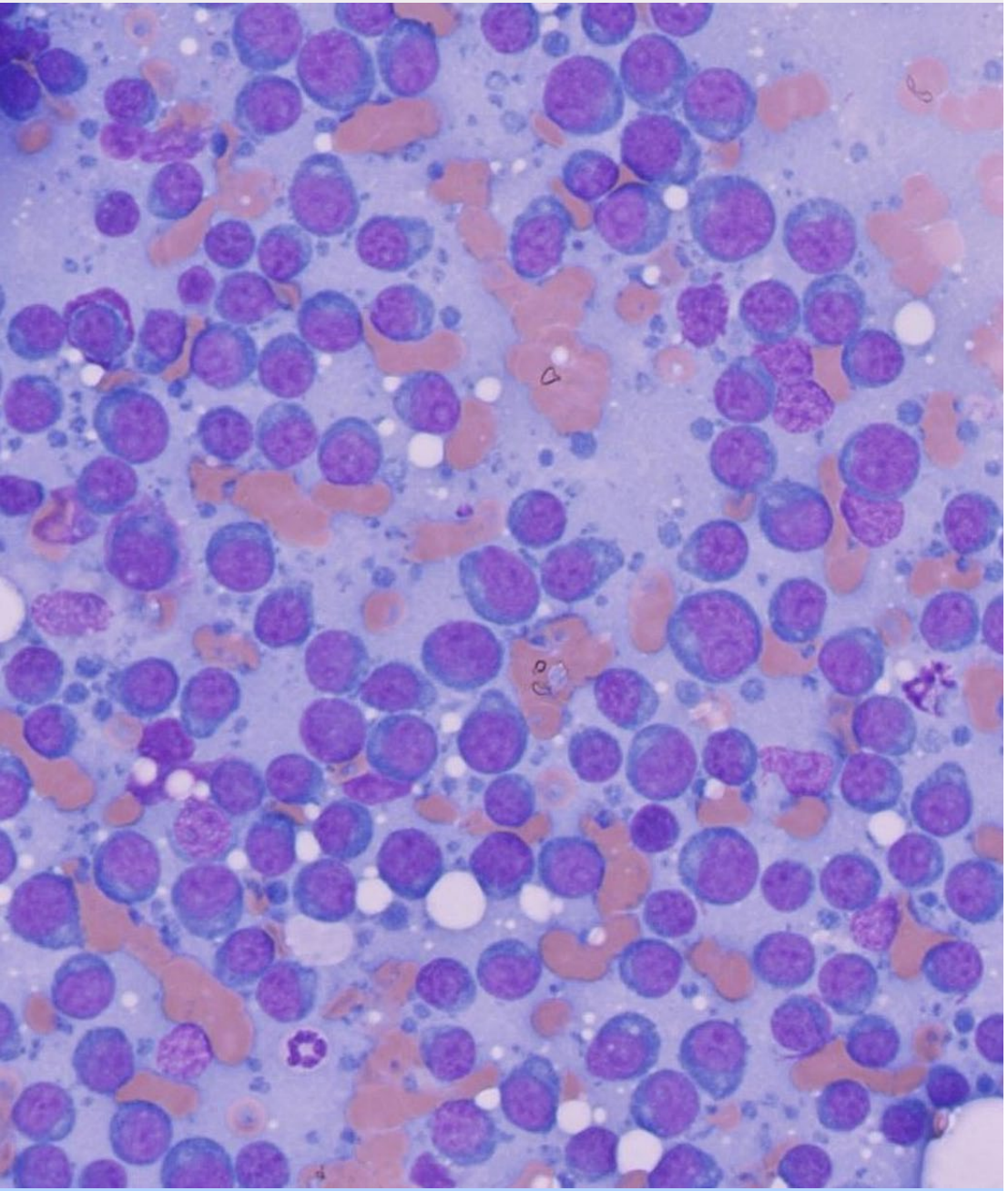

Lymphoma: getting to grips with confusing ancillary cytology diagnostics

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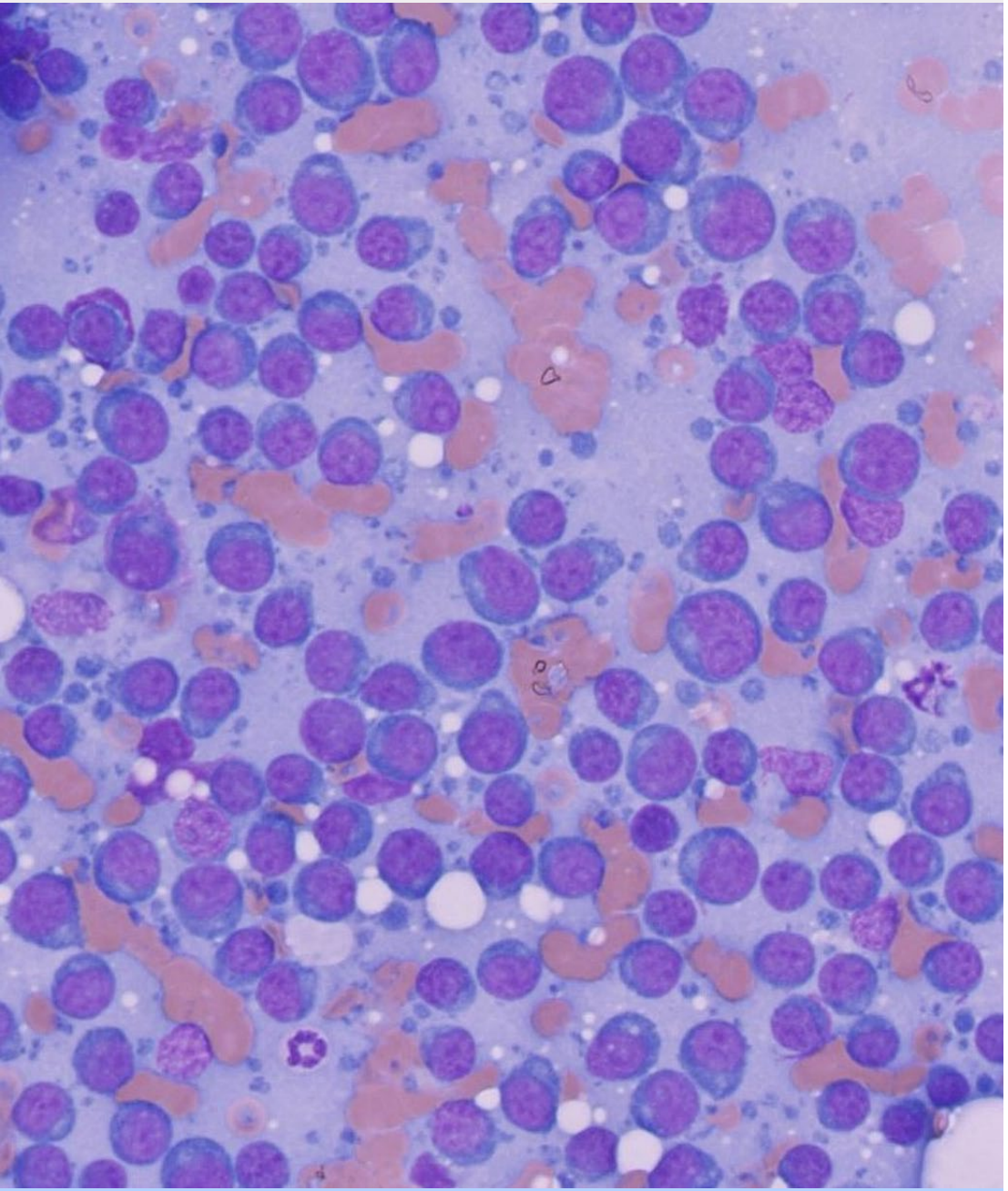
16th November 2023

IDEXX



Disclosure:

I am an employee of IDEXX Laboratories Ltd.

