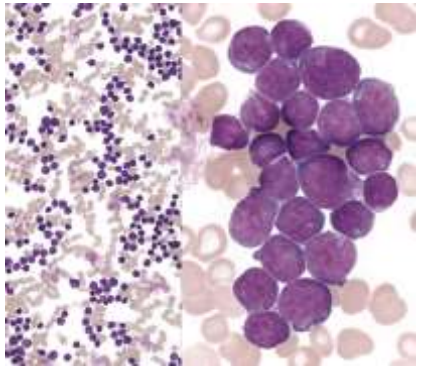




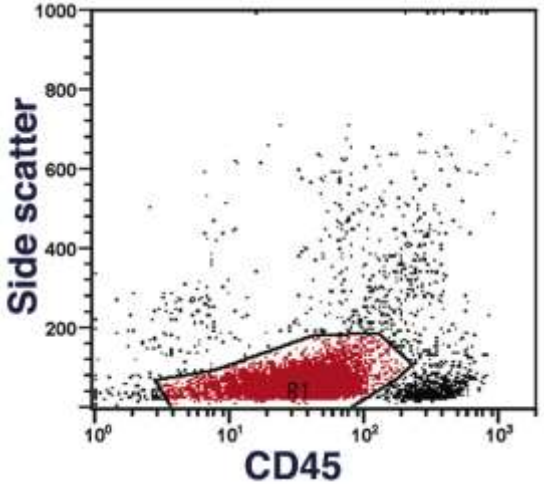


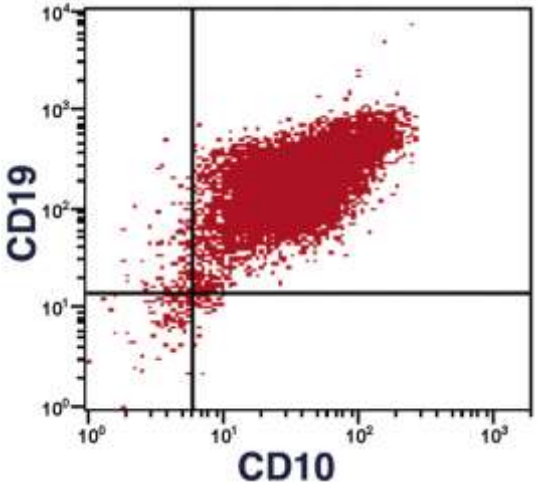


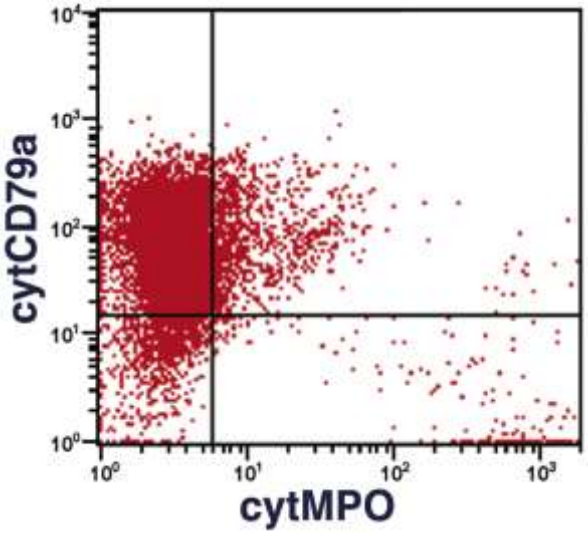
Case 2

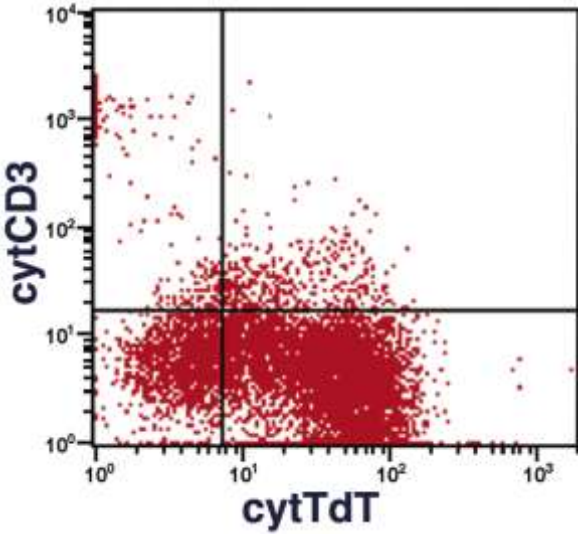


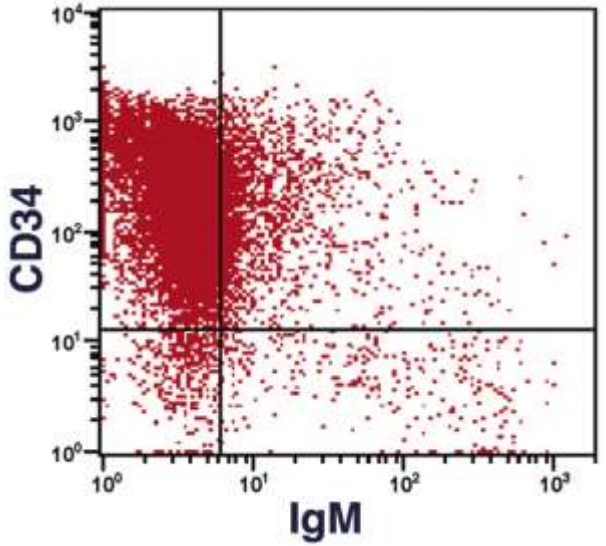
Blast cell population









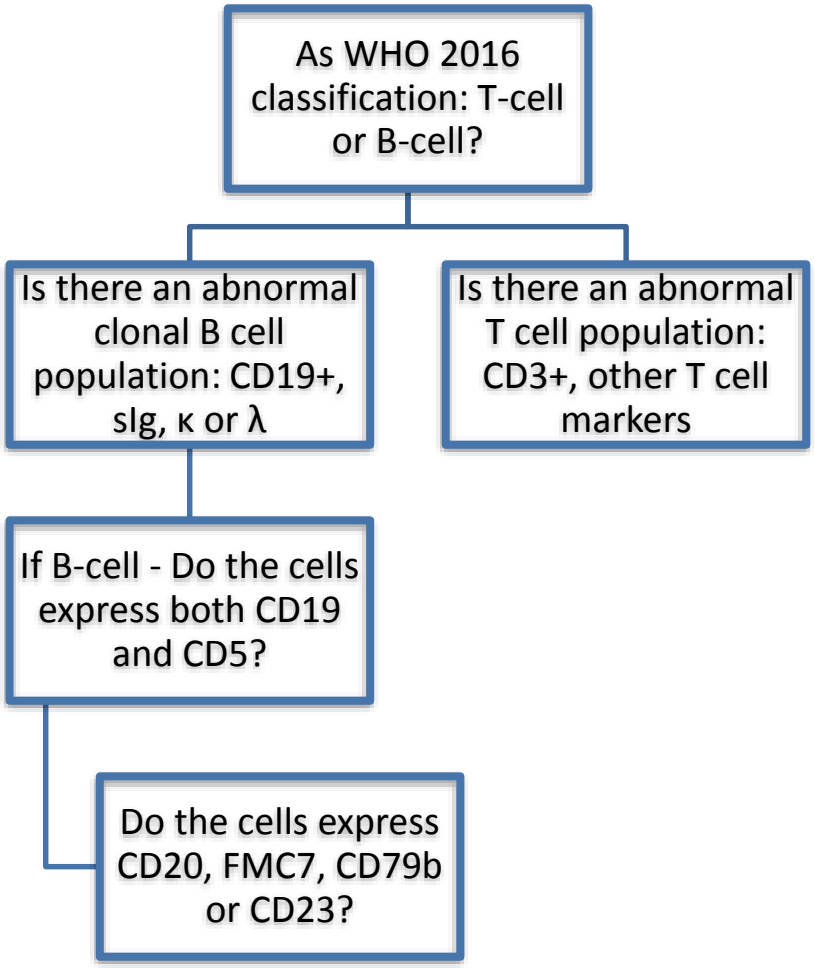


Case 2 B-cell precursor lymphoblastic leukaemia/lymphoma

