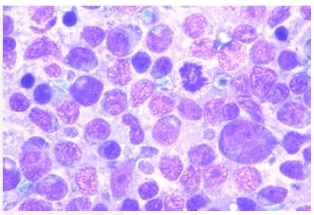
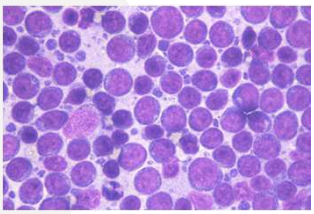
THE CYTOLOGY OF HEMATOPOIETIC NEOPLASMS IN DOG AND CAT





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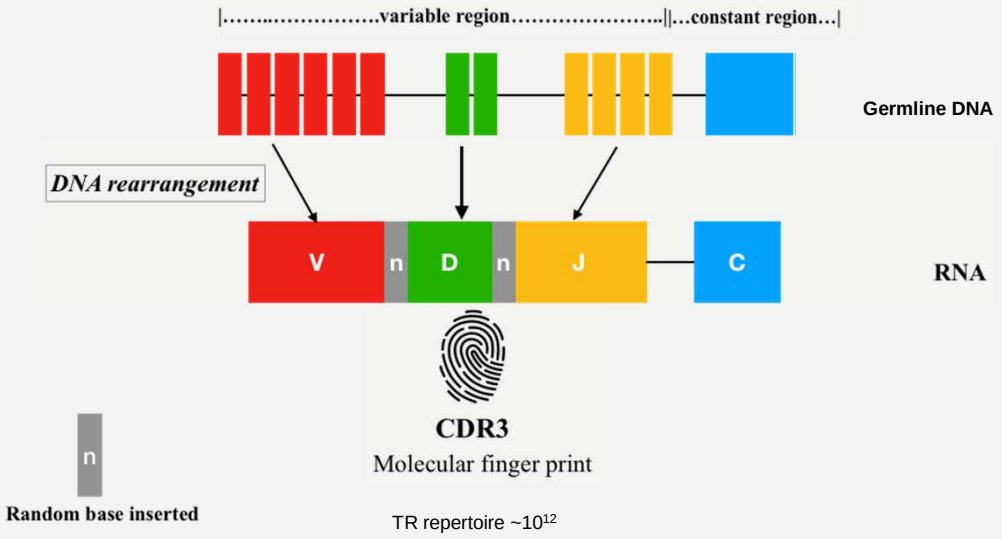
CYTOLOGY OF LYMPHOMA

- WHO classification: recognize distinct entities on the basis of morphology, immunophenotype, genetic, and clinical features.
- The cytological diagnosis based on the presence of a monomorphic cell population in lymph nodes.
- Cytology can be highly suggestive for some WHO lymphoma entities but not definitive with the use of cytology alone
- IHC/ICC and Clonality

Leukocyte markers of diagnostic importance to lymphoma

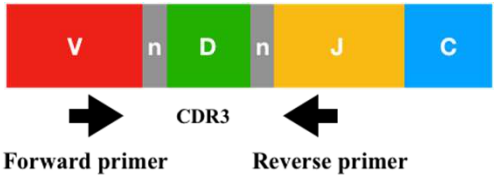
- **CD3ε**: Signaling component of the T cell antigen receptor
- **CD79a**: Signaling component of the B cell antigen receptor, expression reduce in plasma cells
- **CD20**: Surface molecule expressed at all stages of B cell differentiation except for plasma cells
- **PAX5**: Transcription factor essential for maintenance of B cell differentiation
- **MUM/IRF4**: Transcription factor essential for plasma cell differentiation
- **CD18**: Leukocyte adhesion molecule. Expressed on all leukocytes
- **CD45**: leukocyte common antigen
- **C-Kit**: Expressed by most hemopoietic progenitor cells and by mast cells.
- **E-cadherin**: Adhesion molecule expressed by epithelial cells and by some leukocytes
- **Granzyme B**: Serine proteas located in the granules of cytotoxic T cells and NK cells.