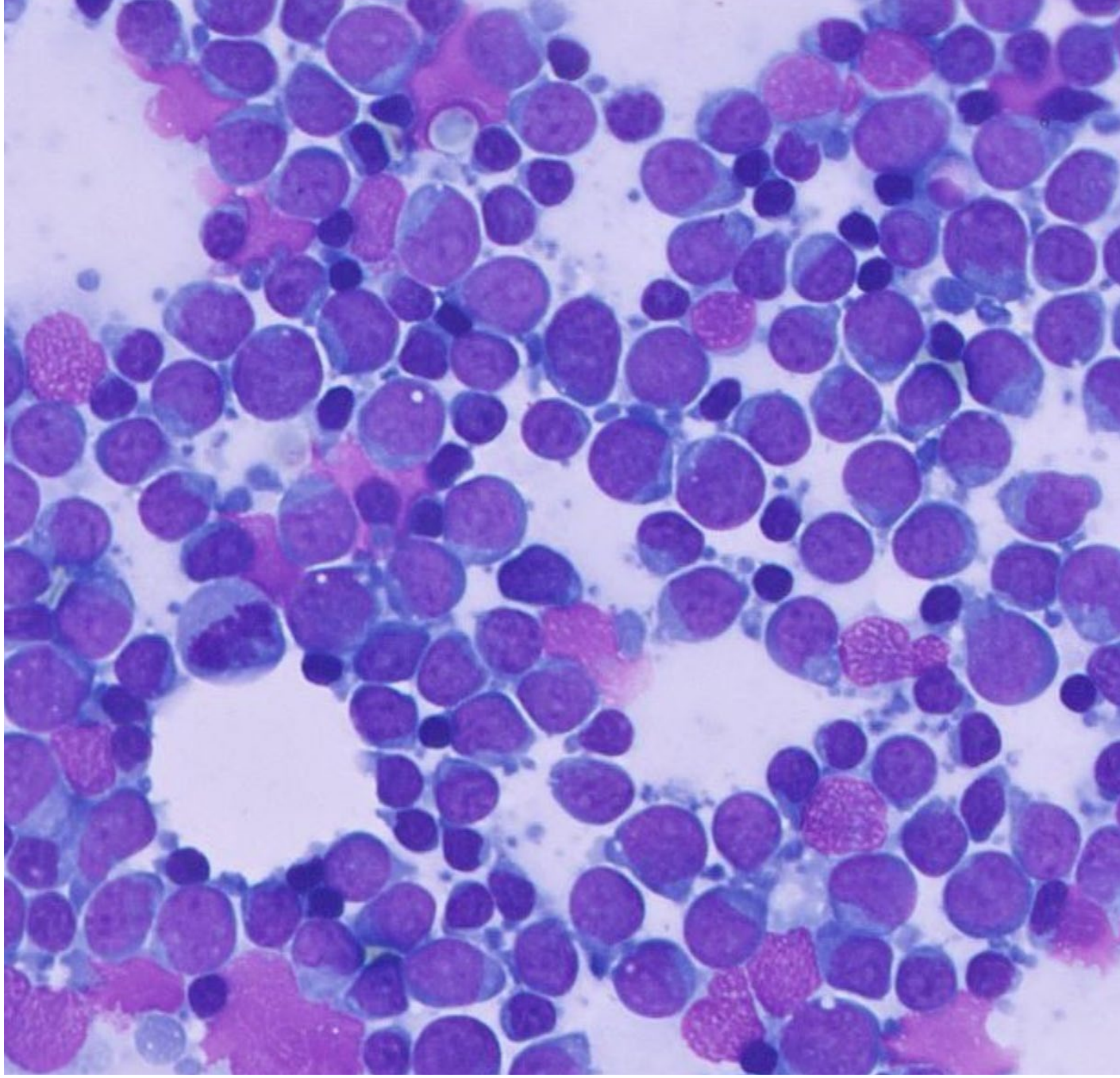
Disclaimer:

The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation, and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions, and cautions. Diagnosis and treatment decisions are the ultimate responsibility of the primary care veterinarian.

We will review:

- Immunocytochemistry (ICC)
- Flow cytometry
- Clonality testing (PARR)

1. What they are
2. What information they provide (confirm a lymphoma suspicion, or further characterise)
3. Sample requirements
4. Pros and cons of each test

