Flow Cytometry Competency Requirements for Haematology RCPA Exams

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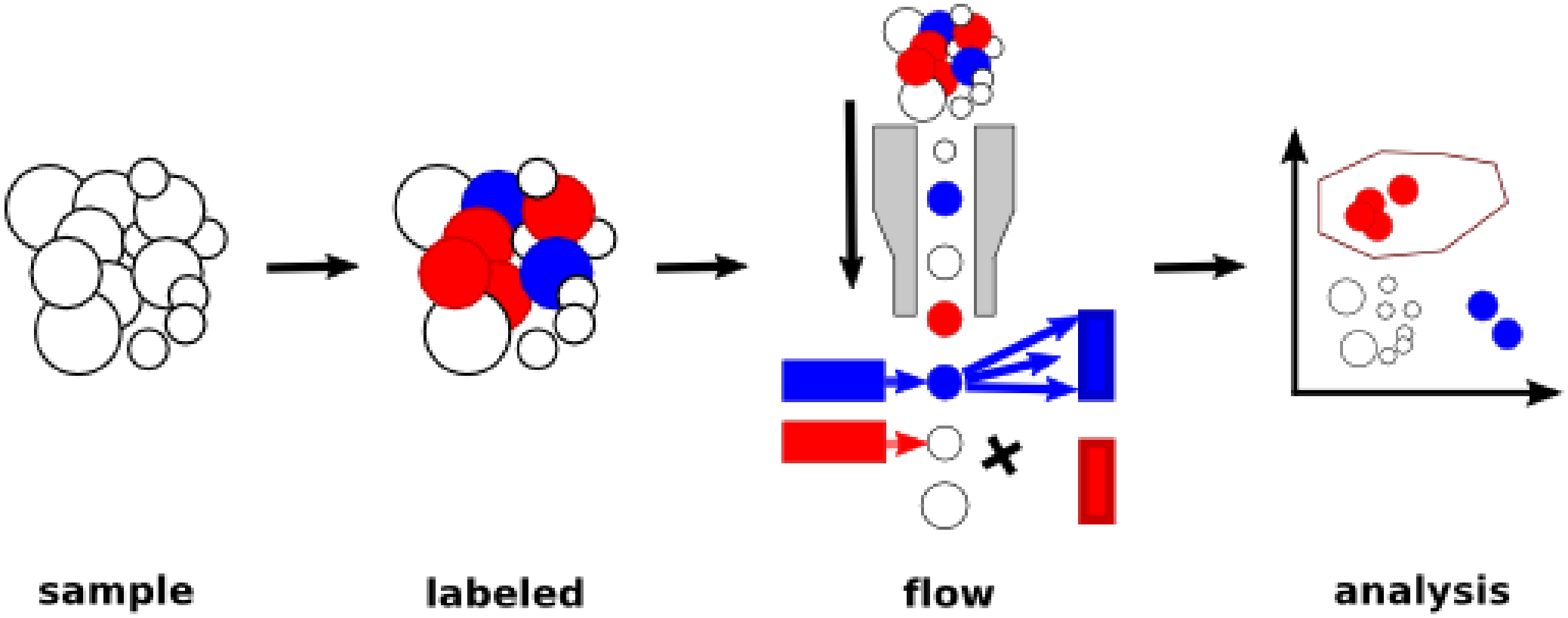
What does the RCPA say you need to know?

- Flow cytometry technologies
- Acute leukaemia and MRD
- Lymphoproliferative disorders
- CD34 cells
- Paroxysmal nocturnal haemoglobinuria
- Assessment of platelet disorders
- Feto-maternal haemorrhage
- Red cell membrane disorders
- Myeloma

What is flow cytometry?



http://www.bioinformin.net/cytometry.php

How does a flow cytometer work?

- Fluidics
- Lasers
- Detection
- Electronic conversion
- Analysis

Fluidics

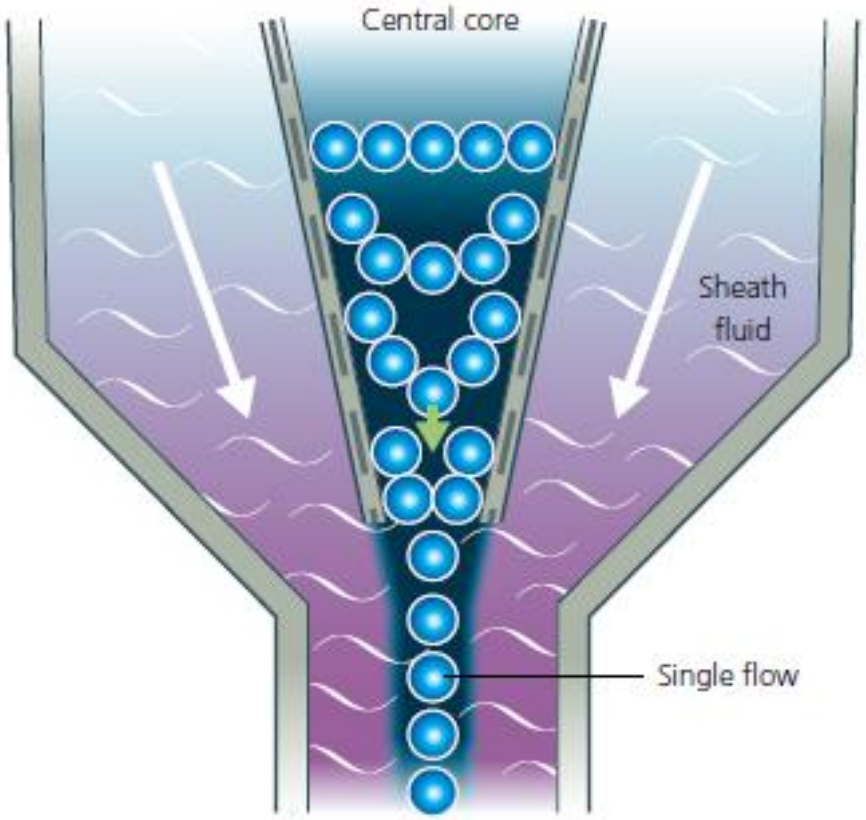
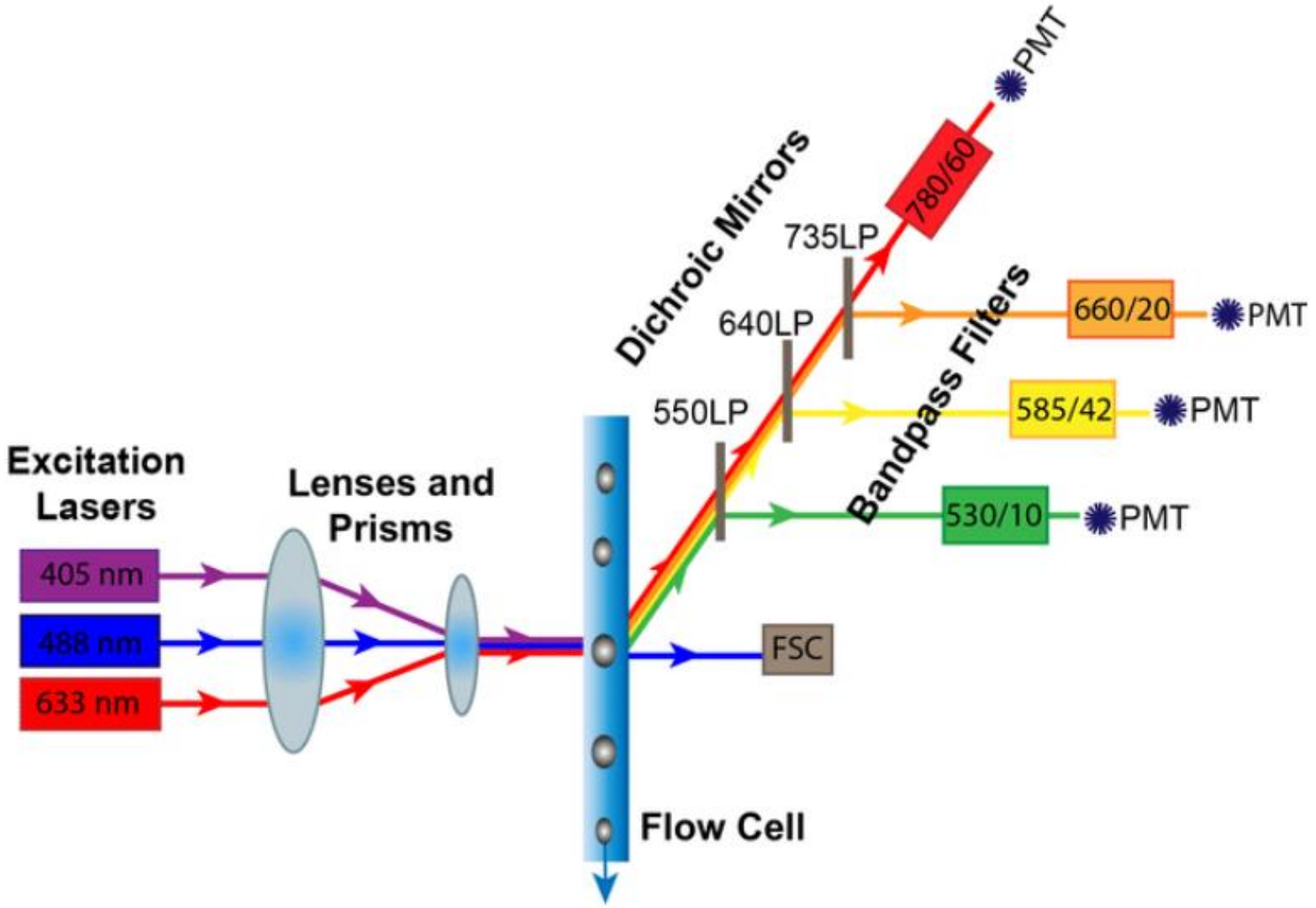