Generation of diversity in antigen receptor genes

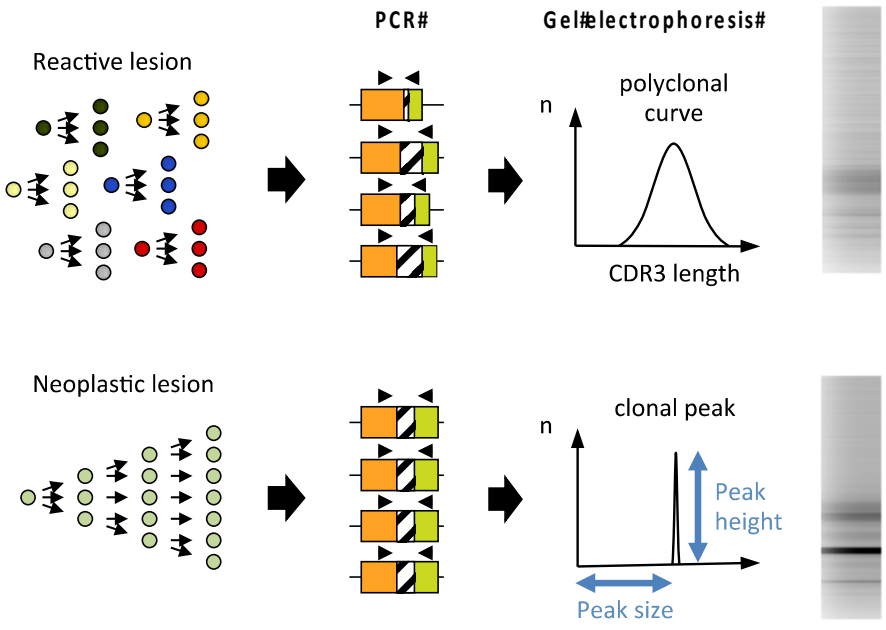


Molecular clonality or PCR for antigen receptor gene rearrangement (PARR)

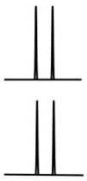
- A PCR based technique to detect the diversity of antigen receptor gene rearrangement of lymphocyte population(s) in a given lesion
- Amplification of the CDR3 followed by gel electrophoretic size separation of amplicons
- Difference in CDR3 length resulting in amplicons of variable size



T cell clonality targets TRG gene
B cell clonality targets IgH gene



Keller, 2016

Electrophoresis profile: (in duplicate)	
Technical description:	**Clonal** One or more reproducible peak(s)
Most indicative of:	Neoplastic process

Keller, 2016

Clonality testing in Veterinary medicine

When to use..

- Morphological and cytological properties are inconclusive

What to be concerned…

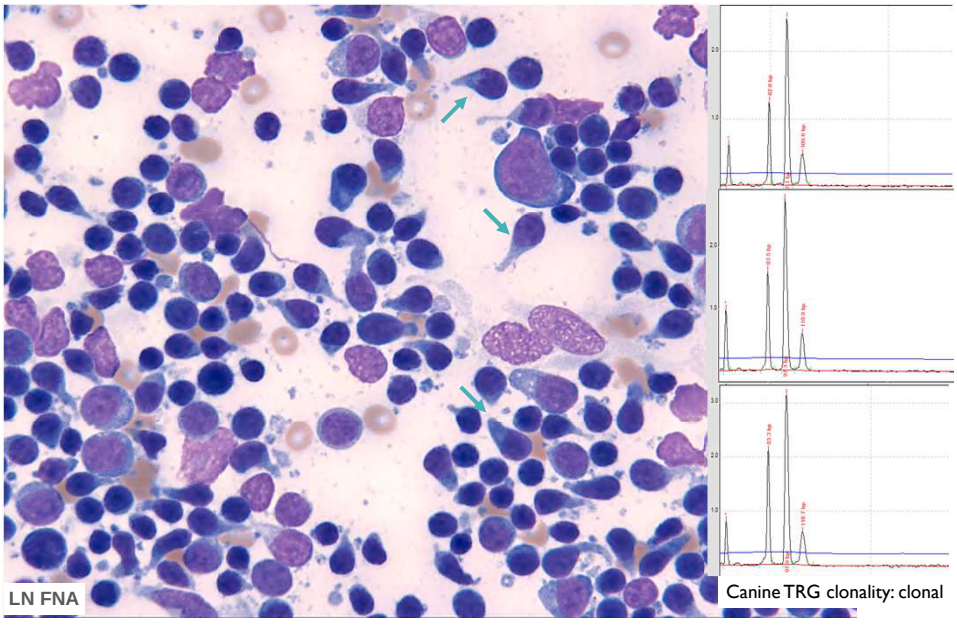
- False negativity: inadequate primer coverage, primer site mutation, polyclonal background
- False positivity: clonal expansion in response to antigen stimulation
- Cannot be used as a lineage marker