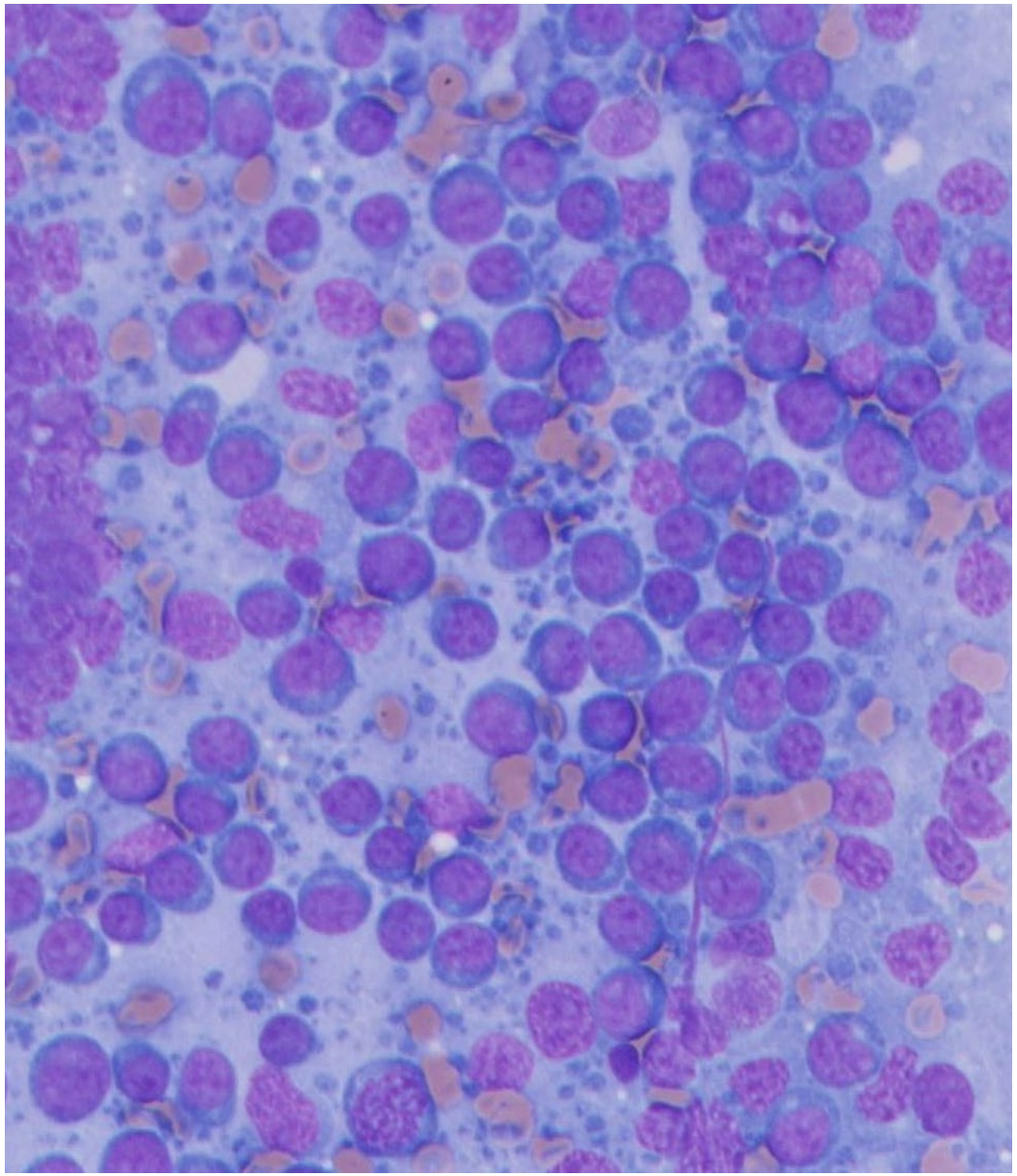


Cytology of lymphoid neoplasms

Sometimes cytological diagnosis is certain

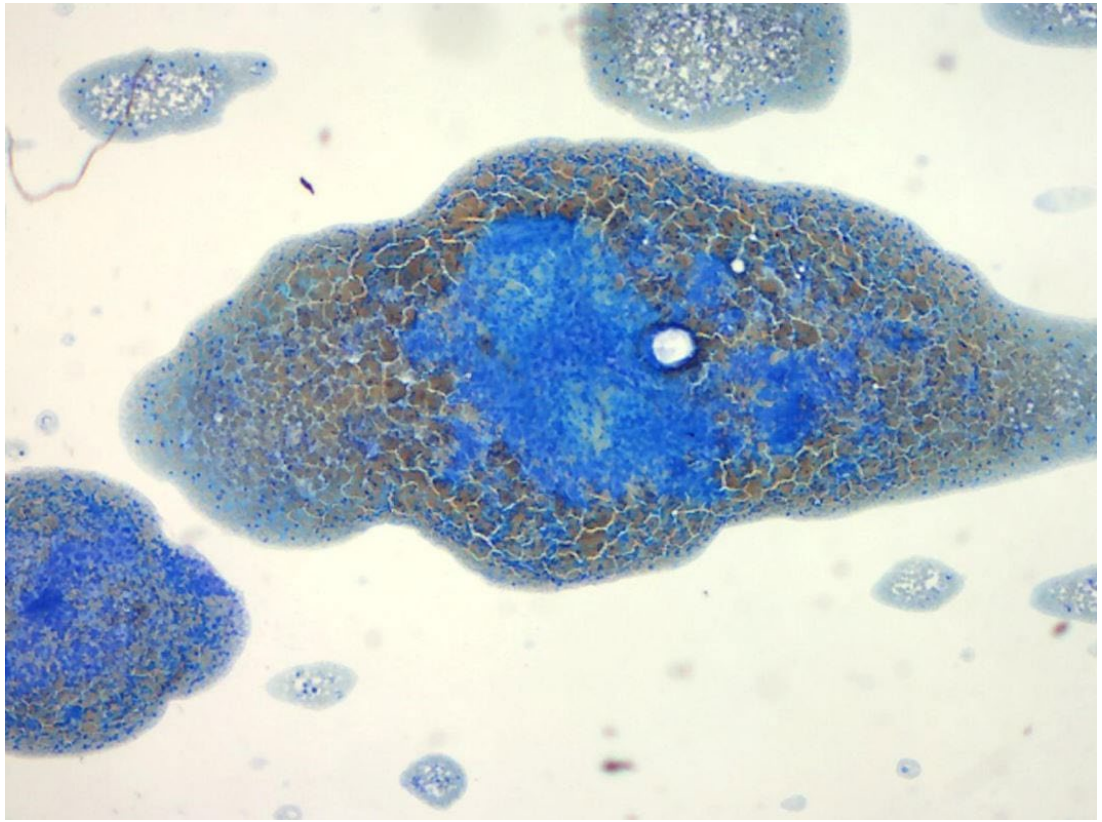
→ Ancillary tests to further characterise

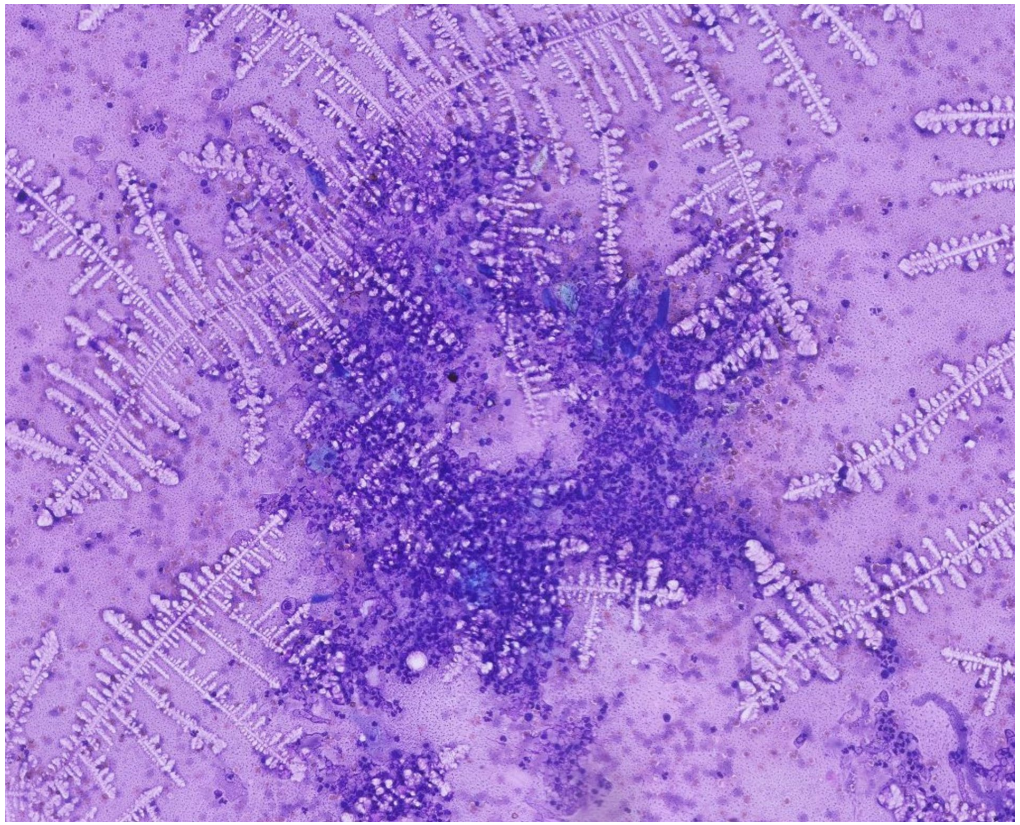
+ Immunocytochemistry (ICC)
+ Flow cytometry
+ (Histopathology and immunohistochemistry)



Cytology of lymphoid neoplasms

Sometimes cytological diagnosis is NOT certain

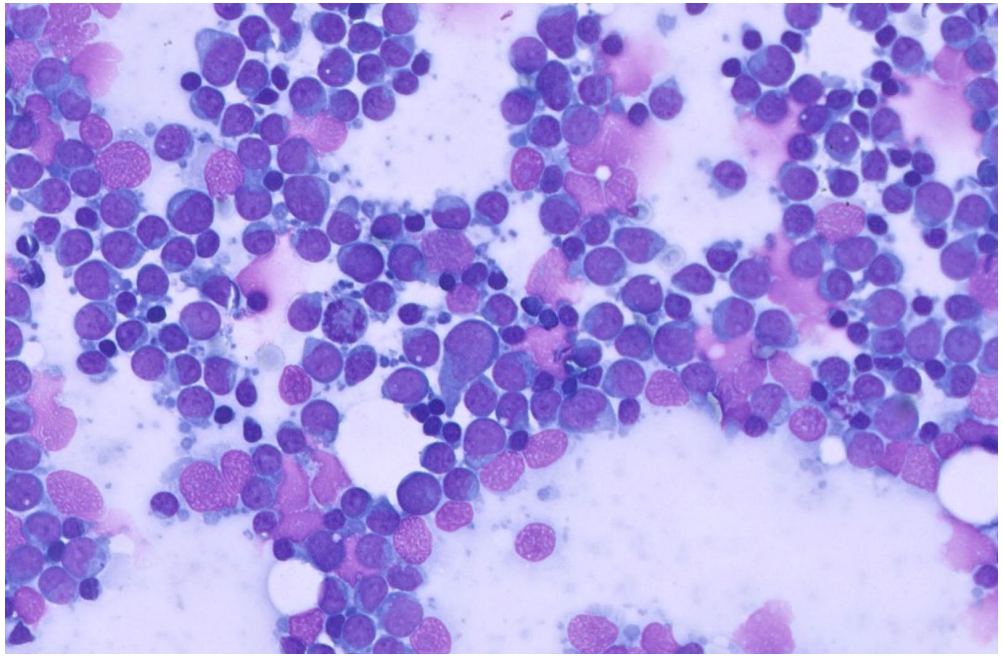


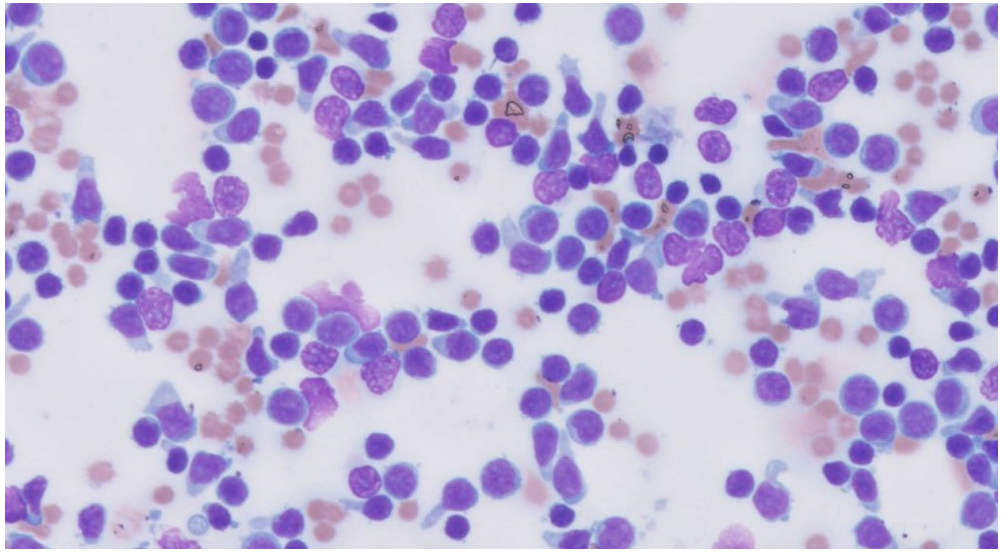


Cytology of lymphoid neoplasms

Sometimes cytological diagnosis is NOT certain

- Heterogeneous lymphoid population
- Small cell lymphoma
- Expansion of monomorphic population with reactive/hyperplastic background