Characteristic markers:

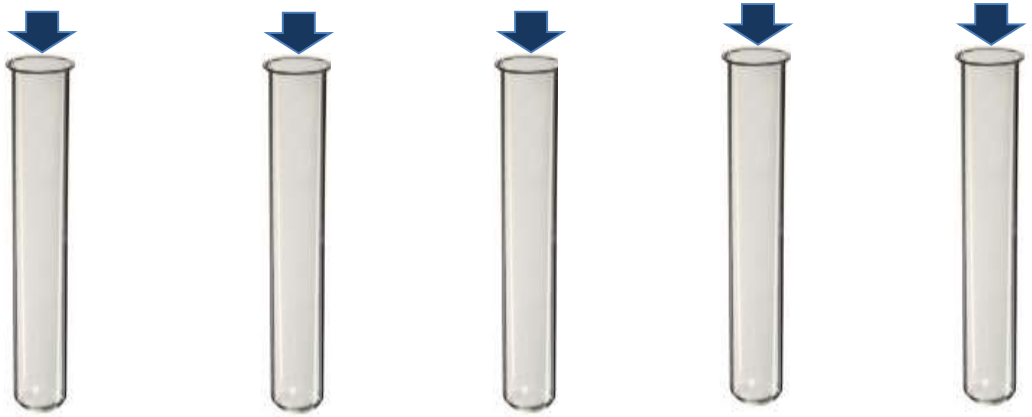
CD117 negative
CD19 positive
CD10 positive
CD79a positive
TdT positive
MPO negative
CytCD3 negative

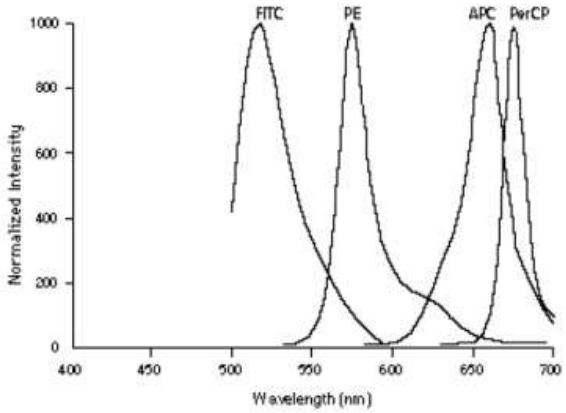
Chronic lymphoproliferative disorders



Mantle cell lymphoma and Chronic lymphocytic leukaemia are the 2 major B cell chronic lymphoproliferative diseases that express CD5 and CD19.