Clonality is not a standalone test

Case 1

- ★ A 5 year-old intact male German Shepard
- ★ Owner noticed swelling at right mandibular area.
- ★ Rt. mandibular ln: 4 cm and firm, lt. mandibular ln: 1.5 cm and firm
- ★ CBC and serum biochemistry were in normal range.
- ★ LN aspirate was submitted

- Predominant small and mature lymphocyte population
- Nuclei ~1.25-1.5 RBC diameter, variable clumped chromatin, inapparent or small nucleoli and a low to moderate amount of cytoplasm (stained pale blue)
- Cytoplasm is sometimes present as a unipolar tail (hand-mirror cell)
- Medium-sized lymphocytes were also seen.



LN FNA

Canine TRG clonality: clonal

- **Dx: T-zone lymphoma (TZL)**
- In dog is indolent, low grade, low mitotic rate
- Single (common) or multiple nodes, typically in head area
- CD3+, CD79a-, CD45-
- Cytology can be highly suggestive of TZL

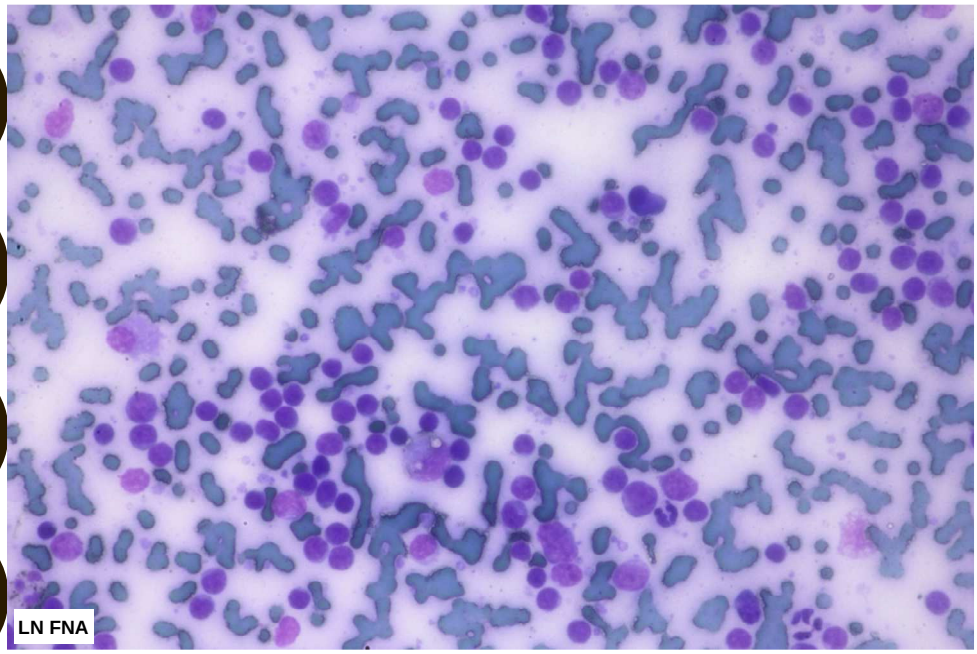
Case 2

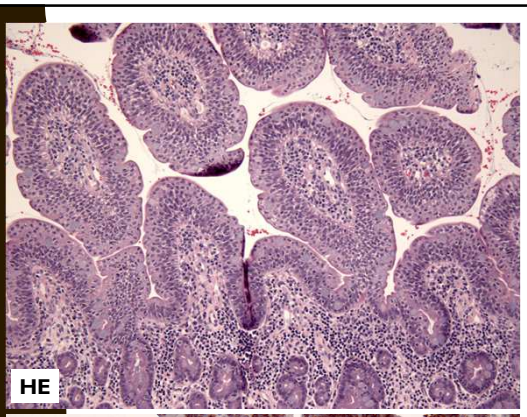
★ A 13 year-old neutered female DLH

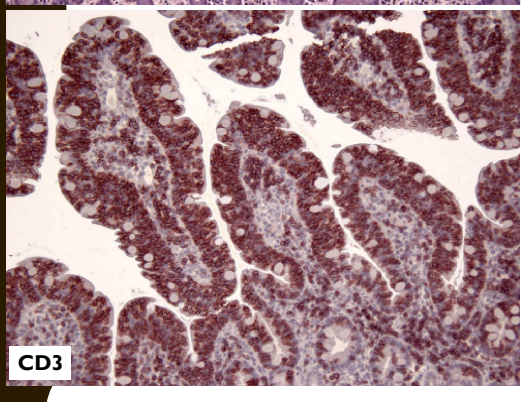
★ Chronic weight loss, diarrhea, u/s found thickening of small intestine