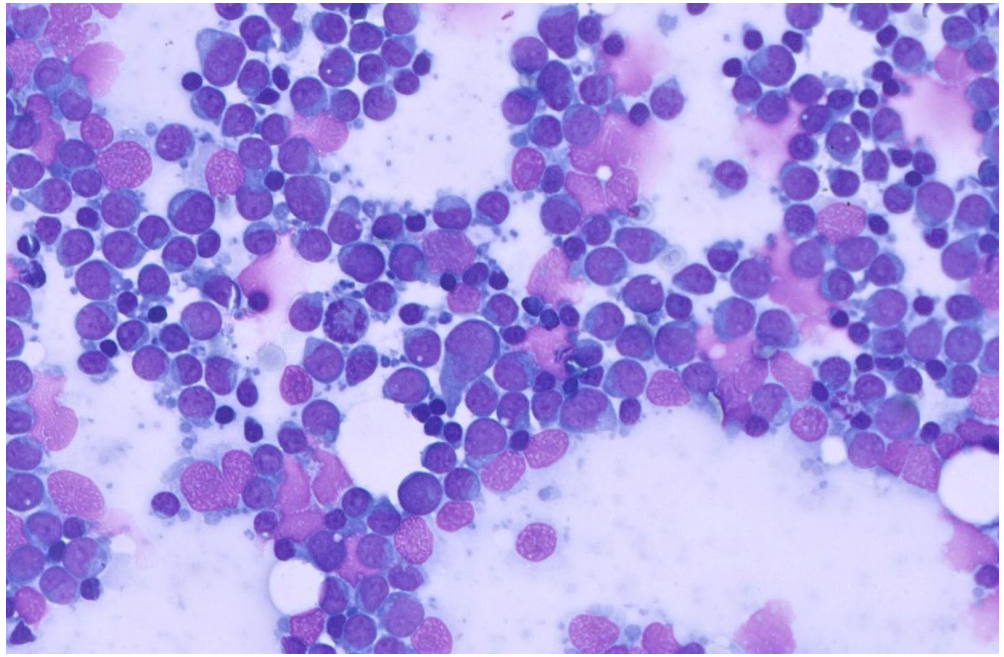


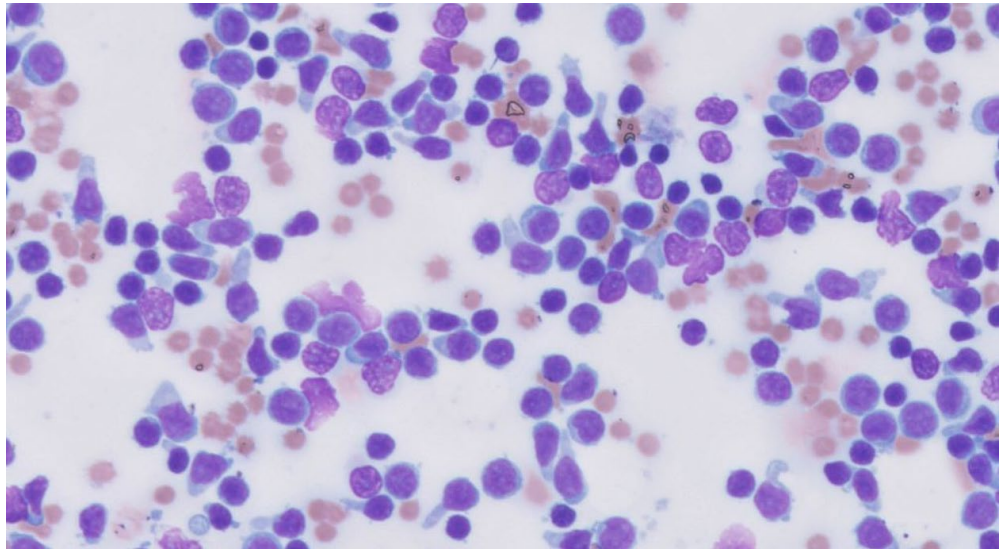
Cytology of lymphoid neoplasms

Sometimes cytological diagnosis is NOT certain

→ Ancillary tests to differentiate neoplastic vs reactive hyperplastic

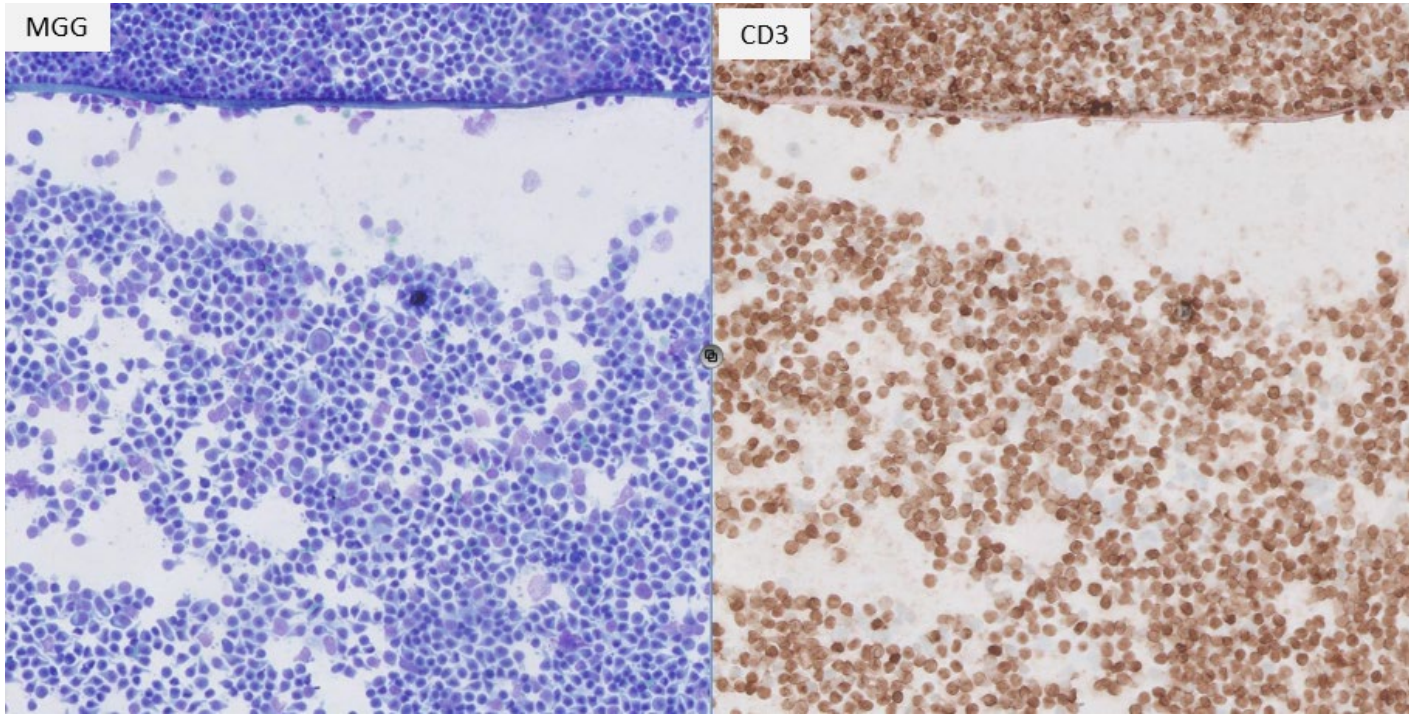
+ Clonality testing (PARR)
+ (Histopathology and immunohistochemistry)