Practicalities: how to perform the chronic lymphoproliferative panel

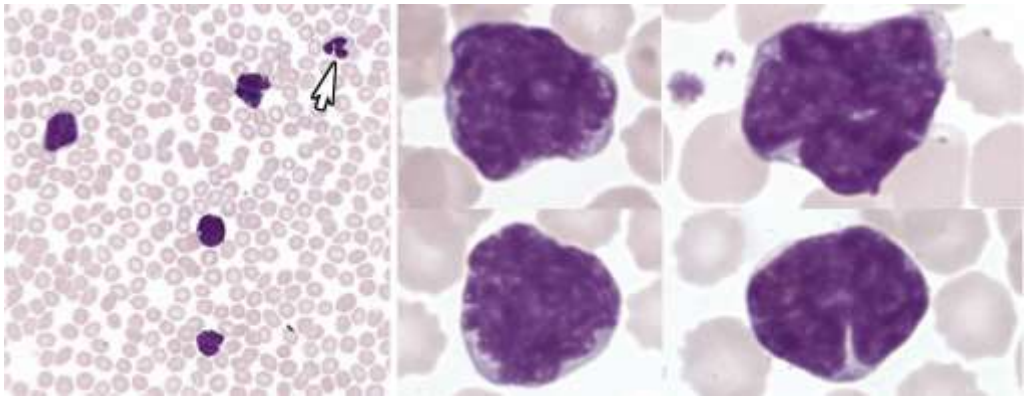


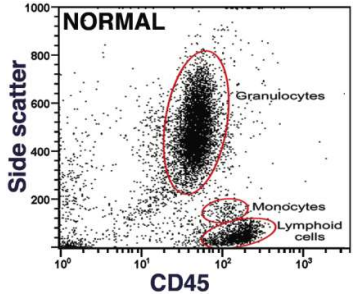


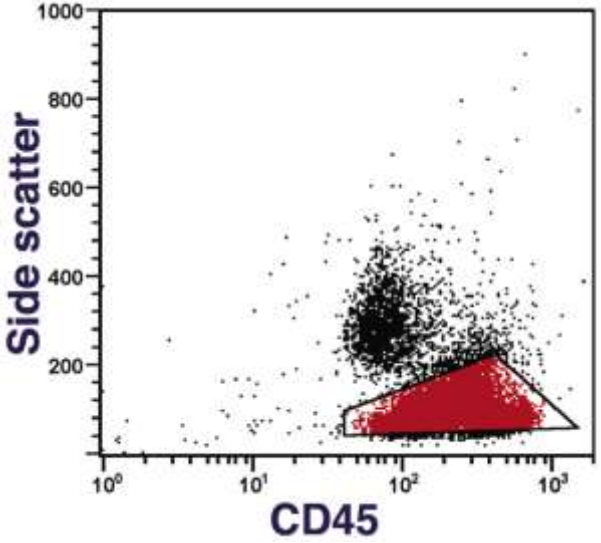
κ *FITC* λ *PE* CD19 *PCP* CD5 *APC* CD45 *APCCY7*	FMC7 *FITC* CD23 *PE* CD19 *PCP*	CD79b *FITC* CD20 *PCP* CD200 *PE*	CD38 *FITC* IgM *PE* CD19 *PCP*	CD5 *FITC* CD10 *PE* CD19 *PCP*

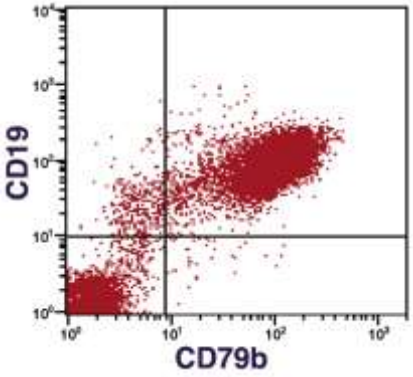
Case 3

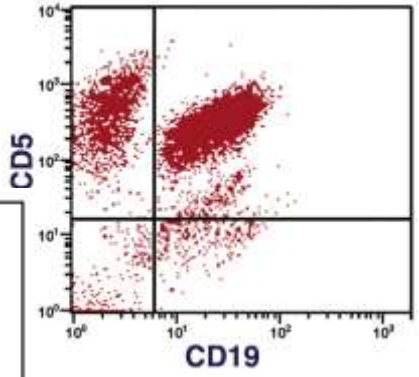
65 year old male, just retired
Lethargic, abdominal fullness
Hb 80, WCC 30, platelets 90