FIGURE 1 **Hydrodynamic focusing produces a single stream of particles**

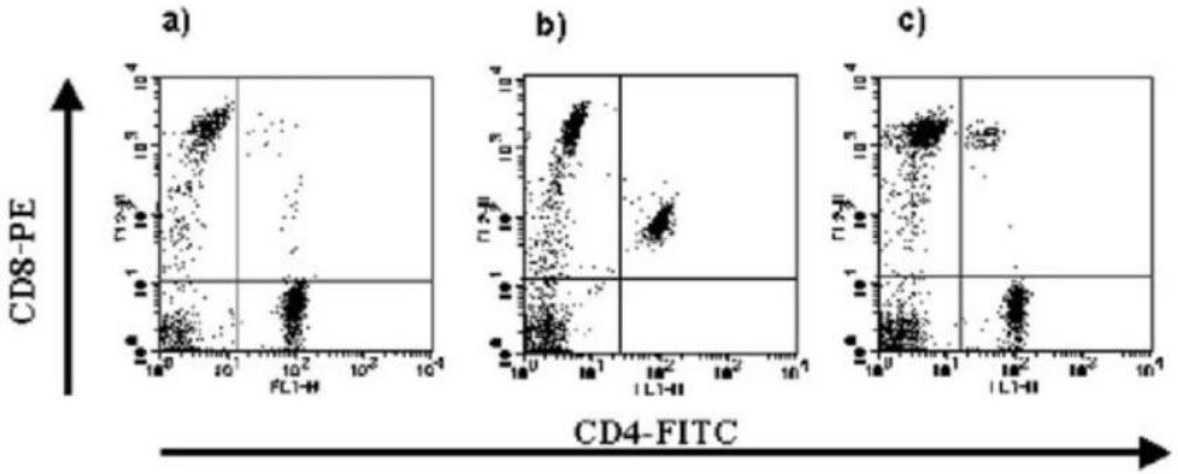
Rahman, Introduction to Flow Cytometry

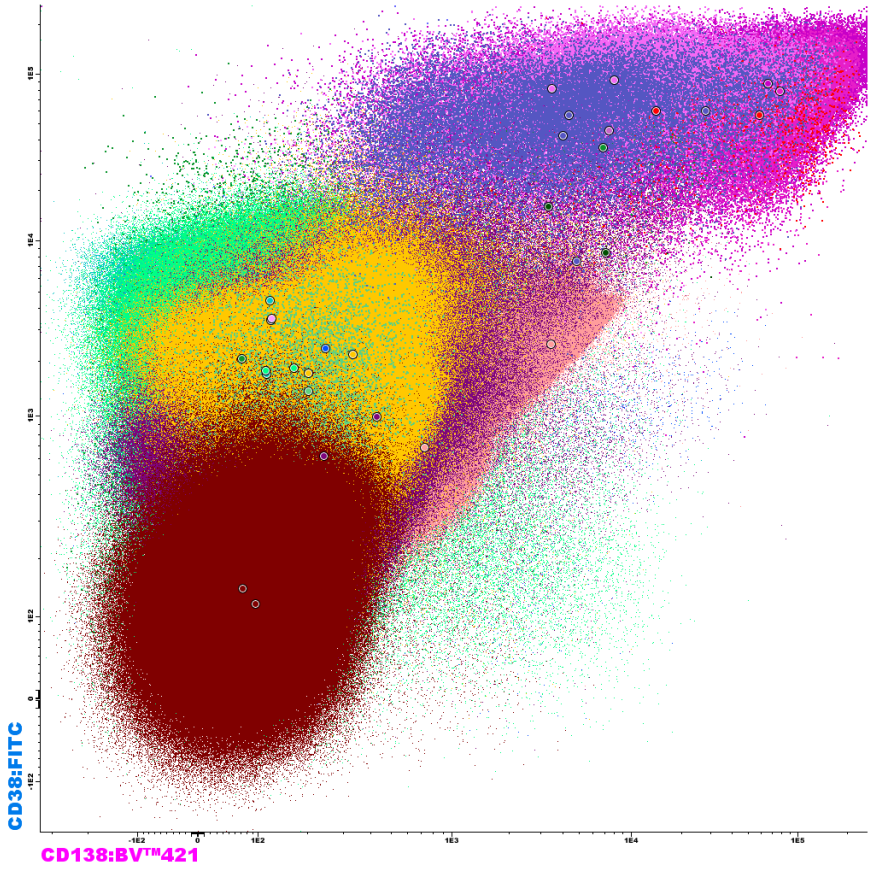
Lasers, Detections and Electronic conversion



Demystifying the Flow Cytometry Optics System: A Peek Under the Hood, Adaptive Biotechnologies

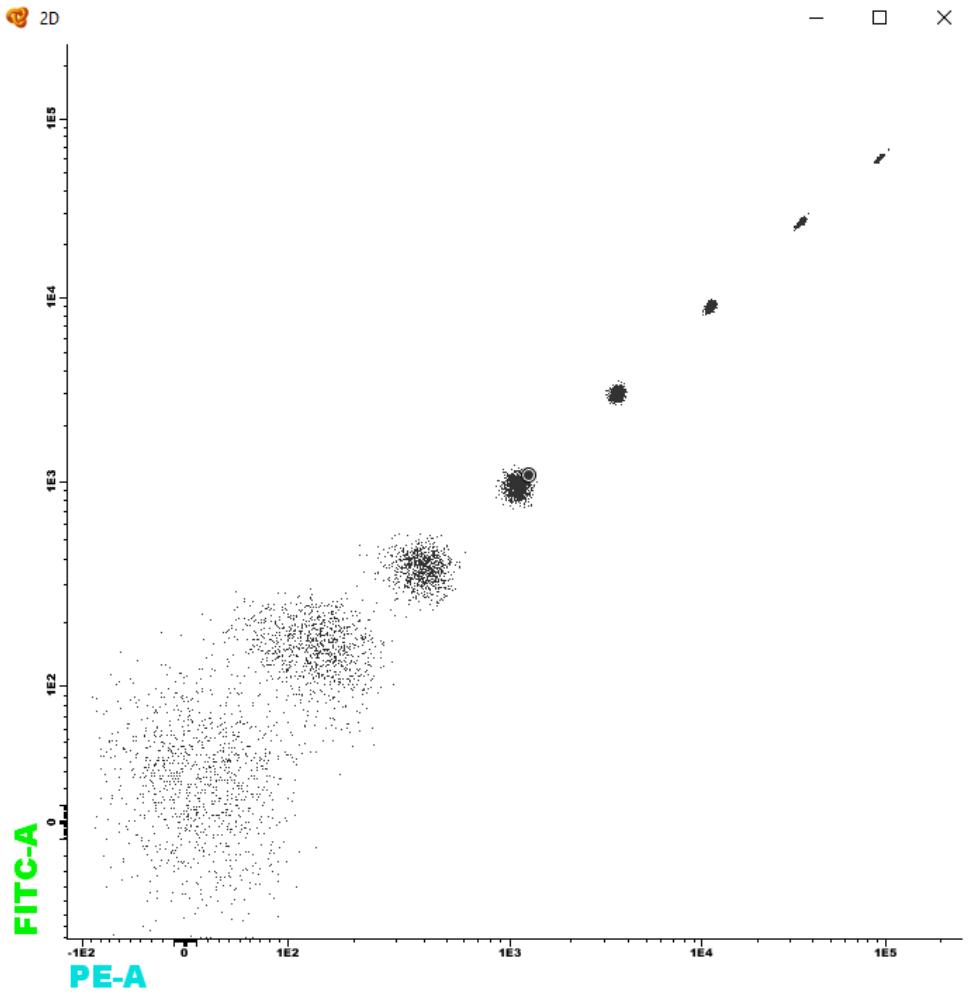
Analysis





Flow cytometry QC/QAP

- Checking the cytometer against a known reference standard (often bead based)
- Ensuring stability of fluoresce for antigens on normal cell populations
- Lot checking antibodies
- Ensure training of staff

