

Clinical Diagnostics in Detection of Monoclonal Gammopathies

ALEXIS DADELAHI PHD

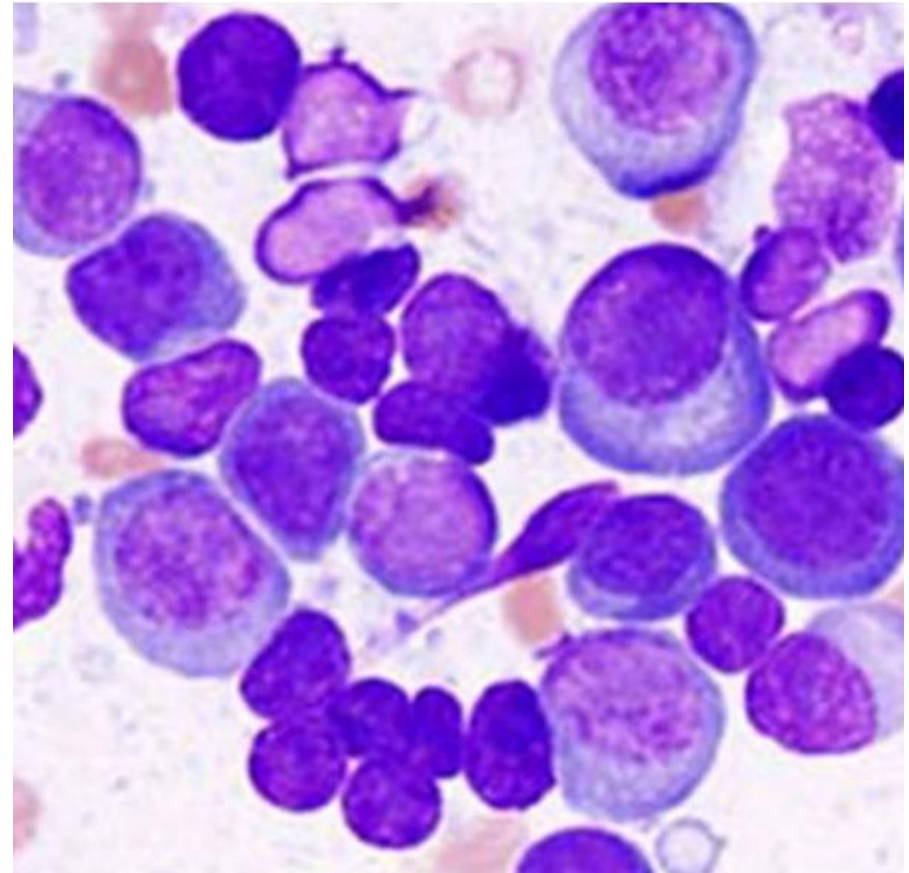
UNIVERSITY OF UTAH, DEPT. OF PATHOLOGY

Plasma Cell Dyscrasias

Neoplastic diseases of the hematologic system

- Incurable
 - But often responsive to therapy
 - Relapse is common

Early DETECTION and MONITORING are key

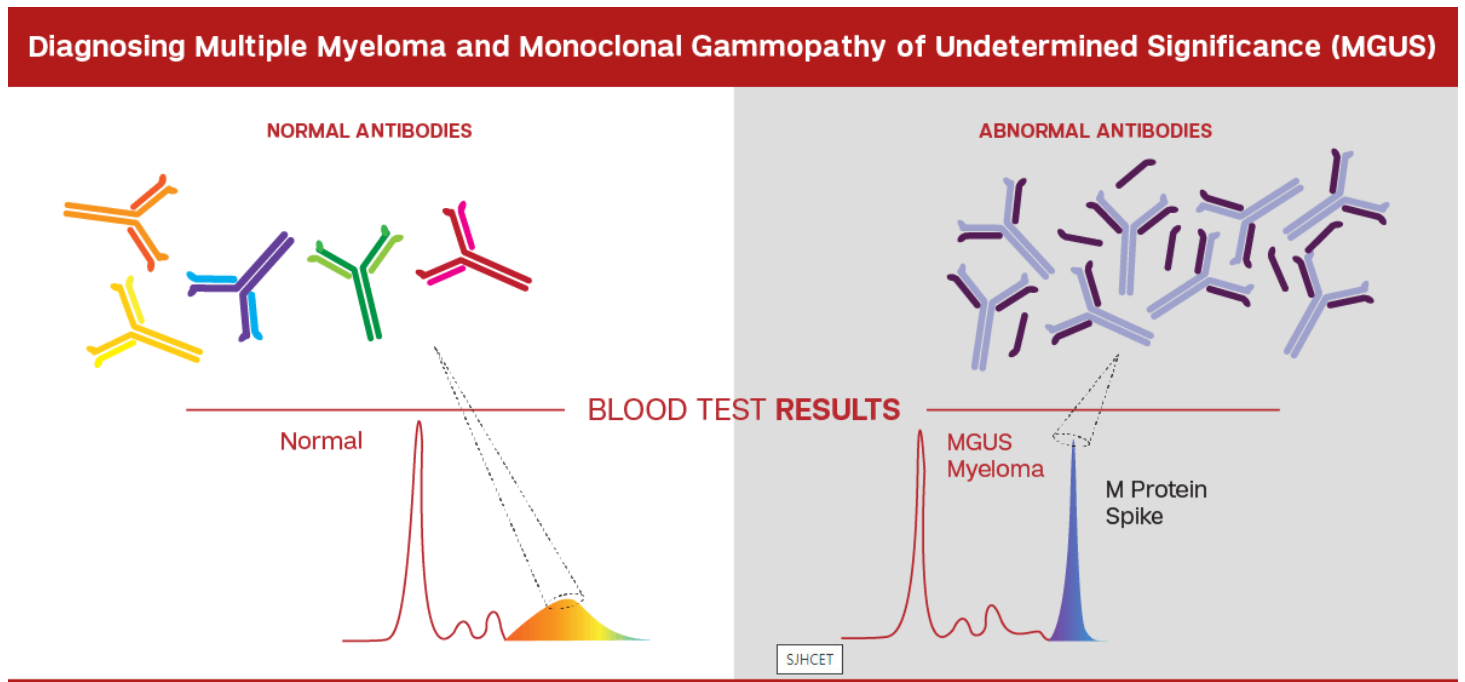


<https://ilovepathology.com/plasma-cell-disorders/>

Plasma Cell Dyscrasias → Monoclonal Gammopathies

Identified by detecting monoclonal immunoglobulin (M-protein, M-spike) produced by cancerous plasma cells

- Multiple Myeloma
- Monoclonal Light Chain Amyloidosis
- Waldenström's Macroglobulinemia



<https://www.facebook.com/WeillCornellMyelomaCenter/photos/a.424429944330390/3438809849559036/?type=3>