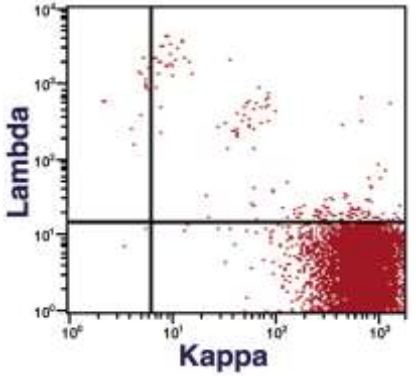


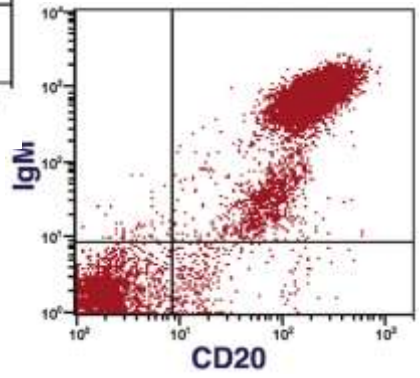


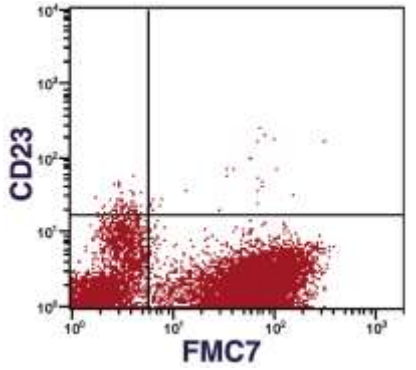










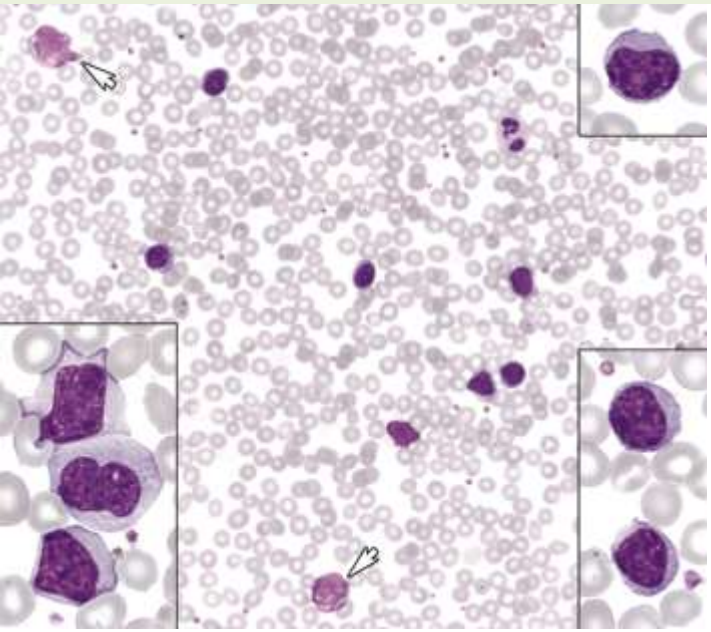


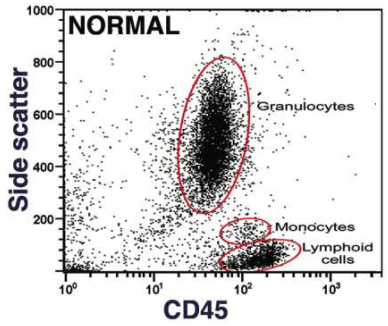
Case 3 Mantle Cell Lymphoma

Characteristic markers:

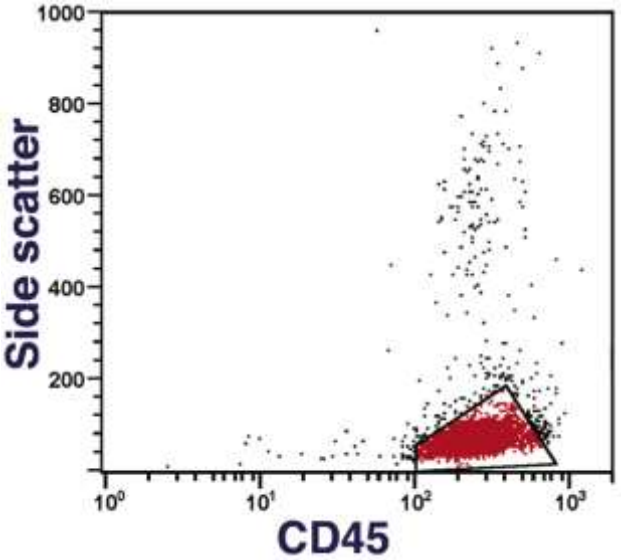
CD19 and CD5 positive
CD23 negative
CD79b positive
FMC7 positive
High level of sIg
Strong kappa or lambda expression