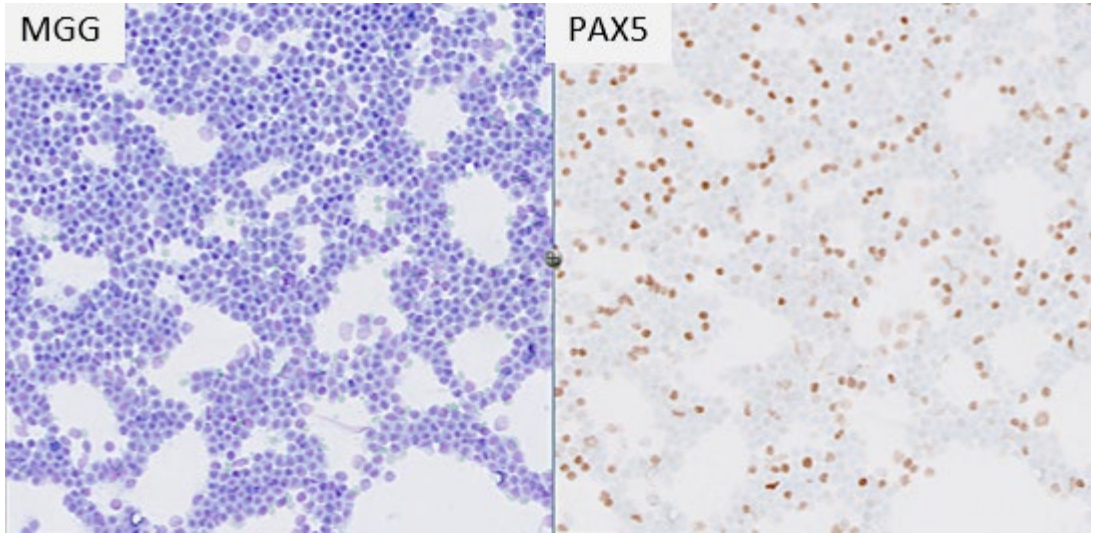




Immunocytochemistry

- Phenotype of cell (B vs T cell)
- Immunolabelling of cells on cytology smears



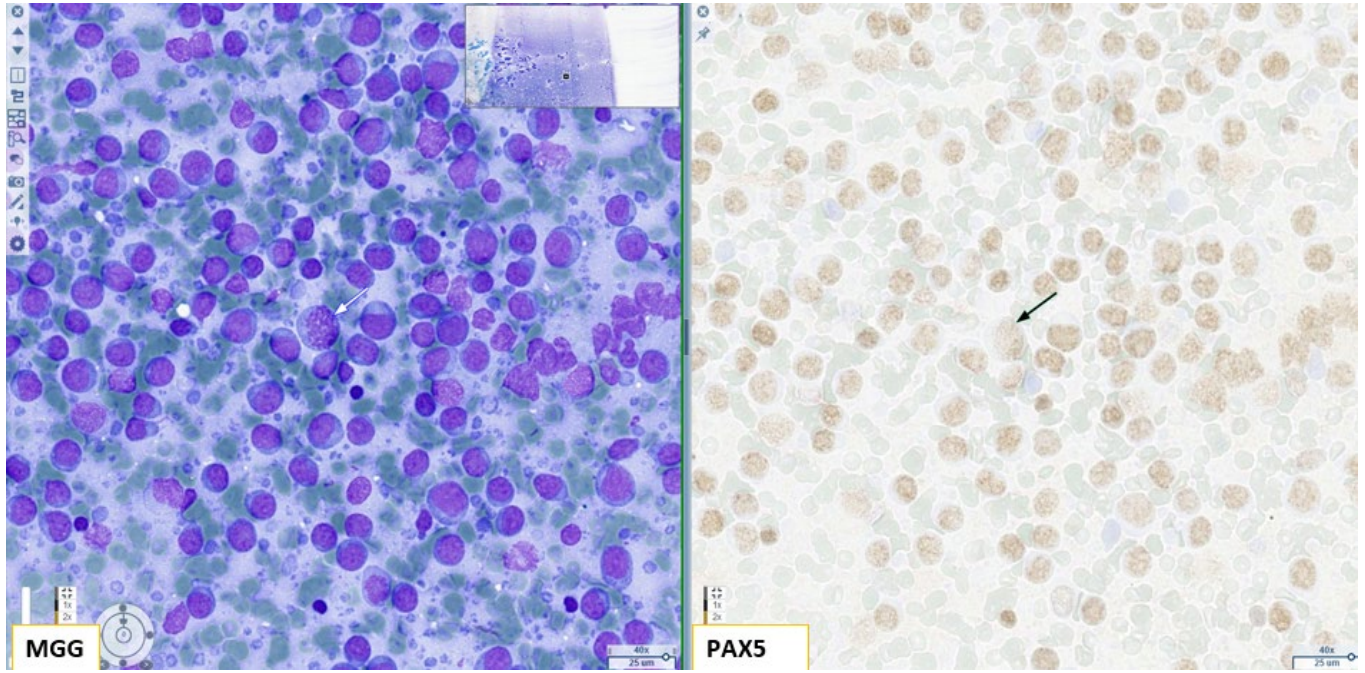


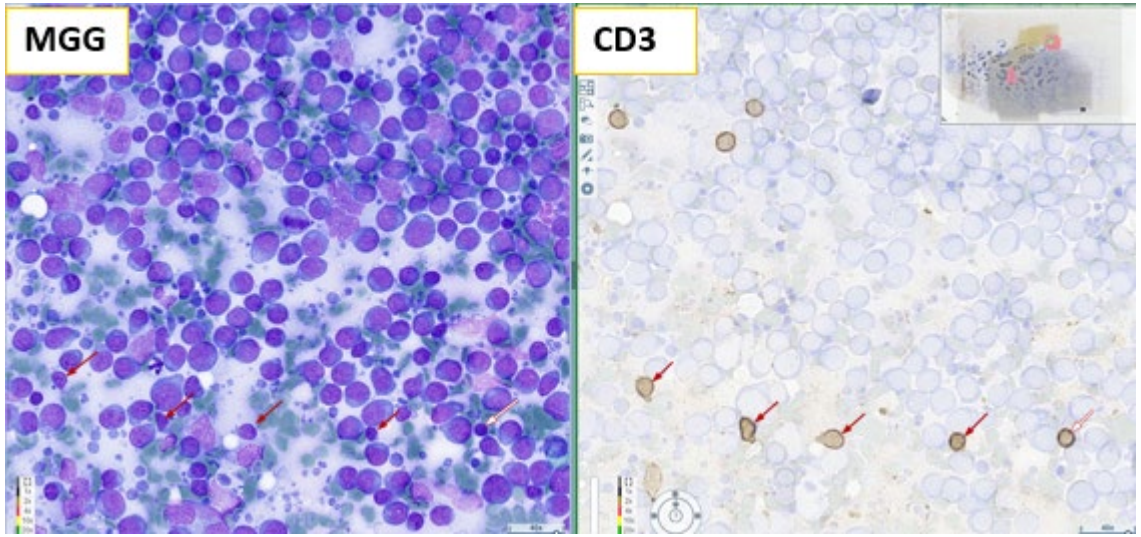
T-Cell Lymphoma

Images courtesy of Nazaré Pinto da Cunha, Vedis, Porto

Immunocytochemistry

- Immunophenotype (B vs T cell)
- Immunolabelling of cells on cytology smears



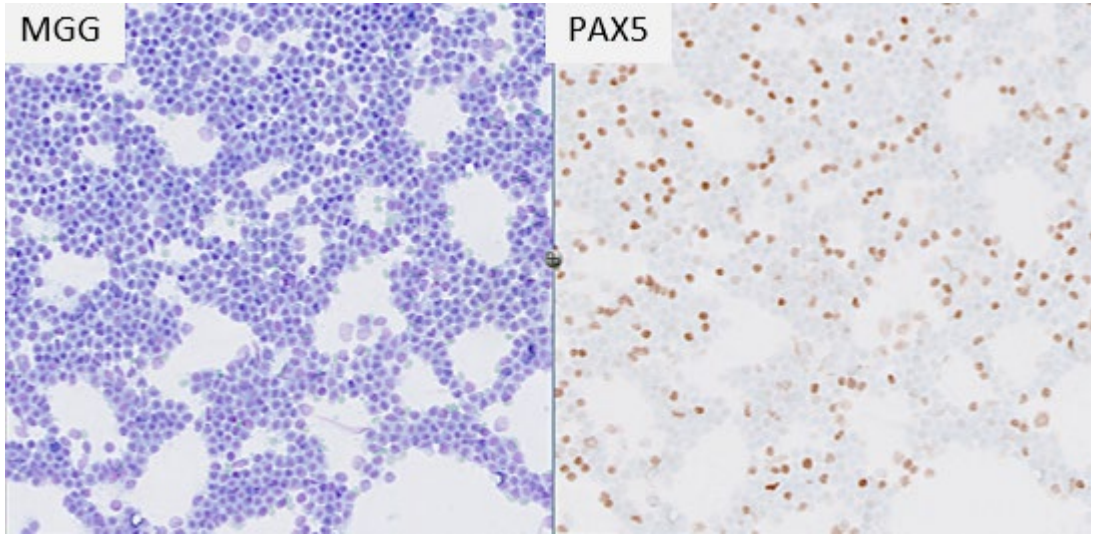


B-Cell Lymphoma

Images courtesy of Nazaré Pinto da Cunha, Vedis, Porto

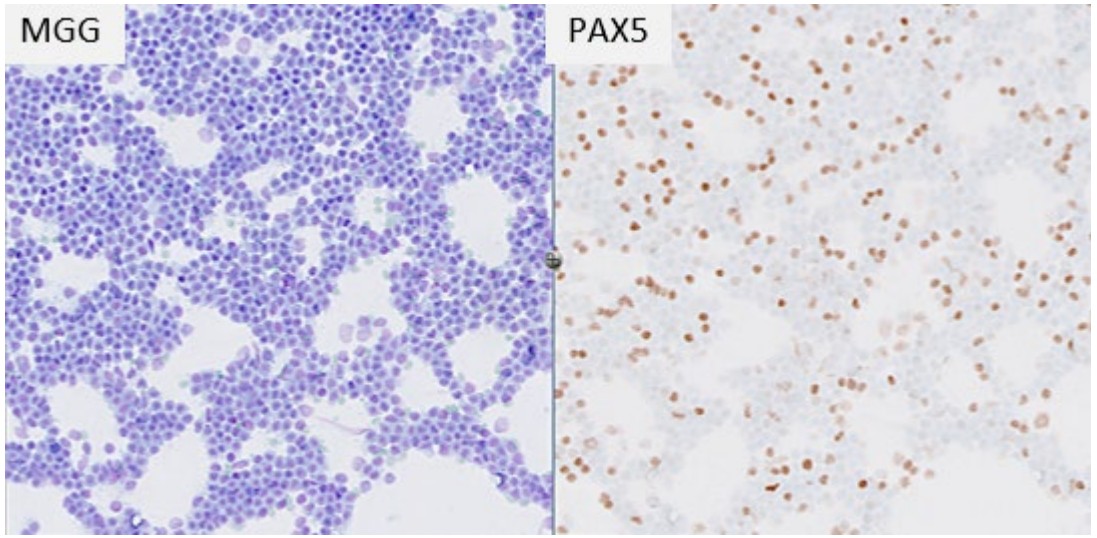
Immunocytochemistry

- Sample requirements:
  - + Good quality cytology smears (minimum 3)
  - + Can be performed on previously stained slides



Images courtesy of Nazaré Pinto da Cunha, Vedis, Porto

Immunocytochemistry

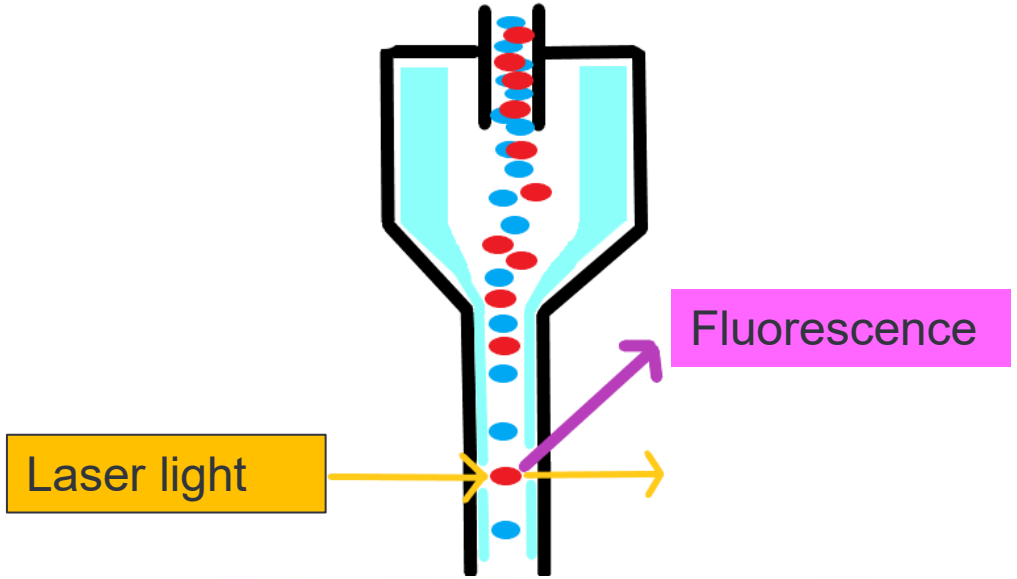


Images courtesy of Nazaré Pinto da Cunha, Vedis, Porto

PROs	CONs
Can be performed on same slides used for initial cytology diagnosis (if adequate)	Limited panel of markers