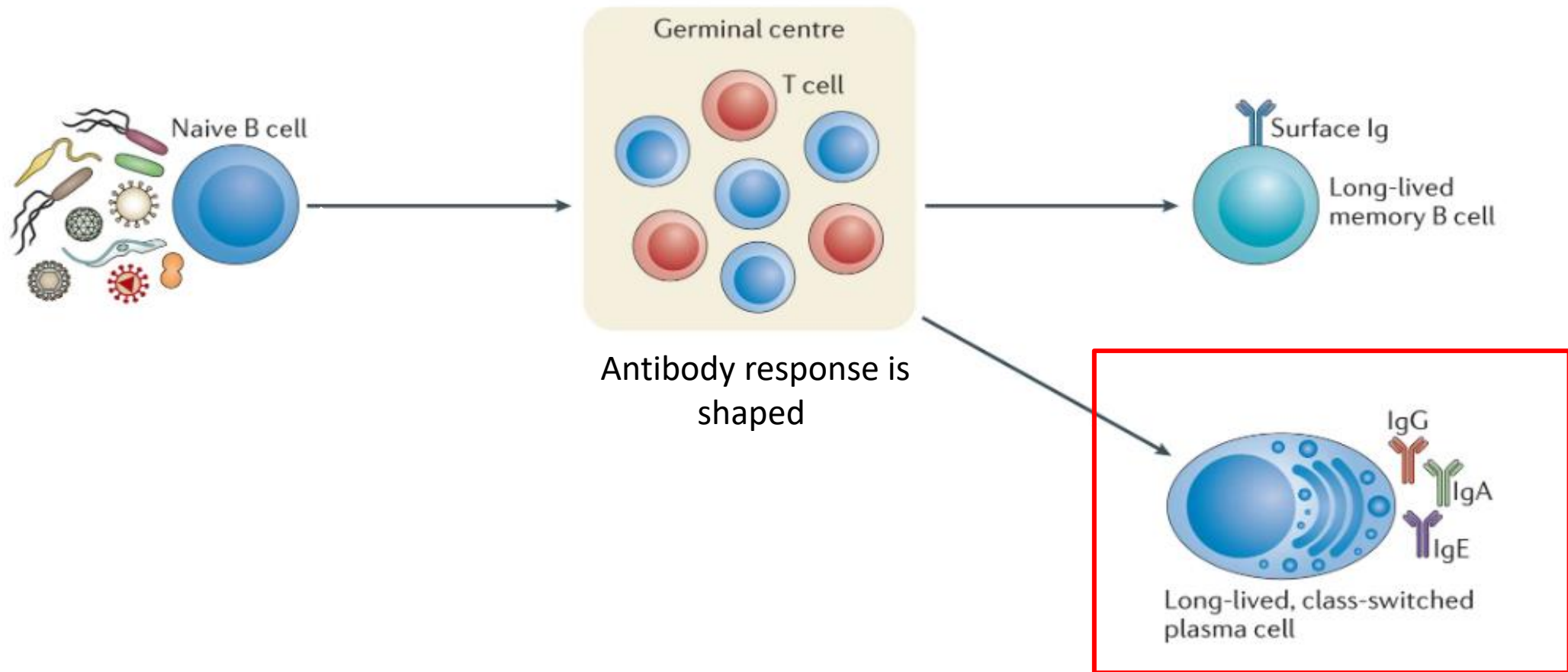
Objectives

Differentiate common diseases associated with monoclonal gammopathy based on clinical and diagnostic findings.

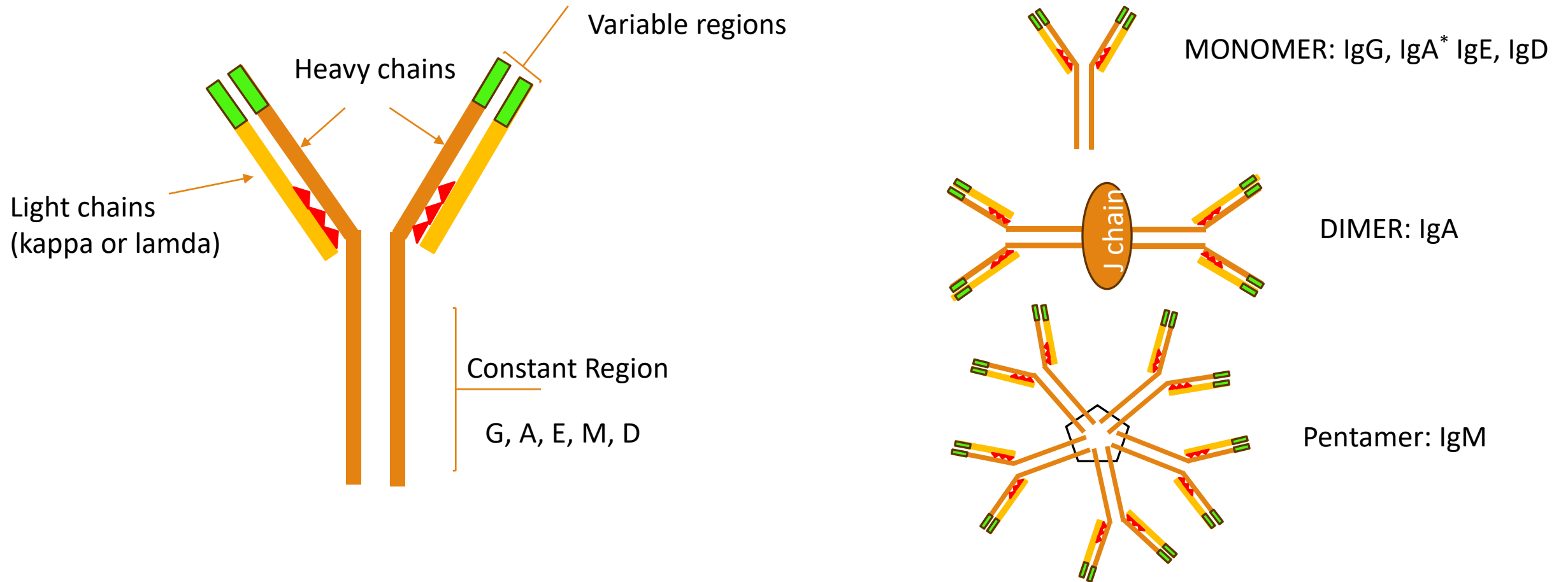
Describe the principles of monoclonal gammopathy detection and characterization, and the utility of testing to the clinician.