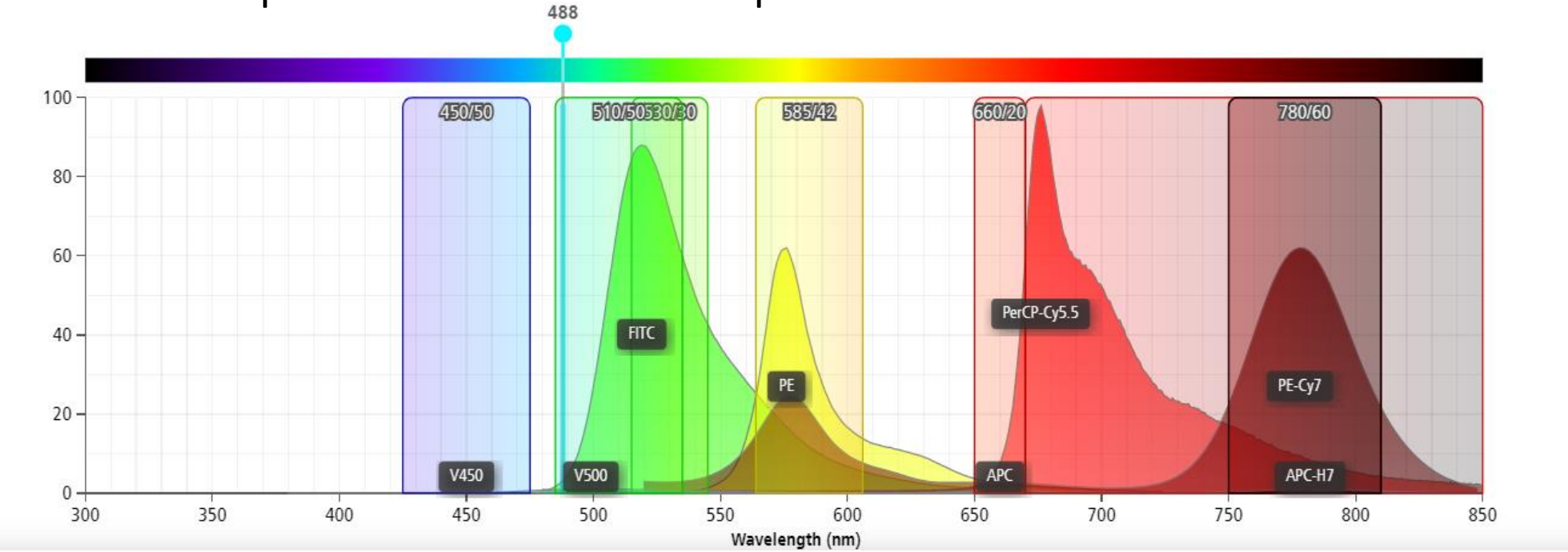


Things to know about flow cytometry instruments

- Number of lasers
- Number of colours (detectors)
- Level of automation
- Flow rates
- Associated software
- Sorting capability

Properties of fluorochromes and panel design

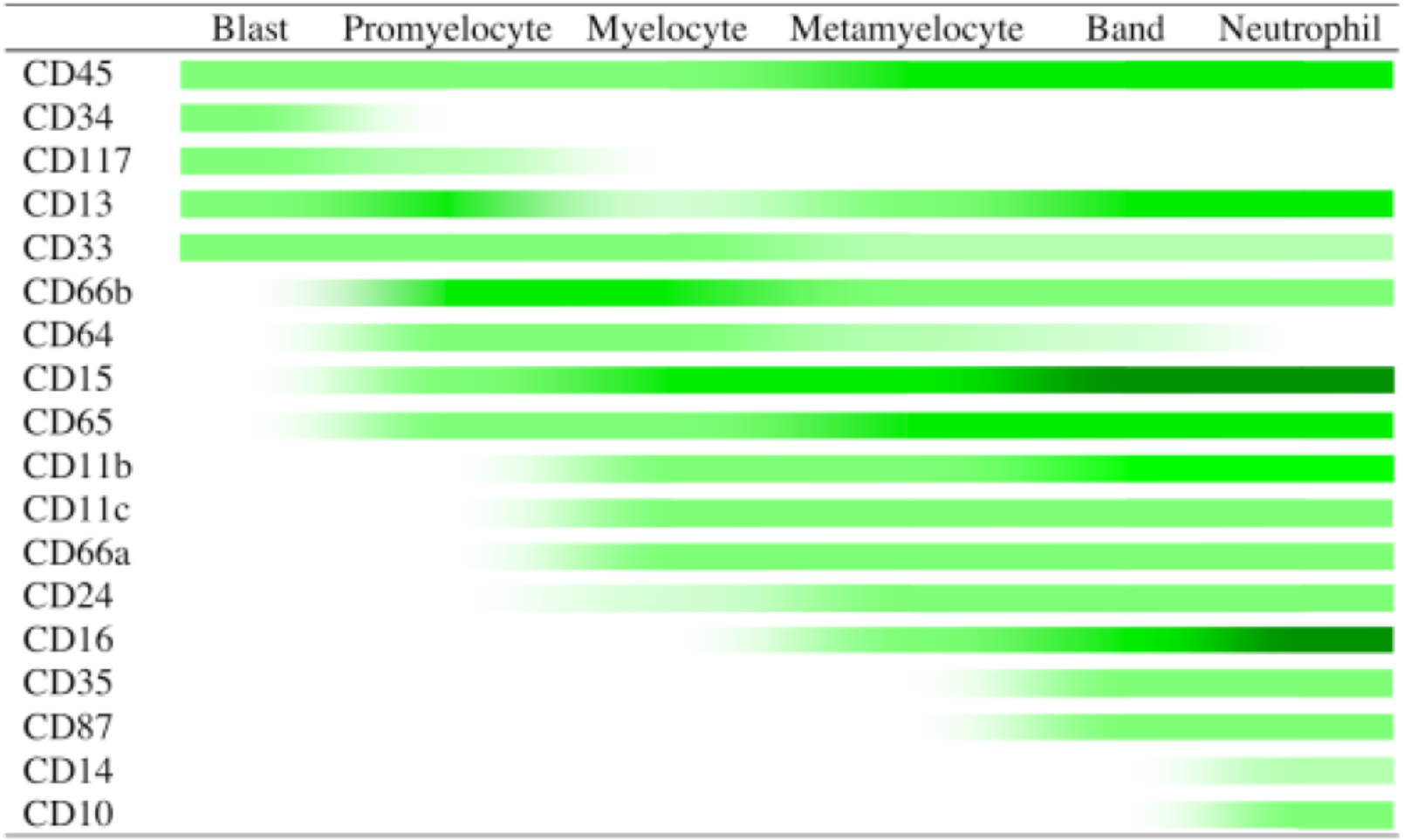
- Know the clinical question and use the right amount of antibodies to answer it
- Match fluorochromes and antigen density (ie bright fluorochromes with low density antigens and vice versa)
- Optimise panel for your instrument
- Minimise spillover and allow for compensation



Basic principles of haematologic malignancies

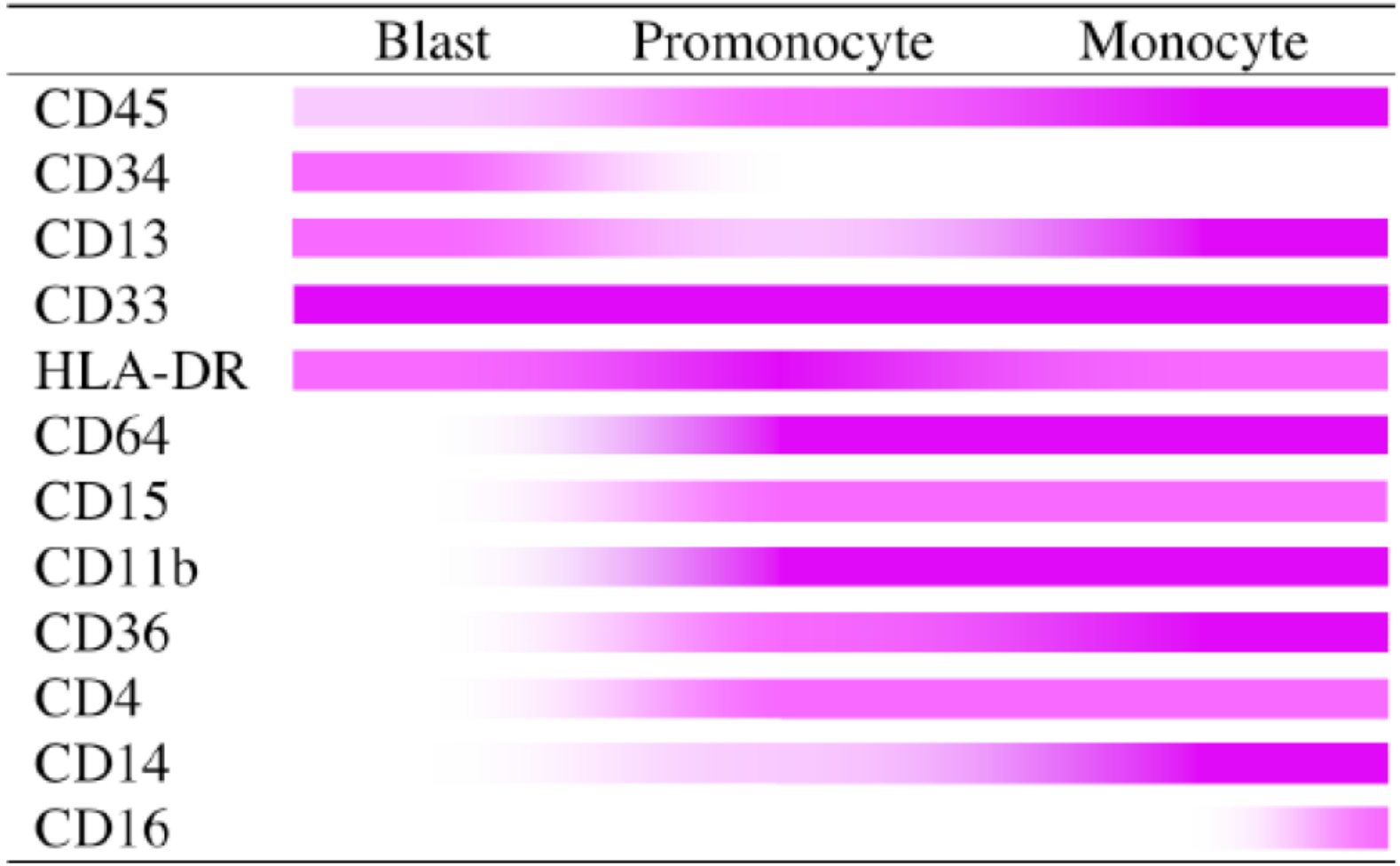
- Normal
  - Antigens expressed in consistent and reproducible patterns with maturation
- Neoplastic
  - Increased or decreased normal antigens
  - Asynchronous maturational expression
  - Aberrant antigen expression
  - Homogeneous expression