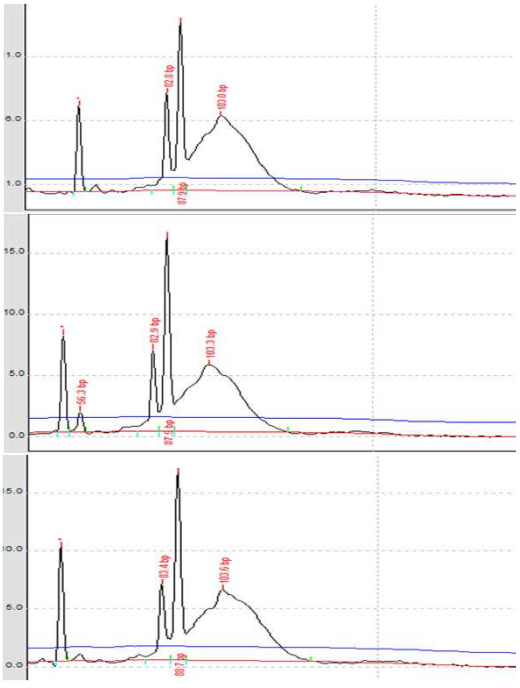
★ Intestinal and mesenteric lymph node biopsied

- LN FNA showed small, mature lymphocytes are predominate.
- Few lymphoblasts, neutrophils and histiocytes —> reactive?



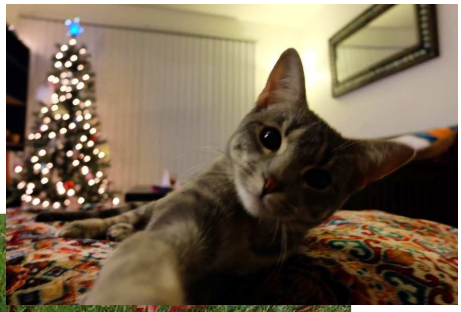


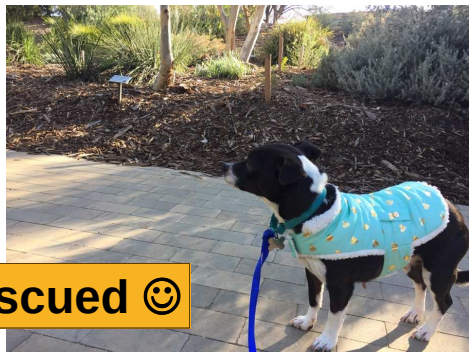




Feline TRG clonality: Clonal

- **Dx: Enteropathy associated T cell lymphoma (EATCL II)**
- Confirmed by HP and TCR clonality
- Difficult to distinguish by cytology especially when reactive lymph nodes are presented
- Small cell, indolent, most common in cats
- Involve small intestines and epitheliotropism is a striking feature