Interpret results and evaluate their clinical significance in monoclonal gammopathy.

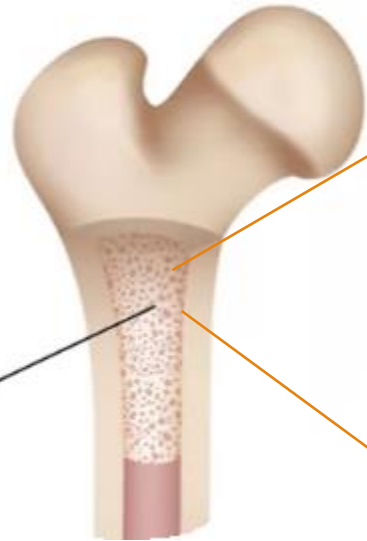
Plasma Cells “The Good”



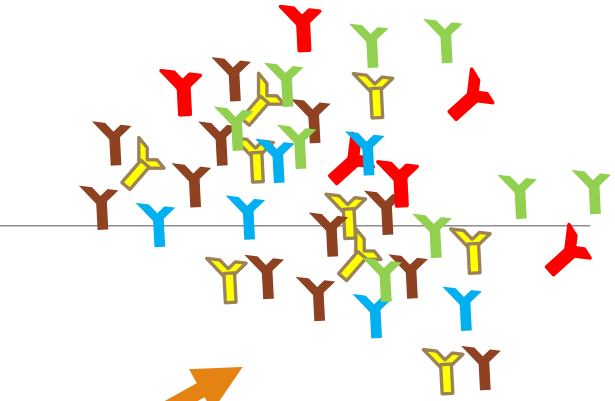
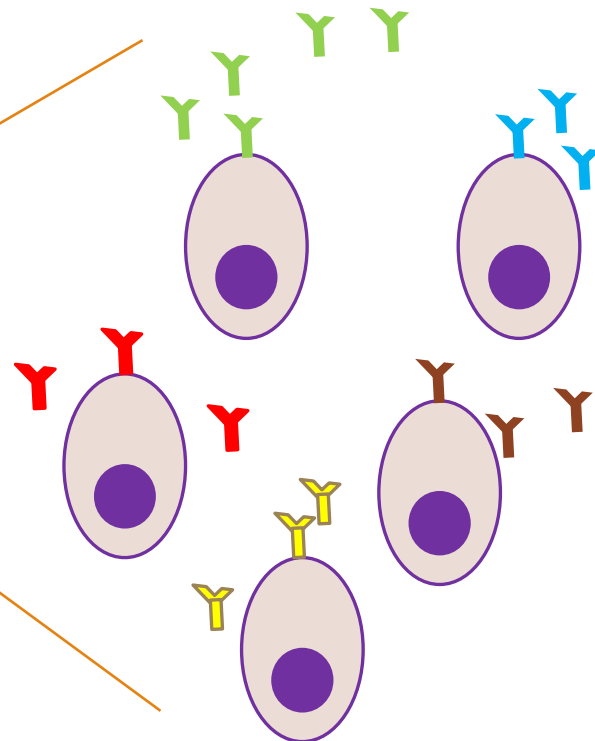
Antibody Structure and Polymerization



Healthy State



Bone marrow



Polyclonal circulating antibody



<http://www.monemotions.com/wp-content/uploads/2012/03/FreeGreatPicture.com-6035-waterfalls-streams.jpg>

Plasma Cell Dyscrasias “The Bad”