Normal Granulocytic Maturation



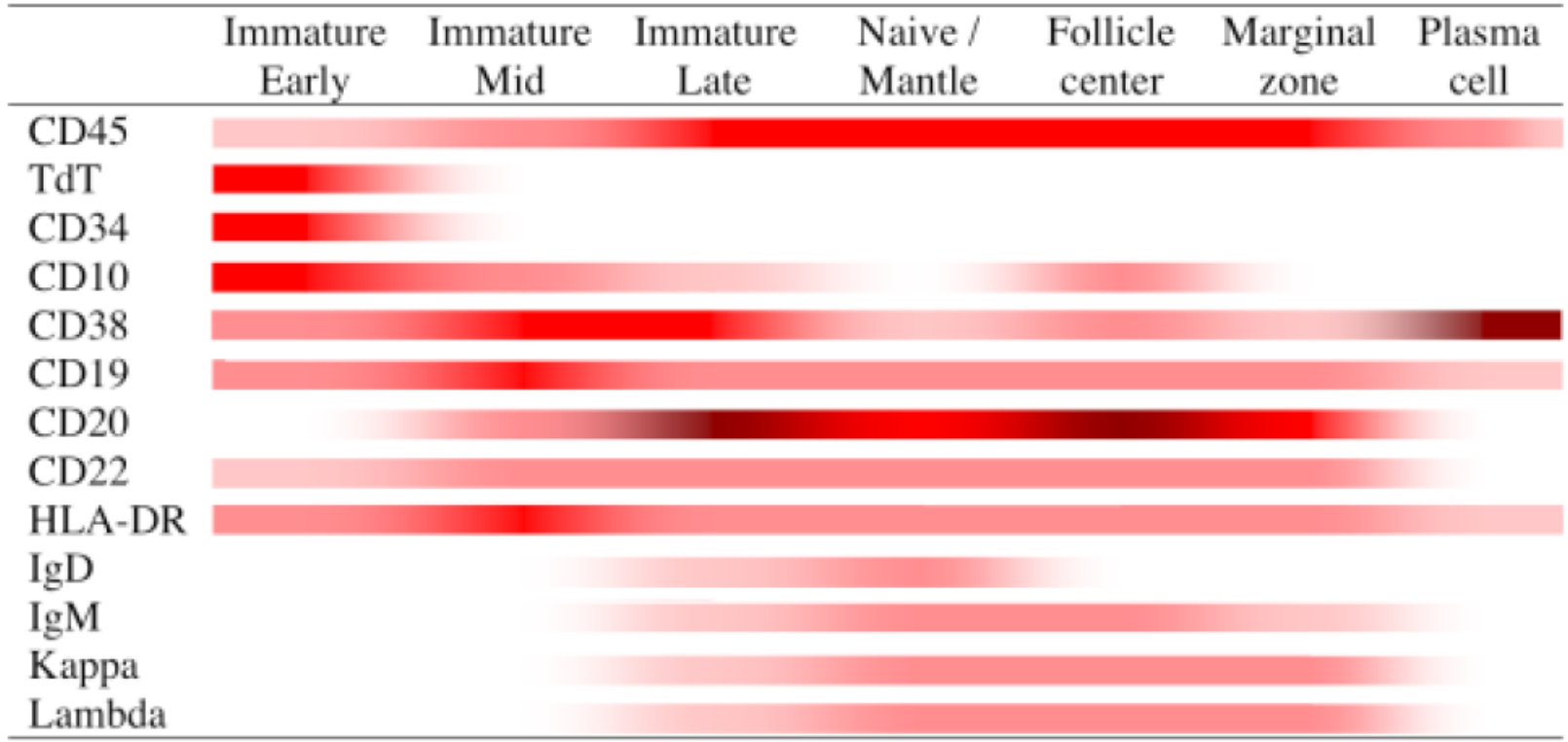
Wood and Borowitz (2006) Henry's Laboratory Methods

Normal Monocytic Maturation



Wood and Borowitz (2006) Henry's Laboratory Methods

Normal B cell Maturation



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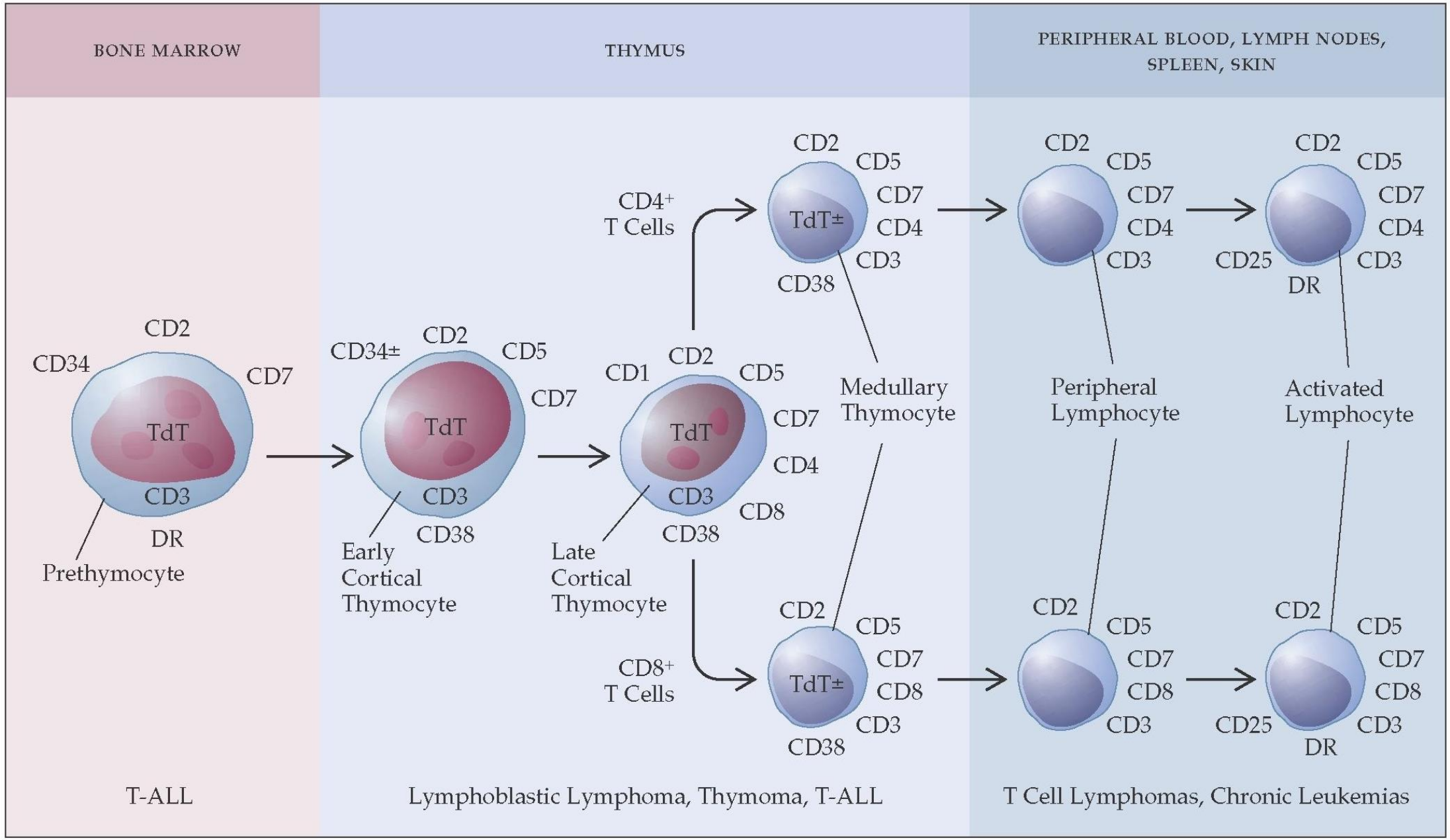
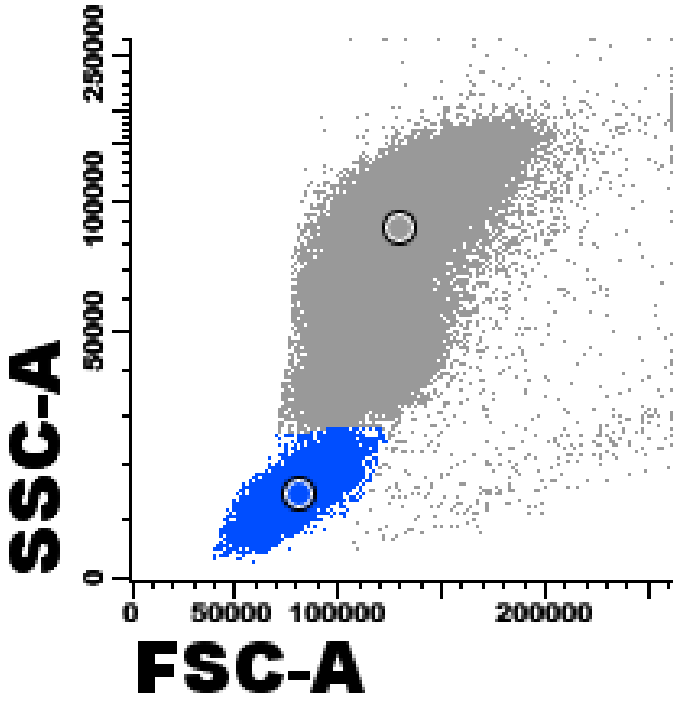
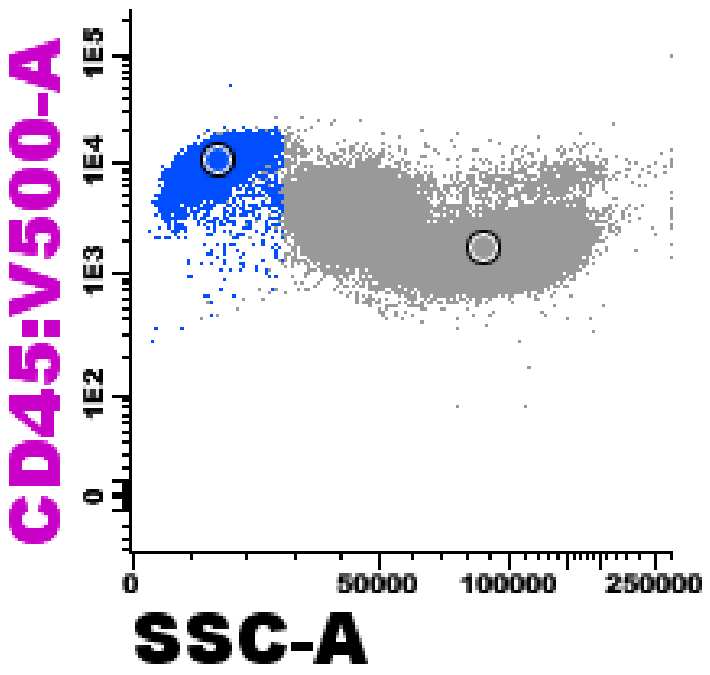
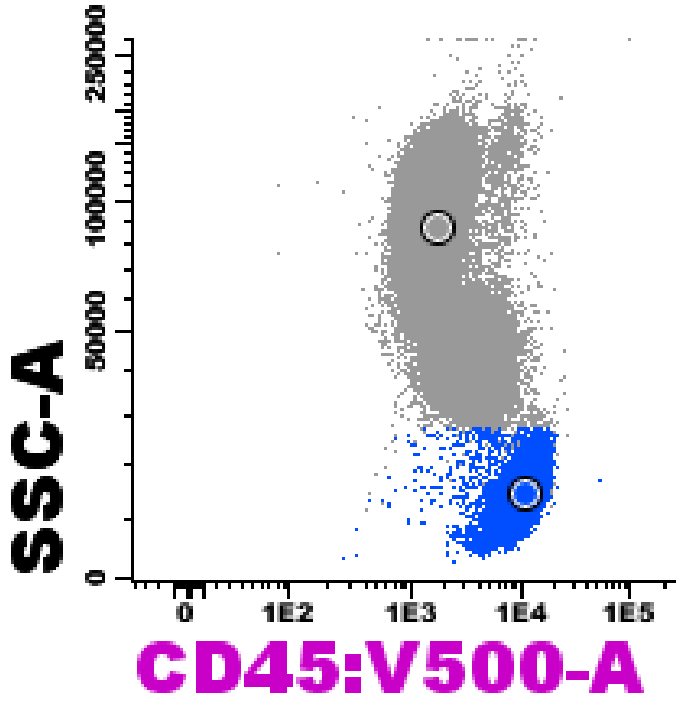