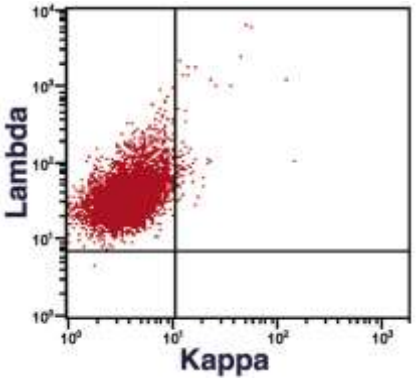
Case 4

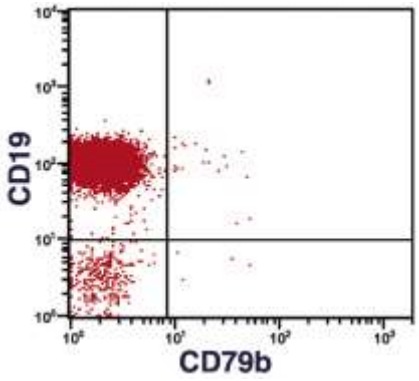


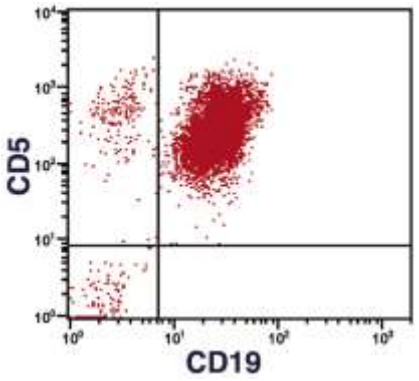


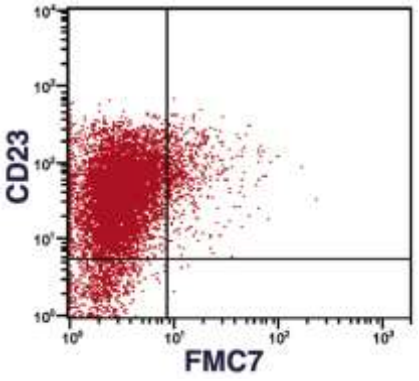
70 year old female
History of HTN
'well person check up' FBC
Hb 130 WCC 20 L 16 Plt 180

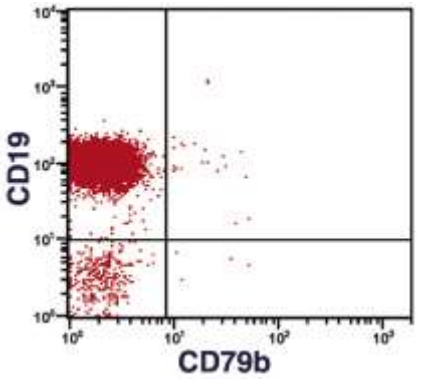










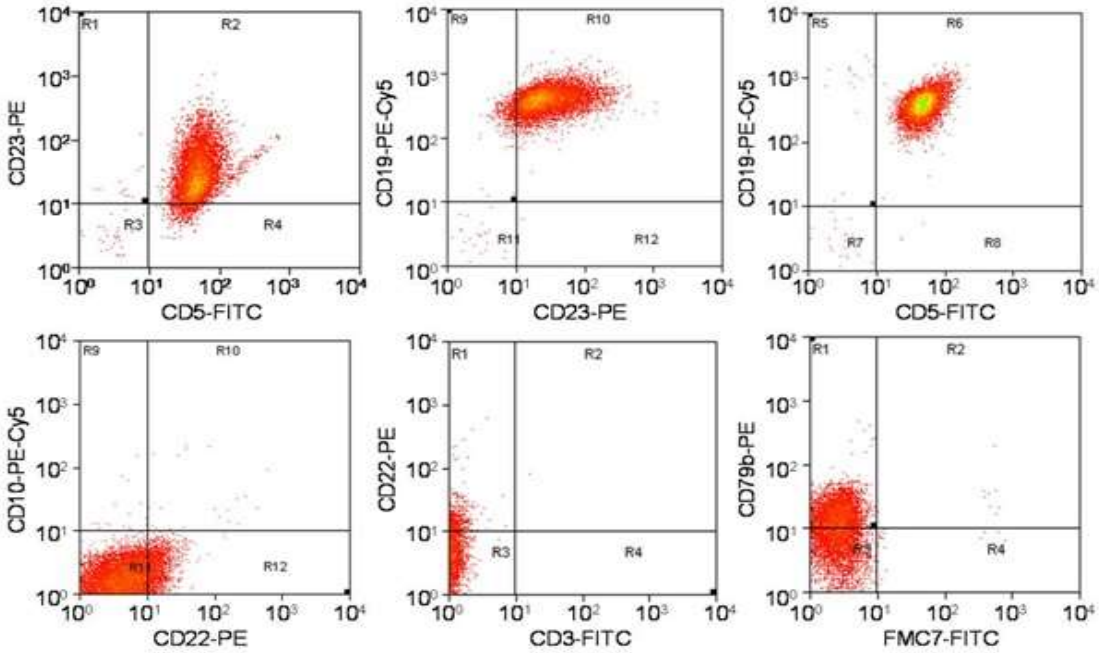


Case 4 : B-cell Chronic Lymphocytic Leukaemia

Characteristic markers:

CD19 and CD5 positive
CD23 positive
CD79b negative
FMC7 negative
Low level of sIg – may be difficult to detect clonality

B-cell CLL Scoring system



- ✓ weak sIg
- ✓ CD5 +ve
- ✓ CD23 +ve
- ✓ Absent/low CD79b
- ✓ low/absent FMC7.

92% of CLL score 4 or 5, 6% score 3 and 2% score 1-2.

Moreau et al, 1997; Okaly et al, Journal of Clinical and Diagnostic Research, 2013.