No need for special sample handling (air dried slides)	Cannot differentiate indolent vs high grade forms (correlation with cytological findings essential)
Quick turnaround time	

Flow cytometry

- Analysis of cells in a fluid
- Fluorochrome conjugated Ab to evaluate phenotype markers
- Also evaluates physical attributes of cells by light scatter properties to ‘gate’ (e.g. size)



Specimen type	Lymph node aspirate
FLOW CYTOMETRY	
[[	40% live cells, mostly intermediate in size
CD3 - T cells	81% co-express CD3 and CD21, 6% CD3+/CD21-
CD4 - T helper cells	4% Positiv
CD8 - Cytotoxic T Cells	79% POSITIVE
CD5 - T cells	86% POSITIVE
CD21 - B cells	81% coexpress Cd3 and CD21, 8% are CD21+/CD3-
CD79a all B cells	Negative
CD45- all leucocytes	10% positive
CD34 - early precursors	Negative
MHCII	92% POSITIVE

CLINICAL COMMENTS

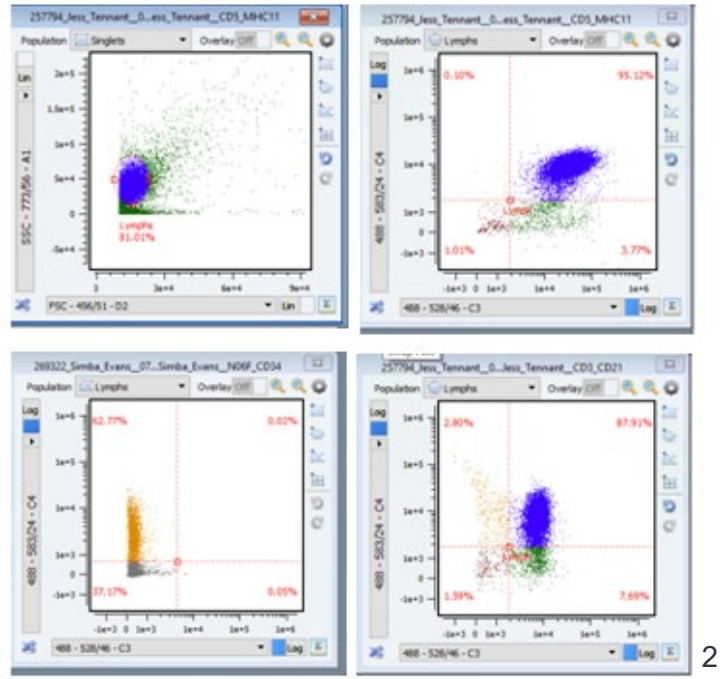
A cytospin of the lymph node consists of mainly intermediate sized cells as previously described.