BONE MARROW
THYMUS
PERIPHERAL BLOOD, LYMPH NODES, SPLEEN, SKIN
CD2
CD34
CD7
TdT
CD3
DR
Prethymocyte
CD34±
CD5
Early Cortical Thymocyte
CD1
CD4⁺ T Cells
TdT±
CD4
CD8
Late Cortical Thymocyte
Medullary Thymocyte
CD8⁺ T Cells
Peripheral Lymphocyte
CD25
Activated Lymphocyte
T-ALL
Lymphoblastic Lymphoma, Thymoma, T-ALL
T Cell Lymphomas, Chronic Leukemias

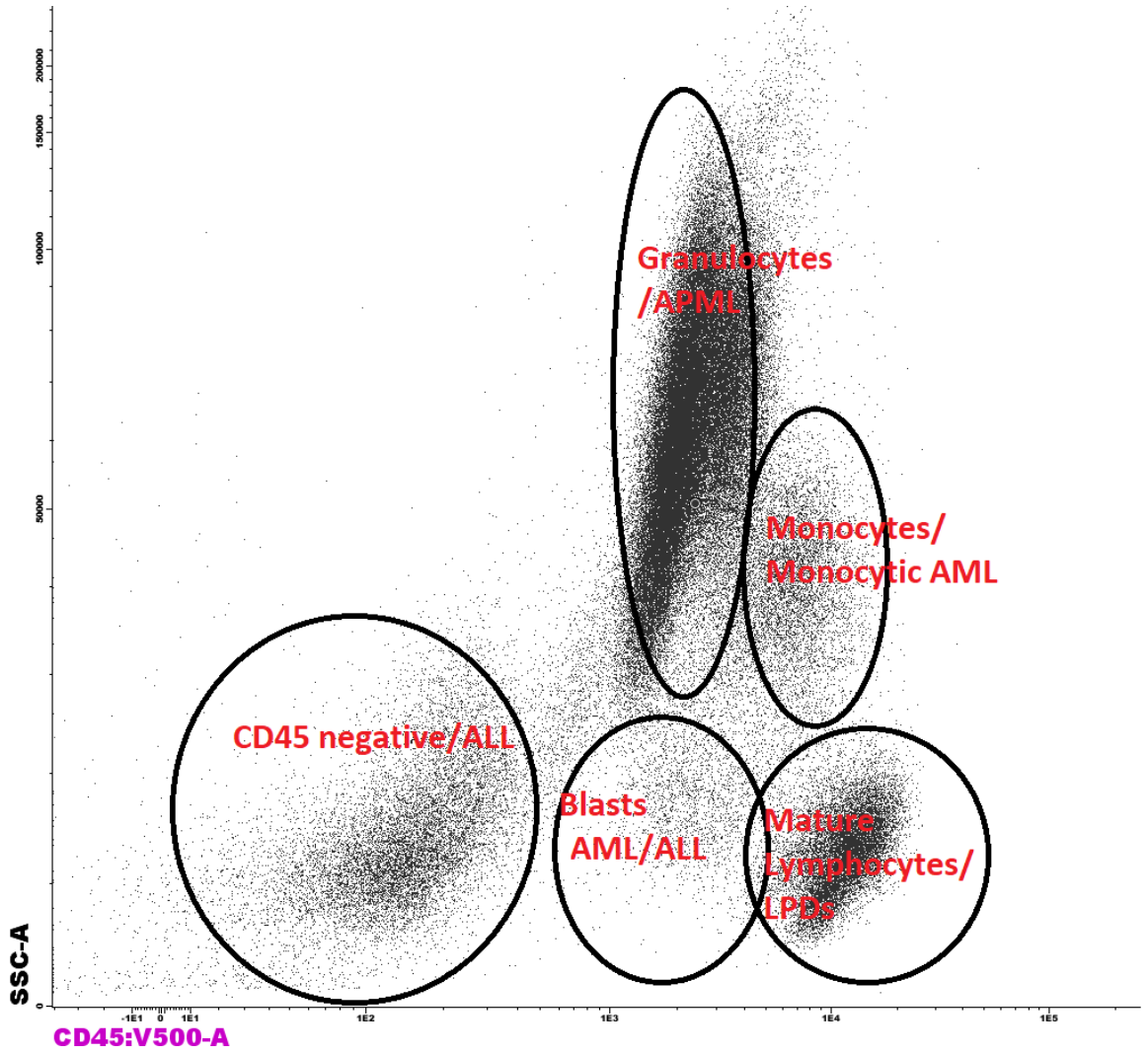
Know your orientation







Know your orientation



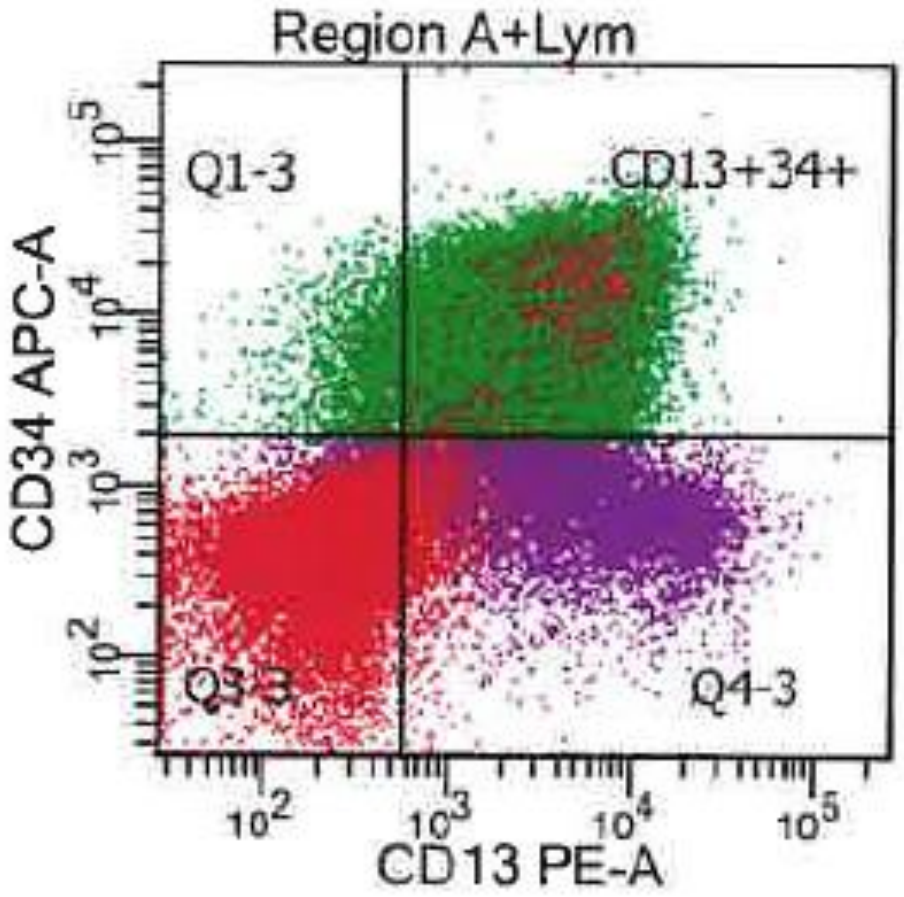
Acute leukaemias

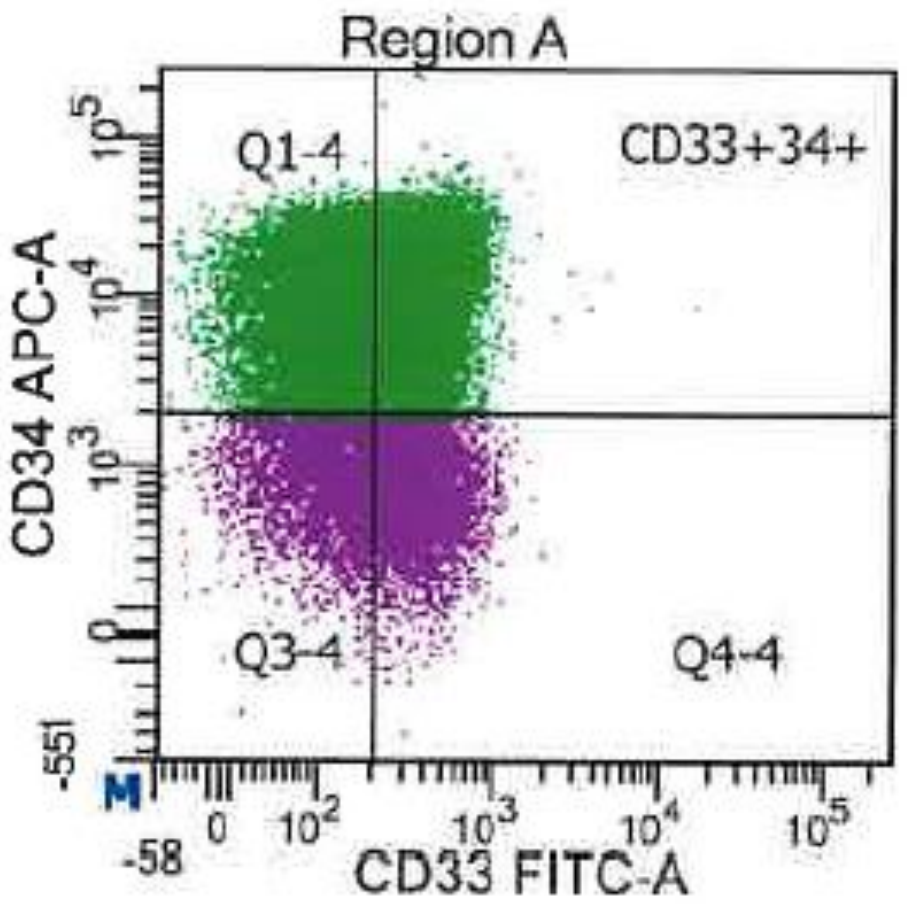
- Acute Myeloid Leukaemia
- Blastic Plasmacytoid Dendritic Cell Neoplasm (rare)
- Acute Leukaemias of Ambiguous Lineage (rare)
- B Lymphoblastic Leukaemia/Lymphoma
- T Lymphoblastic Leukaemia/Lymphoma
- NK Lymphoblastic Leukaemia/Lymphoma (rare)

Acute Myeloid Leukaemia

- Committed to the myeloid lineage
  - MPO or ≥ 2 monocytic markers (CD14, CD64, CD11c)
- Pan Myeloid markers CD13, CD33
- If monocytic differentiation may have and additional or single monocytic population
- Early haematopoietic markers (CD34, CD117)