We are rescued ☺

Case 1

★ A 8 year-old neutered female Boxer

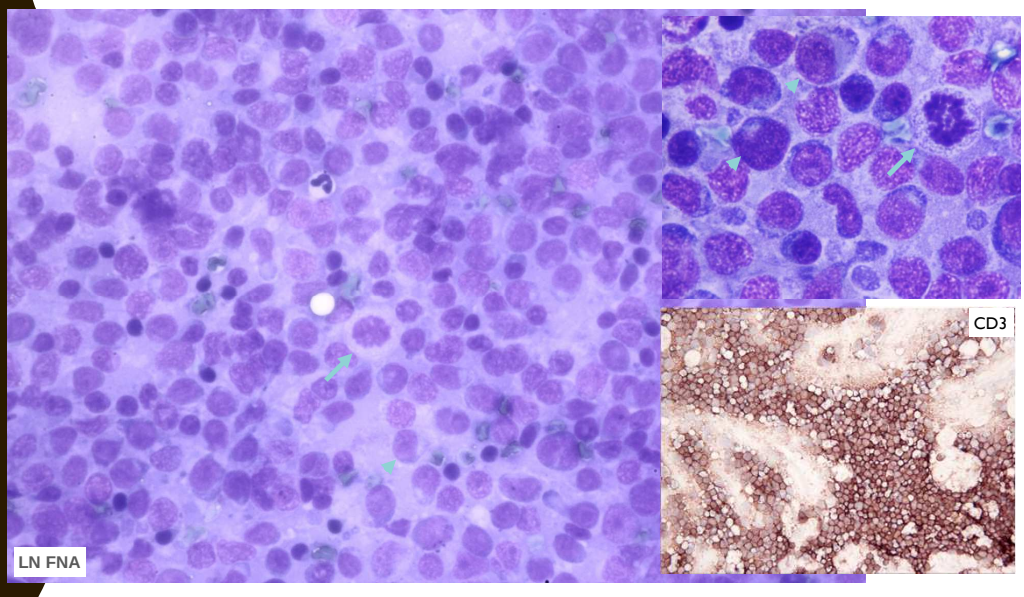
★ Generalized lymphadenopathy, PU/PD, lethargy

★ Leukopenia (4100 cells/ul), lymphopenia (120 cells/ul), mild anemia (Hct 36%)

★ Ca^{2+} 15.6 mg/dl (9.9-11.4), other values were in normal range

★ LN aspirate was submitted.

- Intermediate lymphocytes (nucleus 1.5-2 RBC diameter) have scant amount of light blue cytoplasm and indented to convoluted nuclei with fine, dense and disperse immature chromatin, inapparent nucleoli (arrowheads)
- Note: mitotic figures (arrows) and lymphograndular bodies
- ICC: $CD3^{+}$, $TCR\alpha\beta^{+}$



- **Dx: Lymphoblastic T cell lymphoma (T-LBL)**
- High-grade lymphoma, most frequently of T cell origin
- Involve lymph node (especially mediastinal area), spleen occasionally extranodal sites
- Hypercalcemia is common

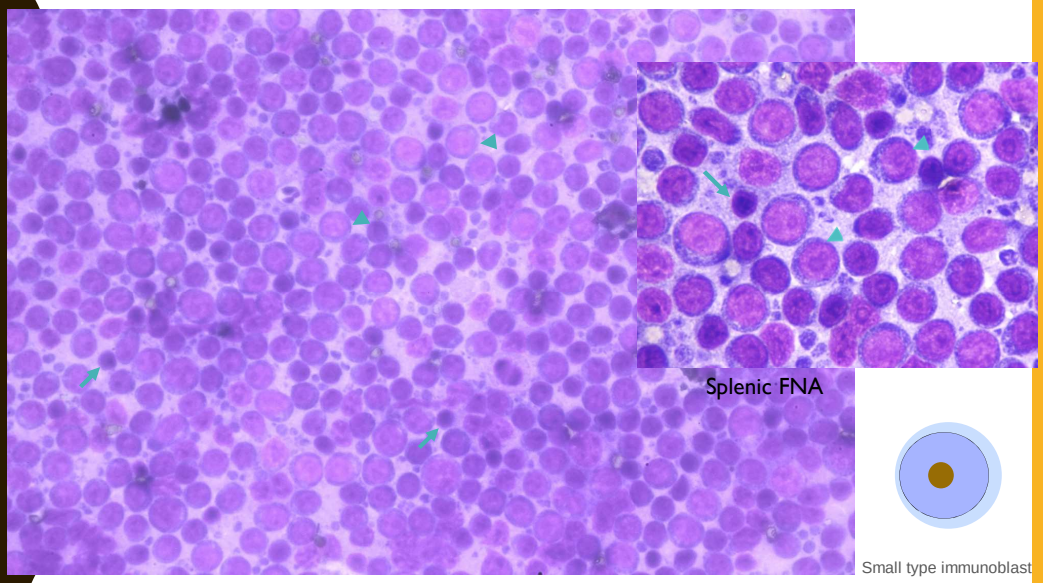
Case 2

★ A 11 year-old neutered male Poodle

★ Chronic weight loss, u/s found a large splenic mass

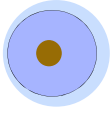
★ Splenectomy performed

- Intermediate lymphocytes dominate with few mature lymphocyte with condensed chromatin (arrows)
- The tumor cells have high N:C ratio, single, large, central nucleoli and a continuous rim of a small amount of grainy blue cytoplasm (arrowheads)
- Note: neutrophil for size comparison and lymphograndular bodies in background