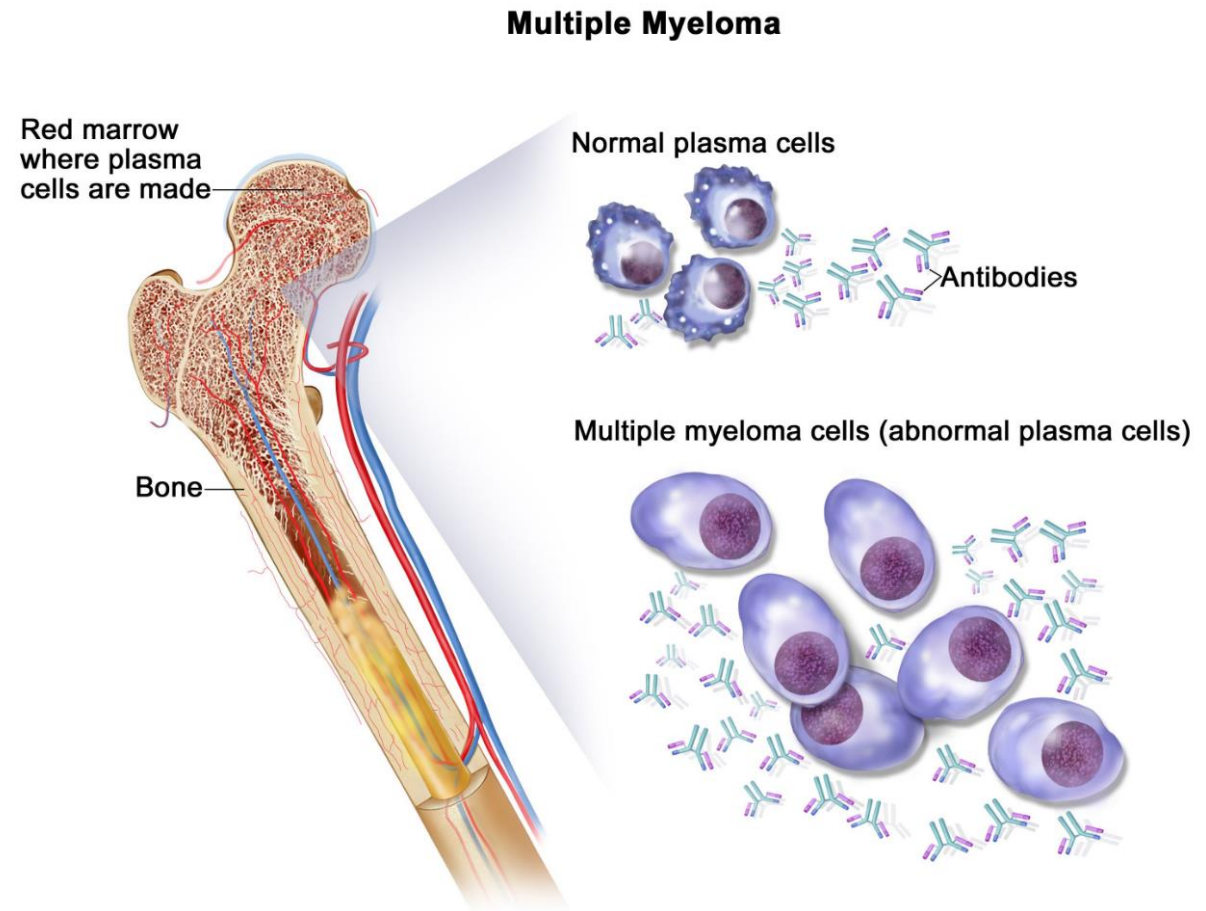
Dyscrasia: abnormal or disordered state of the body

Disease in which abnormal plasma cells proliferate and may form tumors in bones and/or soft tissues