- May have aberrancy of lymphoid markers (CD19, CD7, CD56, CD22)
- Special cases with distinct profiles: acute promyelocytic leukaemia pure erythroid leukaemia, acute megakayoblastic leukaemia

AML contd


Region A+Lym
Q1-3
CD13+34+
Q3-3
Q4-3
CD34 APC-A
CD13 PE-A
10^2
10^3
10^4
10^5


Region A
Q1-4
CD33+34+
Q3-4
Q4-4
CD34 APC-A
CD33 FITC-A
-551
-58
0
10^2
10^3
10^4
10^5

B-ALL

- Committed to the B cell lineage
  - Strong CD19 with ≥ 1 of CD79a, cCD22 or CD10
  - Weak CD19 with ≥ 2 of CD79a,c CD22 or CD10
- Often positive for CD19, cCD79a, cCD22, CD10, TDT, +/-CD34, +/-CD20
- CD13 and CD33 may be positive and suggestive of BCR/ABL positivity
- CD45 may be absent

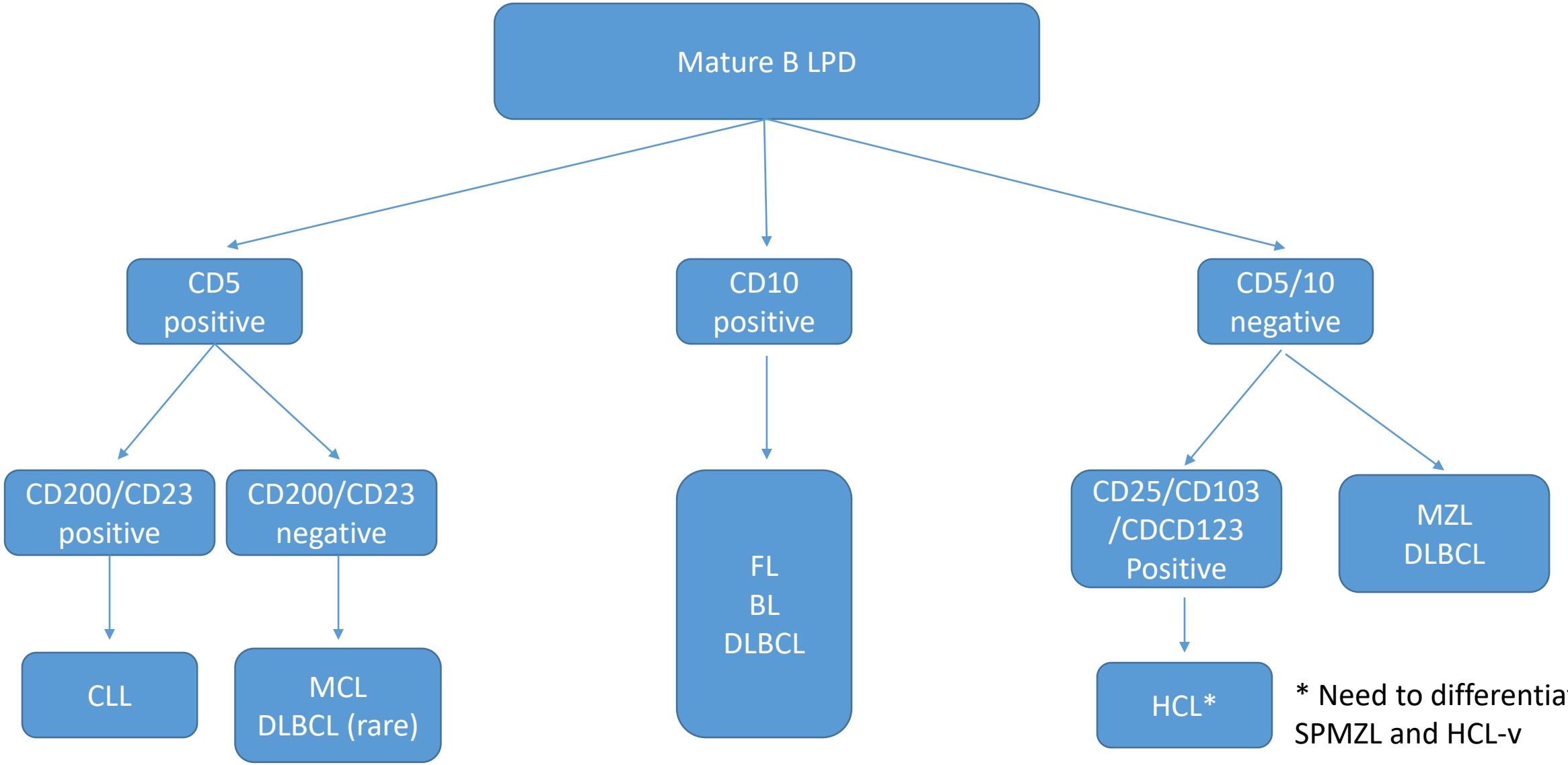
T-ALL

- T ALL
  - CD3 (surface or cytoplasmic)
- Often positive for TDT and CD1a (early markers)
- CD3 is often cytoplasmic
- Aberrancy of T cell markers CD2, CD3, CD4, CD5, CD7
- CD4 and CD8 double positive common but may restricted depending on differentiation (cortical vs medullary)
- May express myeloid markers in early T cell precursor lymphoblastic leukaemia