Small type immunoblast

- **Dx: Marginal zone lymphoma (MZL)**
- Indolent lymphoma, originated from B cell in marginal zone
- Involve lymph node, spleen (a nodule is common) and extranodal sites
- Cytology can be suggestive of MZL, $CD79a^+$ confirm B cell origin
- DDx: immunoblastic type DLBCL lymphoma (high-grade), histopathology is required to definitively confirm

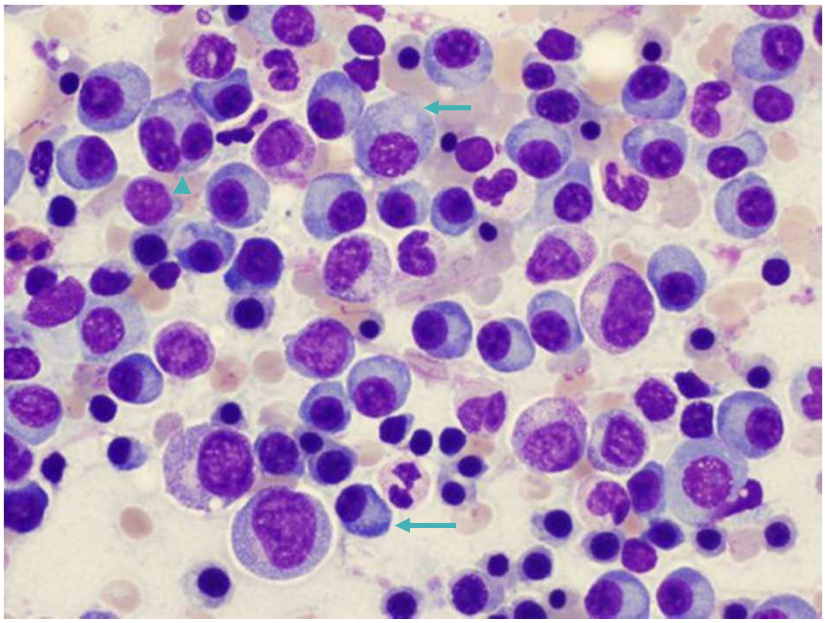


Immunoblast

Case 5

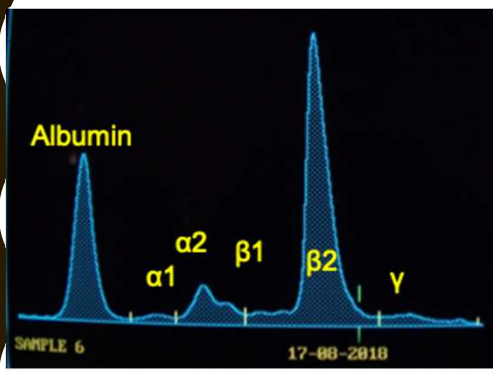
- ★ A 14 year-old neutered male Golden retriever
- ★ History of renal disease and bleeding disorder, azotemia
- ★ Non-regenerative anemia (Hct 34% and 46,900 retics/ul)
- ★ Mild thrombocytopenia (platelets 140,000 cells/ul)
- ★ WBC count was unremarkable
- ★ Hypoalbuminemia and hyperglobulinemia (Total protein = 9.8 g/dl, albumin 2.4 g/dl and globulin 7.4 g/dl)

- High numbers of plasma cell in bone marrow (>20% of nucleated cell)
- High variation in cell size: large immature nuclei with loose chromatin to the small cells with condensed chromatin (arrows)
- Multinucleated cells (arrowhead)

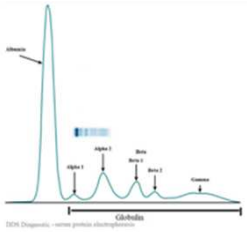


Bone marrow

Monoclonal gammopathy



- Serum protein electrophoresis shows monoclonal spike (immunoglobulin) produced by a neoplastic clone of plasma cells
- Usually IgA and IgG
- Hypercalcemia and localized osteolysis are common
- **Dx: Plasma cell myeloma, multiple myeloma**



Normal SPE