CD5 negative B-cell chronic lymphoproliferative disorders

CD19 positive

High levels of sIg so CD79b positive

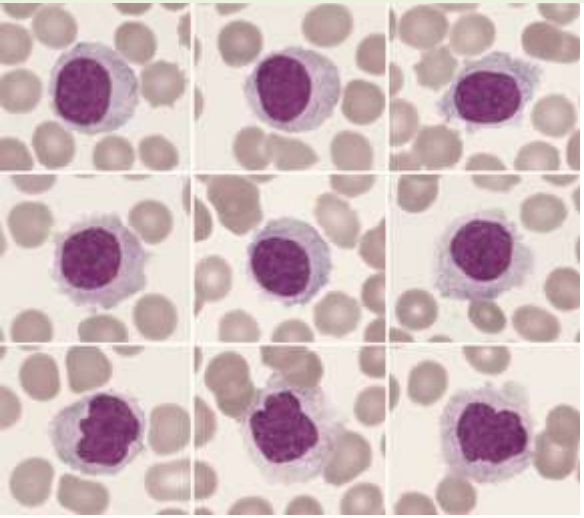
Obvious clonality

High levels of CD20 - FMC7 is positive

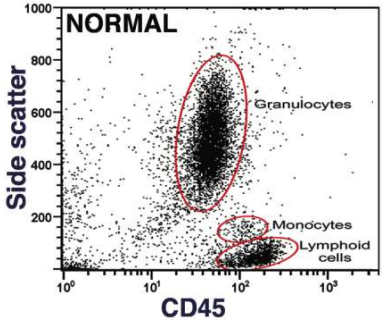
CD10 is positive in FL

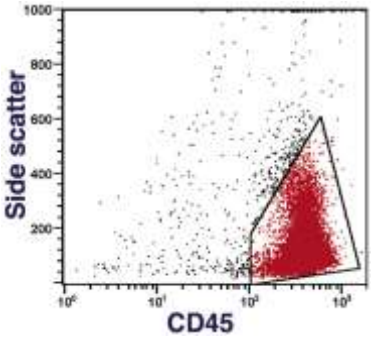
Flow cannot separate cases of LPL vs MZL

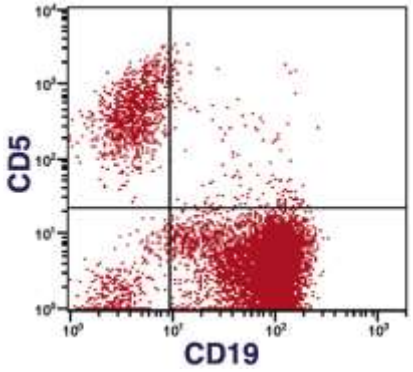
Case 5

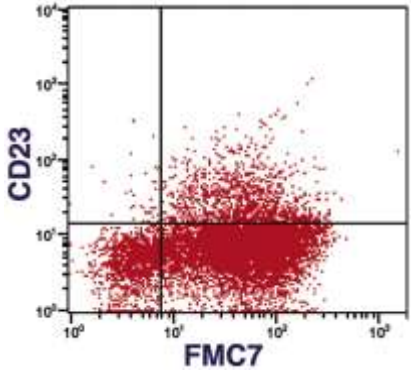


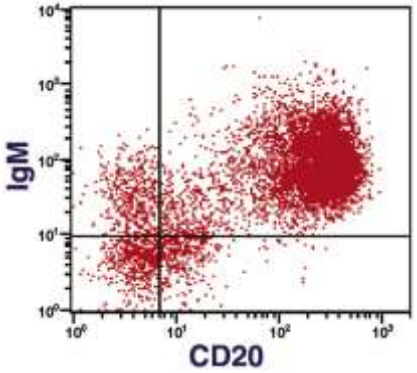
70 year old male
Atypical infection
Pancytopenia
Early satiety
WCC 12
Hb 70
Platelets 45

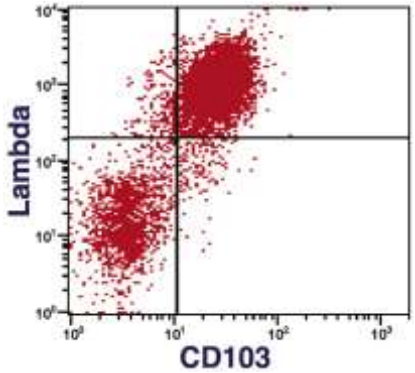


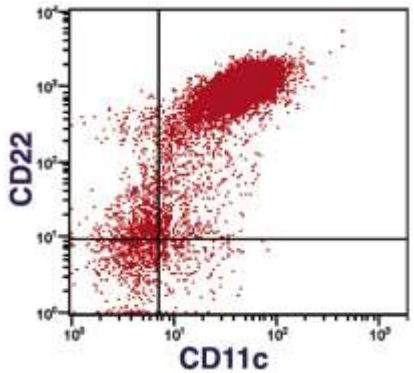


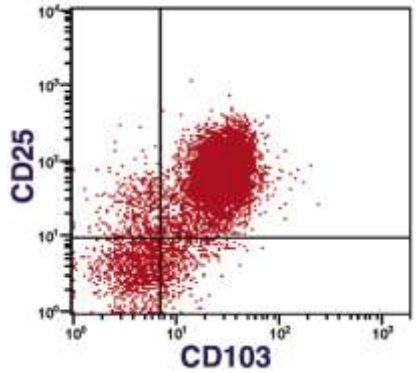












Case 5 : Hairy cell Leukaemia

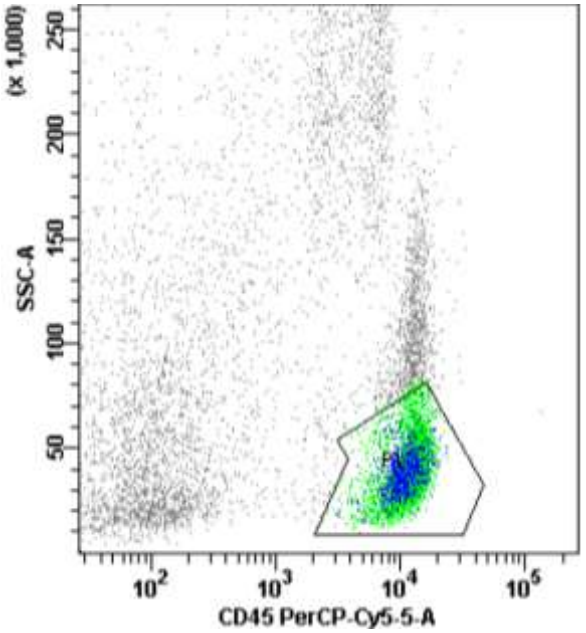
Characteristic markers:

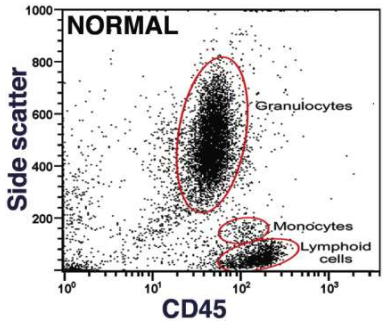
CD19 and CD5 negative
CD79b positive
CD20 strongly positive
FMC7 positive
CD11c, CD103 and CD25 positive

Common phenotypes of B cell lymphoproliferative disease

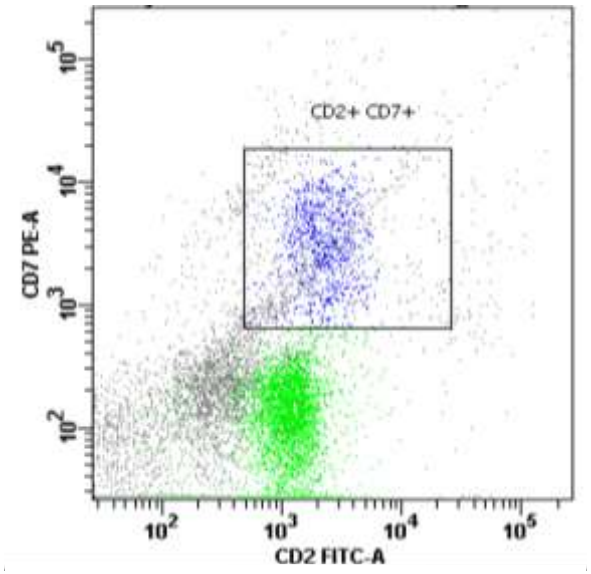
Diagnosis	CD5	CD10	CD19	CD20	CD23	CD79b	FMC-7	CD25	CD11c	CD103
CLL/SLL	+	-	+	+(w)	+	-	-	-/+	+/-	-
Mantle cell lymphoma	+	-	+	+	-	+	+	-	-	-
Follicular Lymphoma	-	+	+	+	-/+	+/-	+/-	-	-	-
Marginal zone lymphoma	-	-	+	+	-	+/-	+/-	-/+	+	-
Hairy cell leukemia	-	-	+	+	-	+/-	+/-	+/-	+	+