- **Clonal expansion**

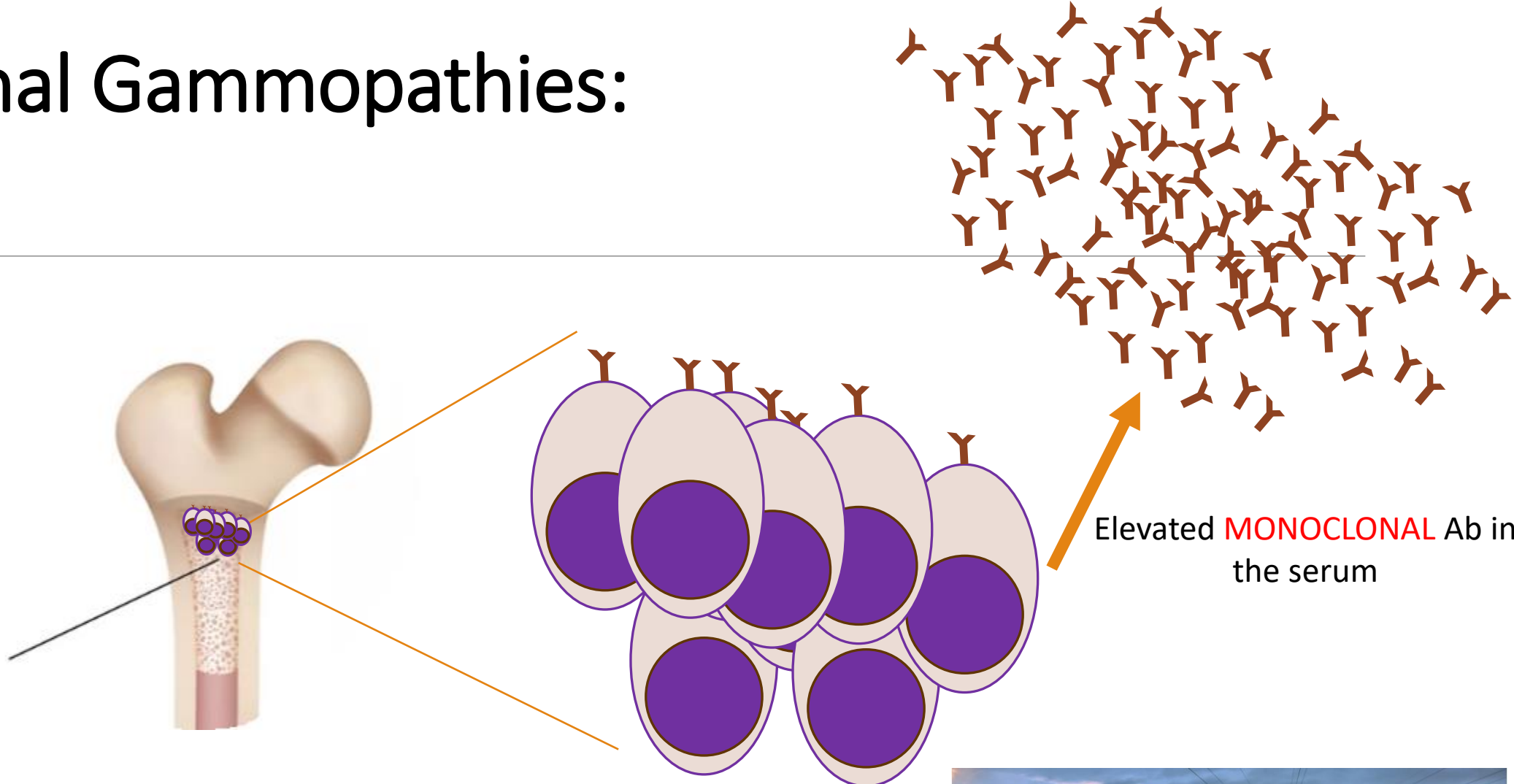
Abnormal plasma cells produce significant amounts of antibody (Ab)

- **Monoclonal**



Monoclonal Gammopathies:

Bone marrow



Elevated **MONOCLONAL** Ab in the serum



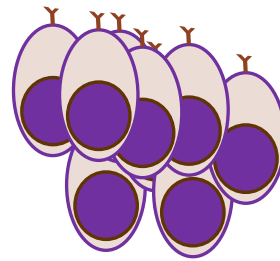
<https://www.ctpublic.org/2021-10-14/floods-threaten-to-shut-down-a-quarter-of-u-s-roads-and-critical-buildings>

Indications for Testing

- Recurrent infections
- Renal failure
- Elevated serum protein
- Unexplained proteinuria
- Unexplained peripheral neuropathy
- Suspect hyperviscosity syndrome



- Unexplained anemia
- Bone pain with lytic lesions (back)
- Fatigue
- Age-inappropriate bone fractures





Multiple Myeloma (MM)

Two mechanisms drive pathogenesis:

- Excessive proliferation of plasma cells in bone marrow
- Excessive monoclonal antibody in circulation (M-spike)
- IgG > IgA

CRAB:

Hyper**C**alcemia

Renal dysfunction

Anemia

Bone lesions

Classification of Myeloma

Monoclonal Gammopathy of Undetermined Significance

MGUS

Monoclonal protein spike <3 g/dL
Plasma cells (PC) <10% of bone marrow
No CRAB features