Flow cytometry on the lymph node shows the majority of the cells express CD3, CD5, CD8 and CD21 as well as MHCII and do not express CD45.

INTERPRETATION

T zone lymphoma.

1



2

1, 2: Images courtesy of Elizabeth Villiers, Dick White Referral Diagnostics

Flow cytometry

- Sample requirements:

Needs to be a fluid sample

  + EDTA blood (if lymphocytosis present)
  + For lymph node aspirates:

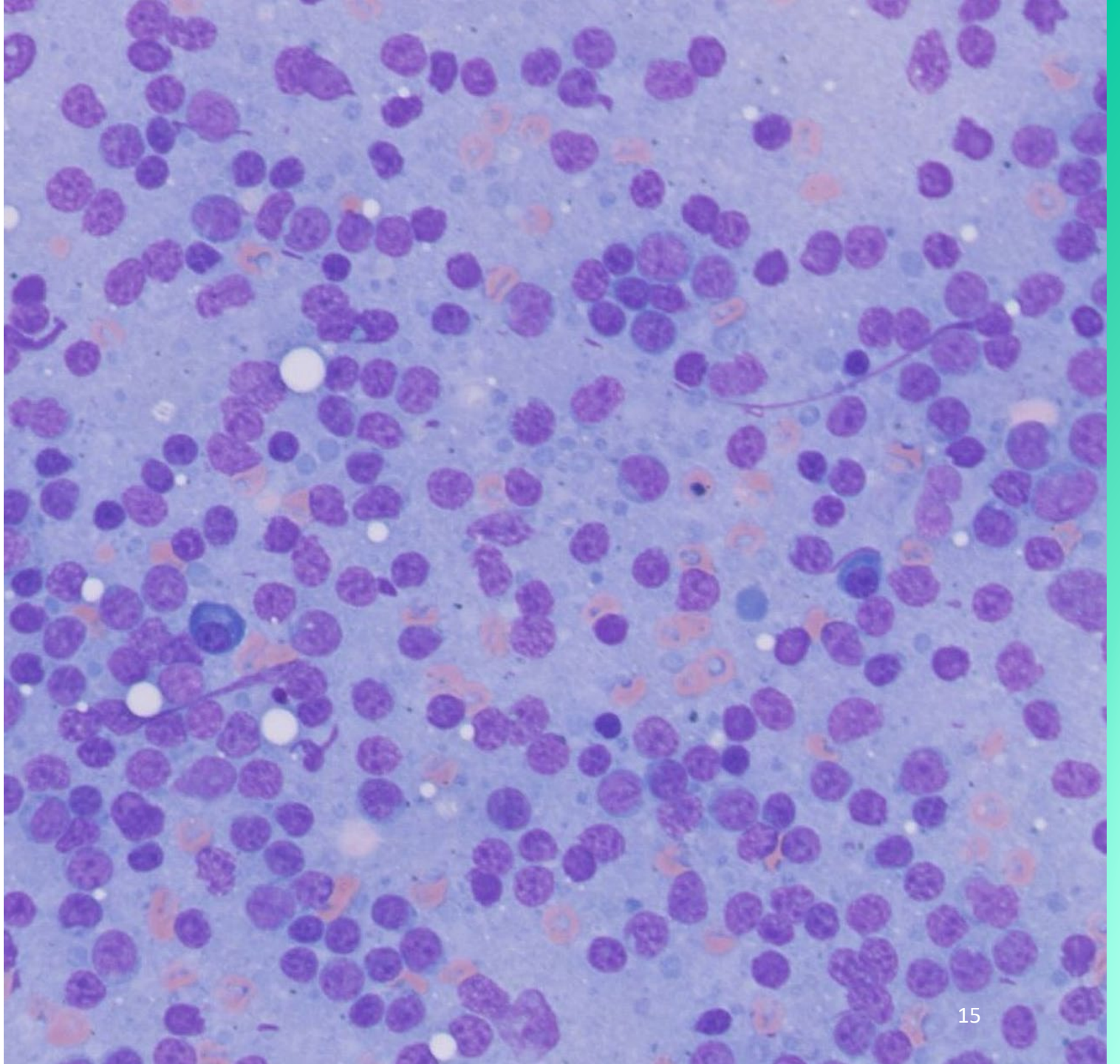
- Aspirates are harvested into a mix of 0.5ml saline and 0.5ml EDTA plasma obtained from the patient, placed into a fresh EDTA tube
- To ensure adequate numbers of cells, 3 or more aspirates should be taken (cloudy appearance of the plasma)
- An aspirate is taken in the usual manner, but the harvested cells are injected into the vial of plasma. Mix gently after each aspirate is injected in the vial to ensure all cells are mixed thoroughly with the plasma
- Samples shipped at room temperature, need to reach the lab within 24hrs

Flow cytometry

PROs	CONs
Comprehensive panel of markers	Requires additional sampling of lymph nodes
May show characteristic profile (e.g., T zone lymphoma)	Strict sample requirements (must be fresh as cells need to be viable at time of analysis)
May provide prognostic information (e.g., CD34 expression, MCH class II and Ki-67)	
Quick turnaround time	

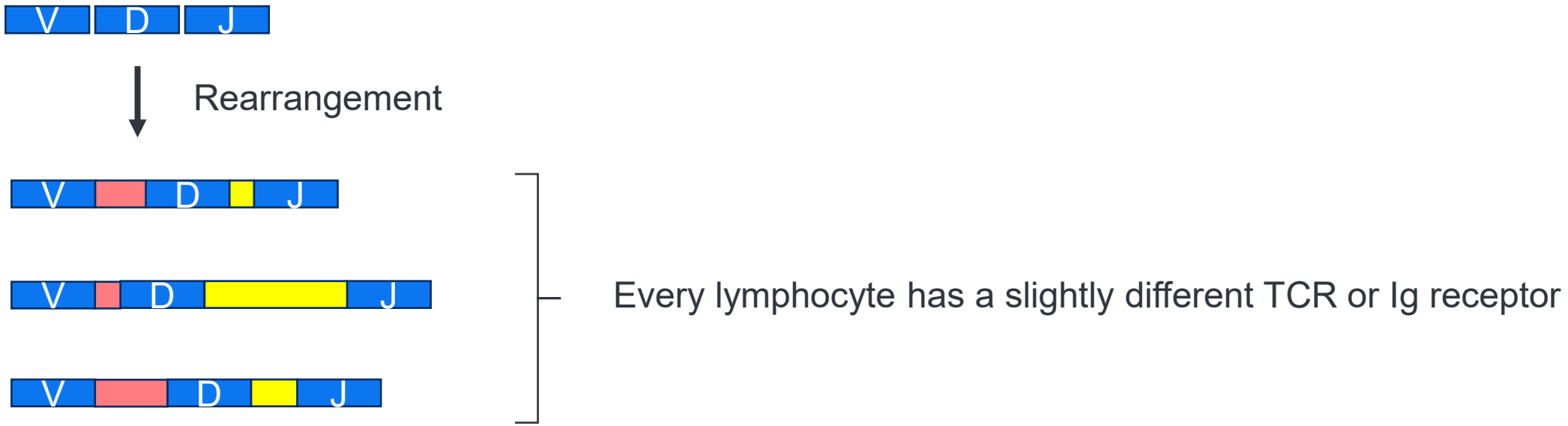
Clonality testing (PARR)

- PCR for Antigen Receptor Rearrangement (PARR)
- Assess lymphocyte antigen receptor rearrangement diversity
- Differentiating a neoplastic from a reactive lymphoid proliferation