Mature lymphoproliferative disorders

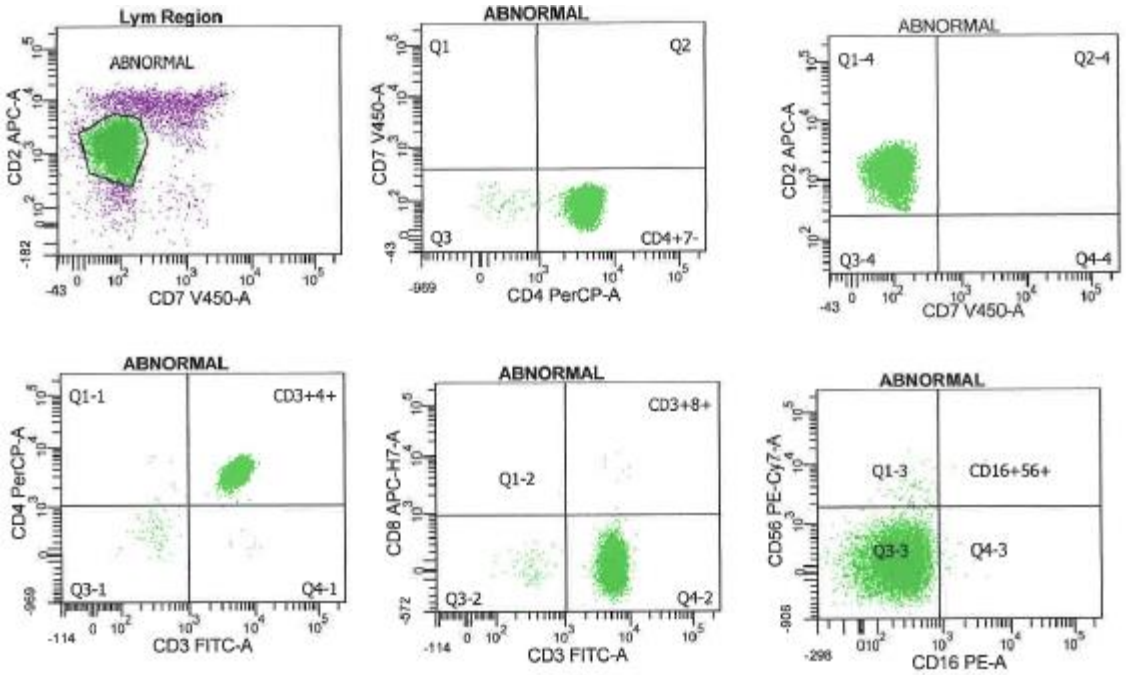
- Located with mature lymphocytes
- Absence of early markers
- B LPDs show an absence of early markers
- T LPDs demonstrate aberrancy of T cell markers

B LPD approach



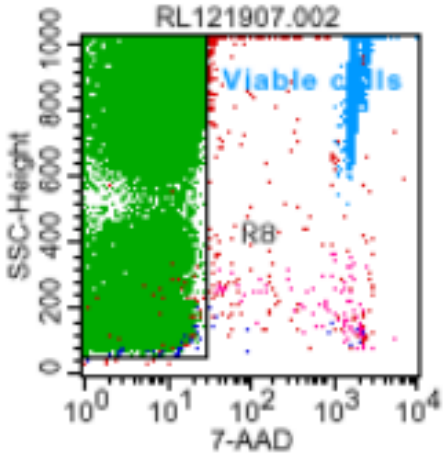
T-LPD

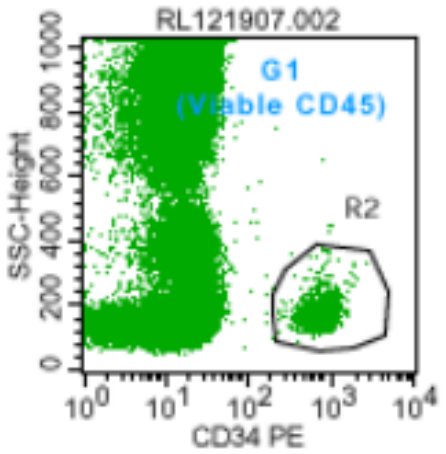
- Many forms with different aberrant expressions
- Key ones to know
  - Sezary syndrome (CD2, CD3, CD4, CD5 with a loss of CD7)
  - AITL (often express CD10)

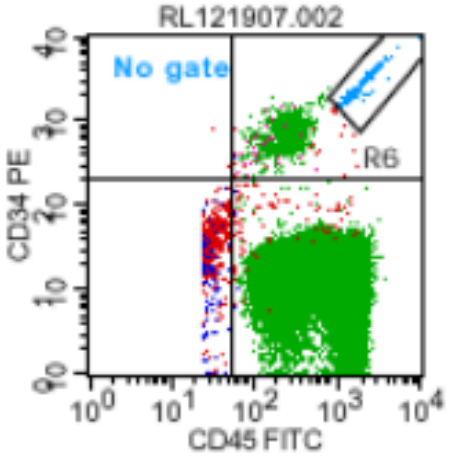


CD34 enumeration

- Principle of a single platform approach
- Viability dye, CD45 and CD34 recognition
- Beads allow for absolute quantification