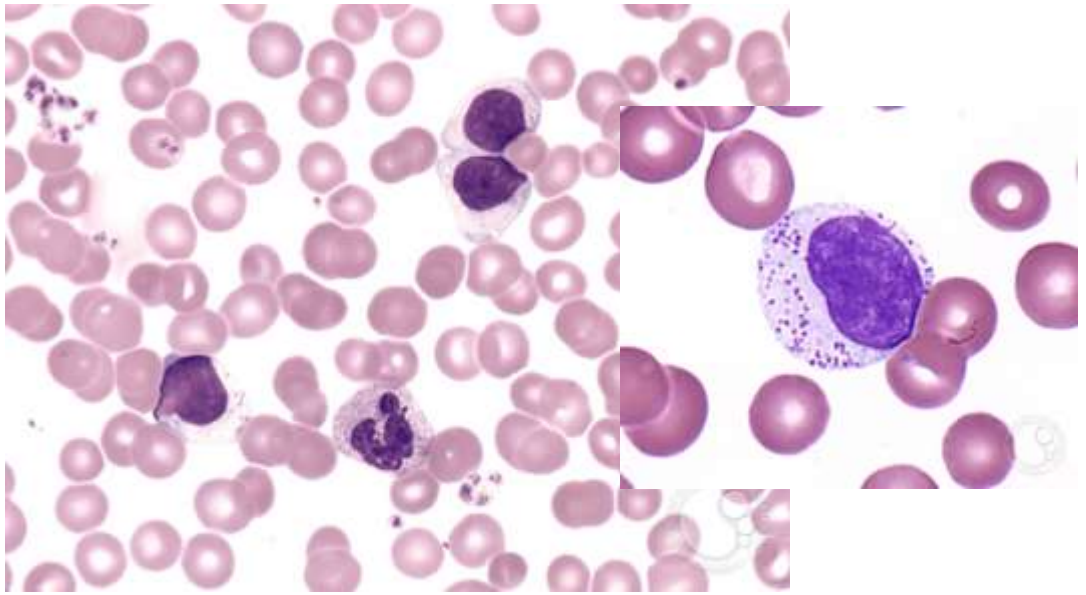
Case 6



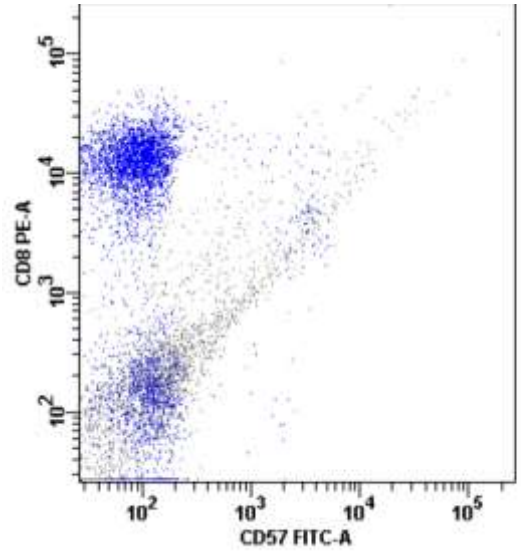


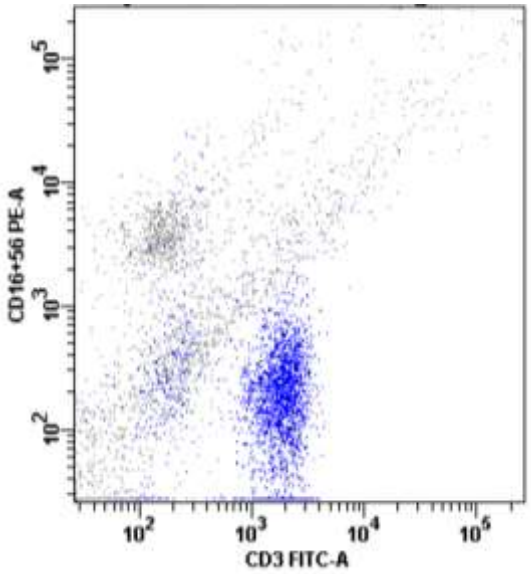
55 year old female
History of Rheumatoid arthritis
RhF+ve
Hb 130, Plts 225, WCC 11
Neutropenia Neut 0.7

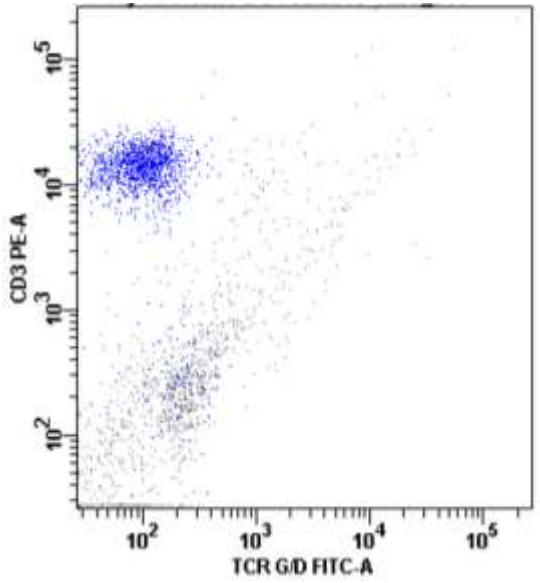


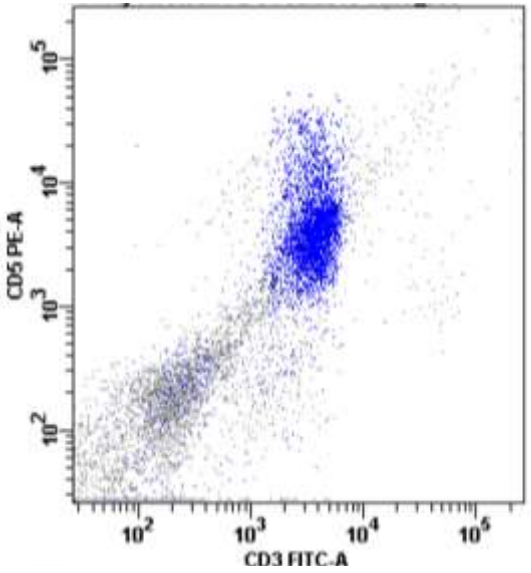


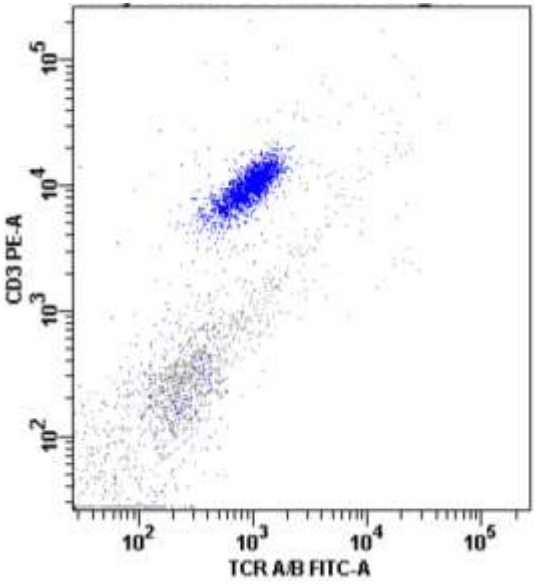
Case 6


CD8 PE-A
CD57 FITC-A


CD16+56 PE-A
CD3 FITC-A


CD3 PE-A
TCR G/D FITC-A


CD5 PE-A
CD3 FITC-A


CD3 PE-A
TCR A/B FITC-A

Case 6: Large Granular Lymphocytosis

Characteristic markers:

CD 2 positive
CD3 positive
CD4 negative
CD8 positive
Often CD16, CD57 positive
TCR A/B or D/G expression