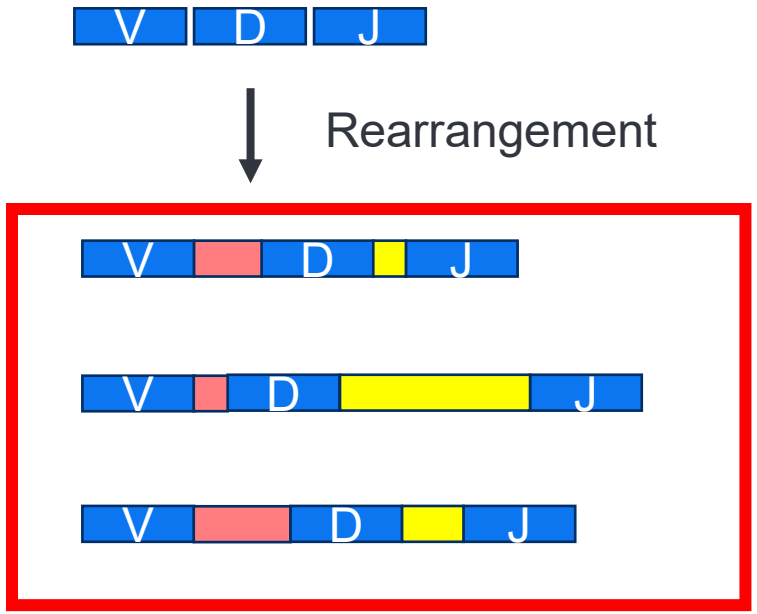
Clonality testing (PARR)

- During development, cells undergo rearrangement of T cell receptor (TCR) and immunoglobulin receptor (Ig) for a specific antigen



Clonality testing (PARR)

+ During development, cells undergo rearrangement of T cell receptor (TCR) and immunoglobulin receptor (Ig) for a specific antigen

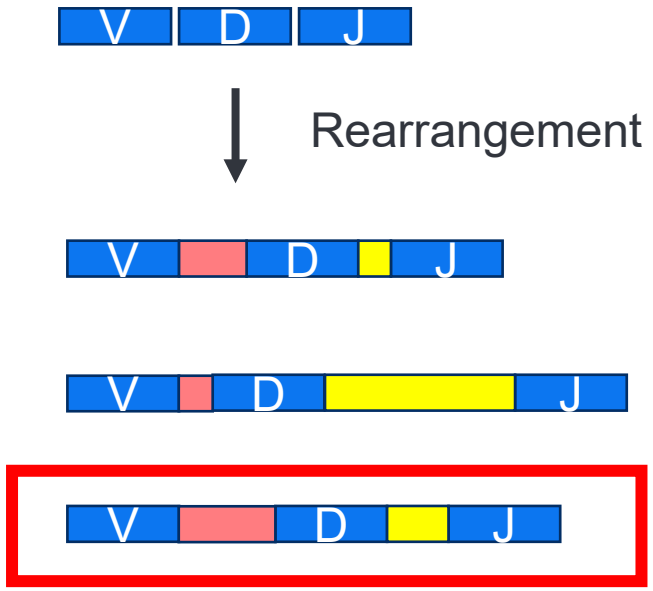


Reactive population:

- Heterogeneous population of lymphocytes
- Multiple-sized products

Clonality testing (PARR)

+ During development, cells undergo rearrangement of T cell receptor (TCR) and immunoglobulin receptor (Ig) for a specific antigen

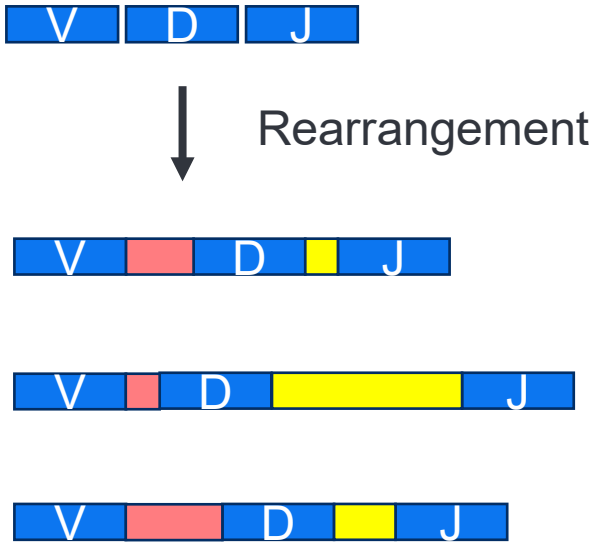


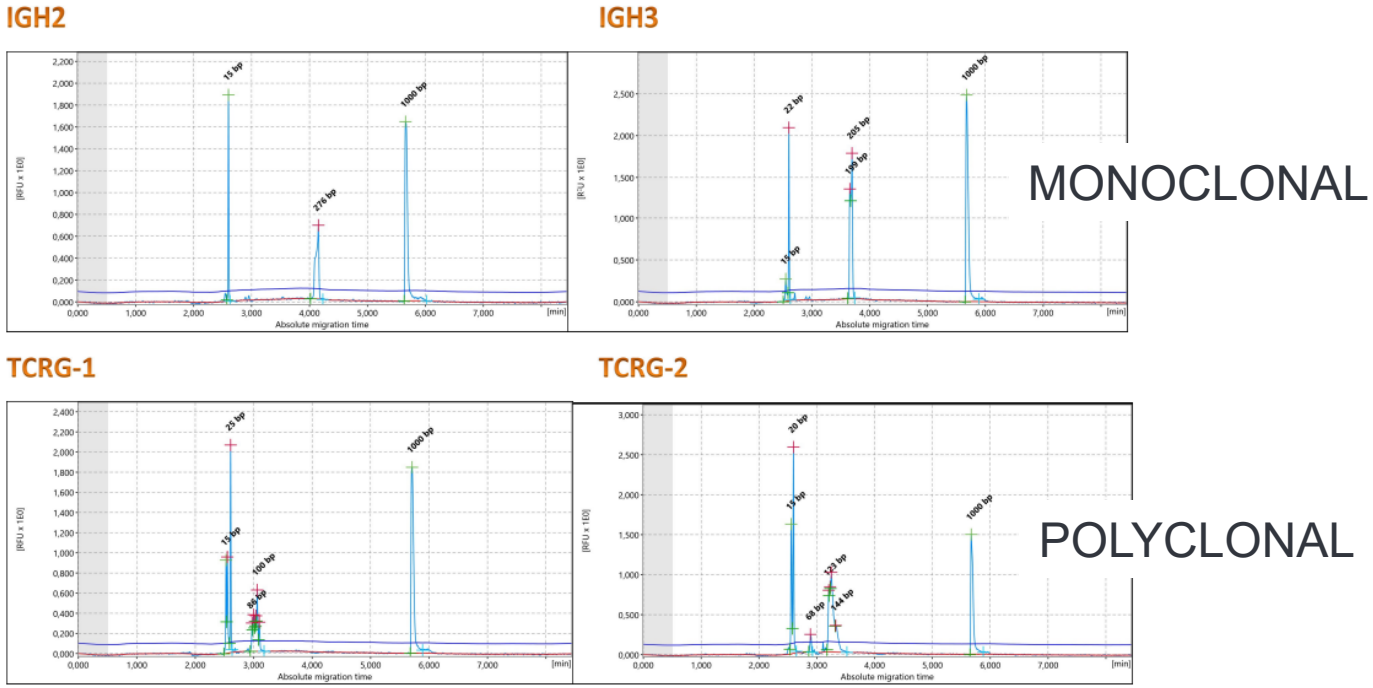
Lymphoma:

- Clonal population
- Single-sized products

Clonality testing (PARR)

- During development, cells undergo rearrangement of T cell receptor (TCR) and immunoglobulin receptor (Ig) for a specific antigen





Images courtesy of Barbara Rütgen, University of Veterinary Medicine, Vienna

Clonality testing (PARR)

- Sample requirements:
  - Can be performed on blood, fluid samples, cytology slides (pre-stained), histology samples
  - Provided there is sufficient lymphoid material

Clonality testing (PARR)

PROs	CONs
Differentiate neoplastic vs reactive	Cannot differentiate indolent vs high grade forms (correlation with cytological findings essential)
Can be performed on samples already submitted e.g., stained cytology slides	Not ideal for phenotype (B vs T) as cross-lineage rearrangement possible
	False negative (e.g., small sample and low numbers of clonal cells in polyclonal background, primer site mutation)
	False positive (chronic infectious disease such as *Ehrlichia*, *Leishmania*, rarely thymoma, non-specific amplification)

Ancillary tests: points to remember

- Should not be used as standalone tests
- Guided by cytological findings to help selecting the most appropriate test for each case
- Need to be evaluated in light of the full history and clinical picture and together with cytological findings in order to obtain an accurate interpretation (e.g., indolent vs high grade lymphoma, phenotype) to guide therapeutic options

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Any questions?