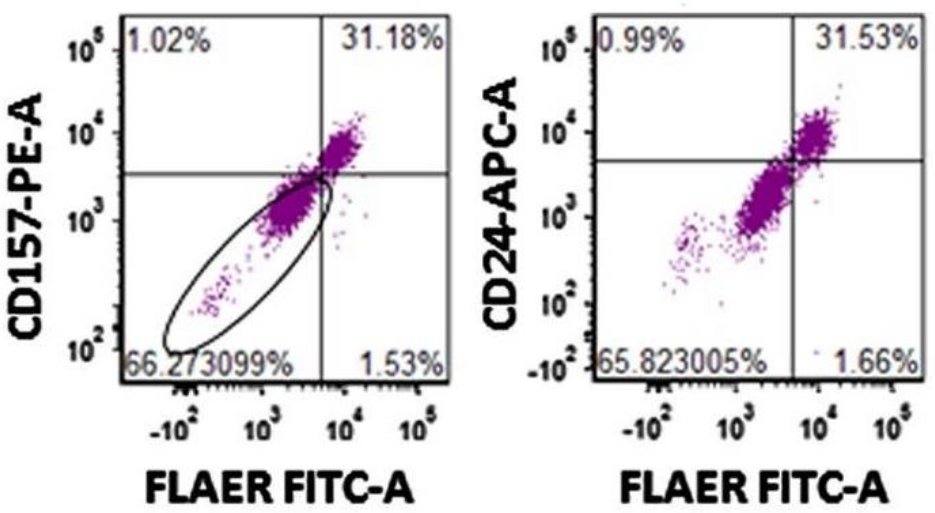






PNH

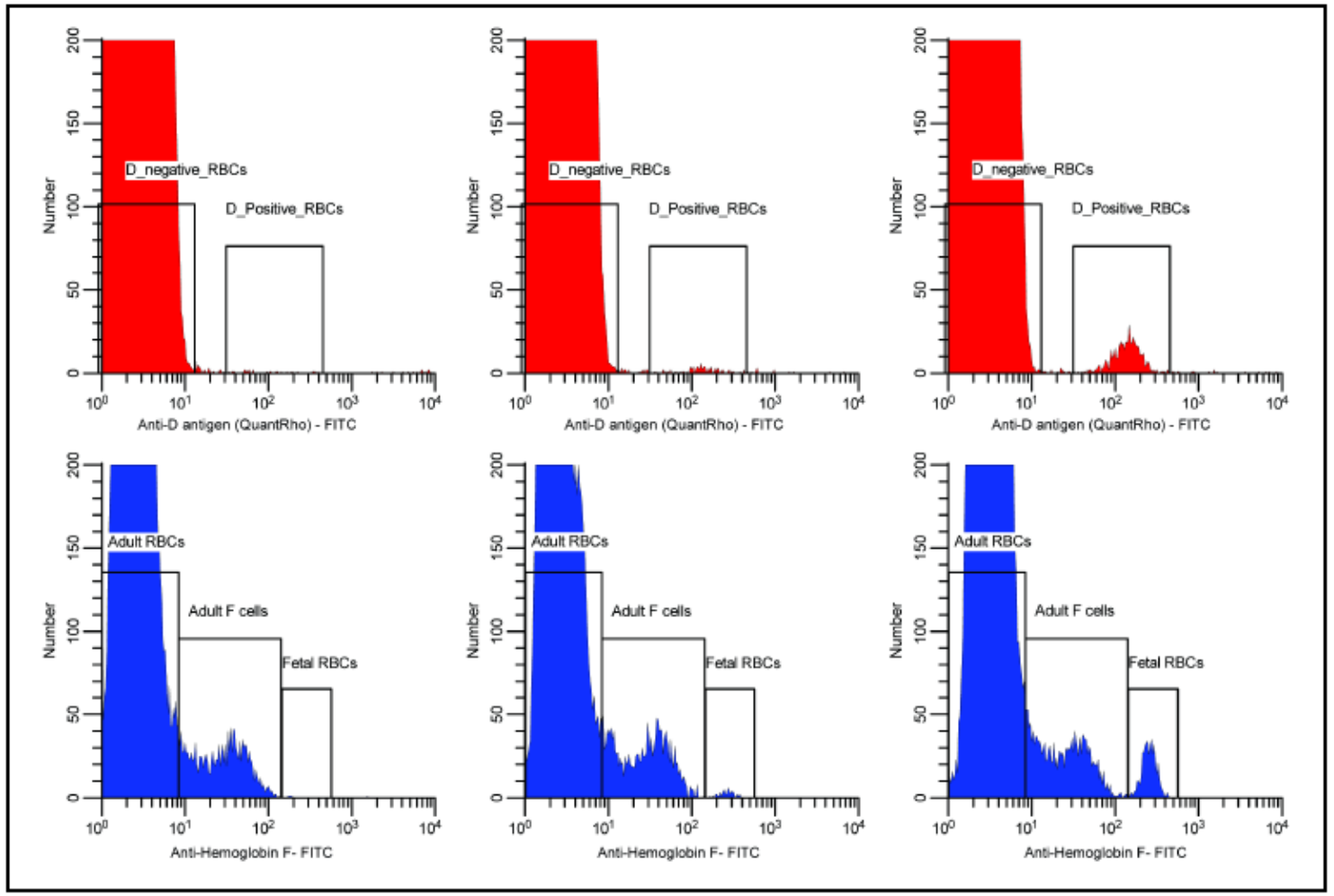
- Determination of a PNH clone is based on the presence detection of absent GPI anchor and associated antigens
  - FLAER – binds GPI
  - Requires lineage non GPI linked markers to identify populations and GPI linked markers to assess for PNH clone and size
  - Populations assessed
    - Erythroid (**CD235a – non GPI** CD59 GPI)
    - Granulocytes (**CD15 – non GPI** CD24+,CD66b, CD157 all GPI)
    - Monocytes (**CD64 – non GPI** CD14 or CD157 – GPI)



Feto-maternal haemorrhage

- Flow cytometry highly accurate method, can be optimised for high throughput
- Determines presence or absence of foetal cells by anti-HbF antibodies (can also include Anti-D)
- Employees external controls
- Allows for calculation of anti-D dosing if required

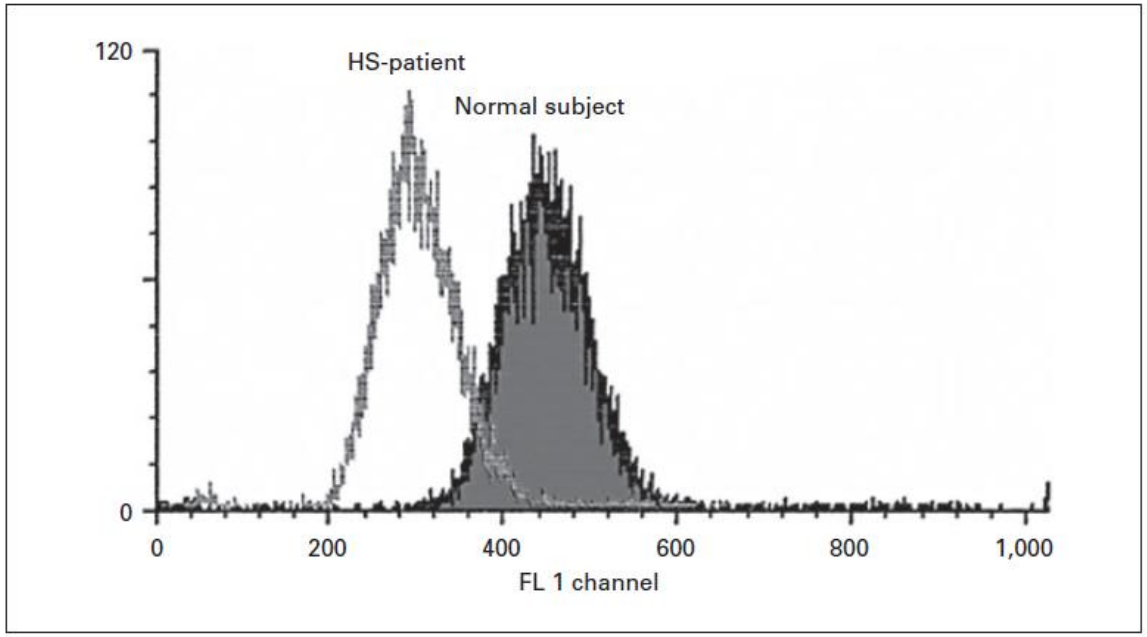
Feto-maternal haemorrhage



Davis, CE Update, 2017

Red cell membrane disorders

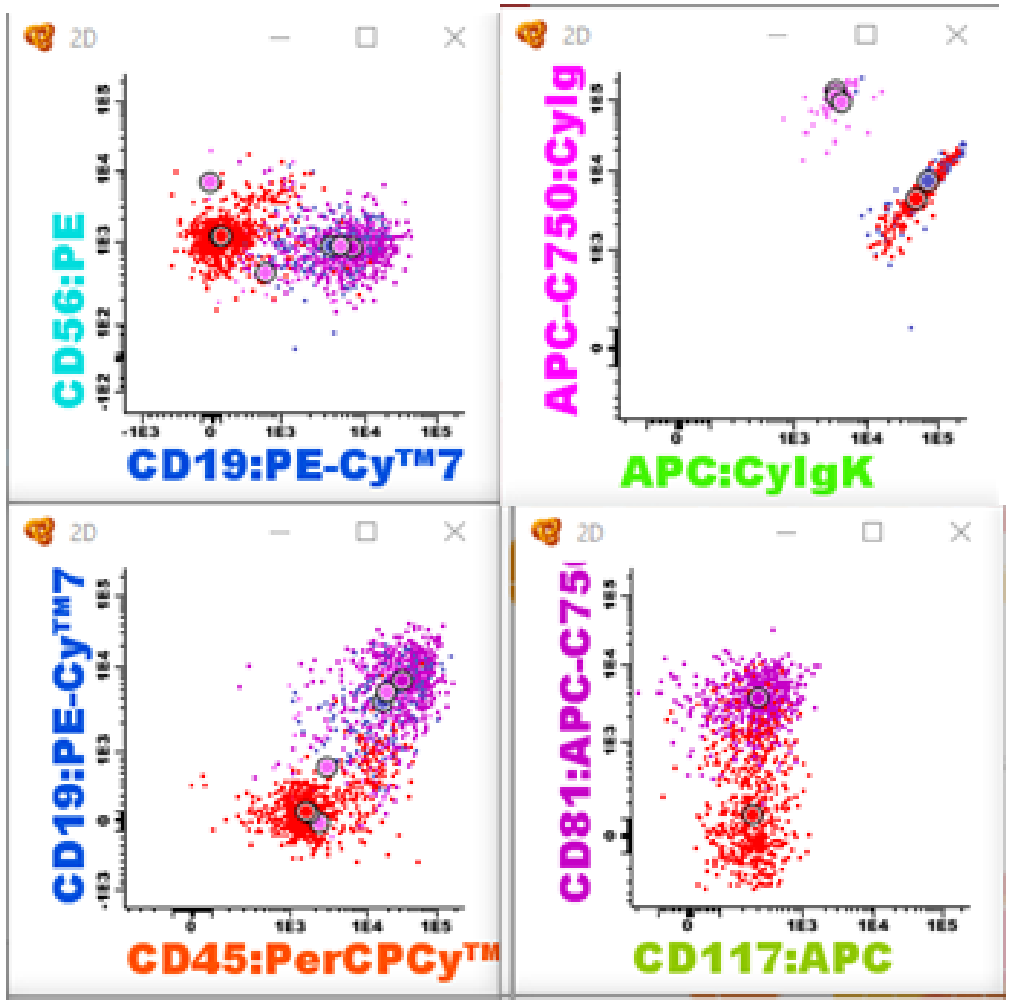
- Hereditary Spherocytosis can be accurately diagnosed using membrane dye quantification
- eosin-50-maleimide (EMA) quantifies erythrocyte band 3 complex and demonstrates reduced binding in HS
- Careful controls required



Stoya, Haematological, 2006

Myeloma

- Flow is used in the diagnosis and MRD tracking for myeloma
- Plasma cells are bright for CD38 and express CD138 in the bone marrow
- Aberrant phenotypes include CD56+, CD117+, CD45-, CD19- and light chain restriction



Minimal residual disease

- Established and emerging approach in flow cytometry
  - Do not need to know specific gating strategies
  - Know the approach, interpretation and implications in some disorders
    - Well established in ALL at induction
    - Routinely performed in AML, CLL, Multiple myeloma

Questions?