Smouldering multiple myeloma

Monoclonal protein spike >3 g/dL
PC >10% of bone marrow
No CRAB features

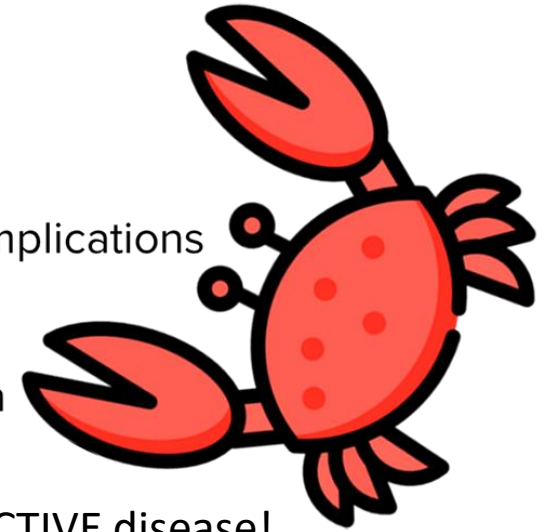


ACTIVE disease!

Multiple myeloma

Monoclonal spike
PC >10% or plasmacytoma
CRAB features
Or PC >60% or SFLC ratio >100
Or more than one focal lesion on MRI

Calcium
Renal complications
Anemia
Bone pain



Factors Influencing MM Risk and Rates of Progression:

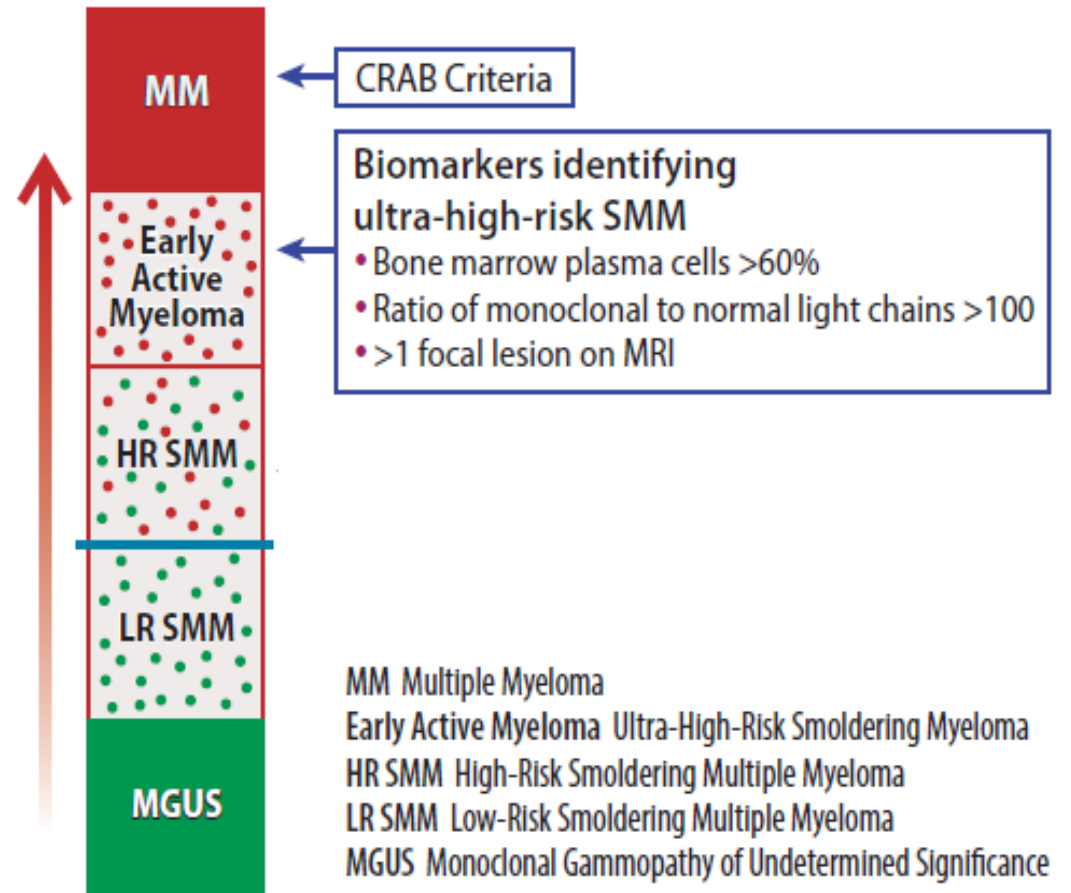
Almost all patients with MM progress from asymptomatic, pre-malignant stages (MGUS/SMM)

Males > Females

→ (>60yrs)

African American 2x > Caucasian

Rate of progression is influenced by underlying cytogenetics of disease (FISH)

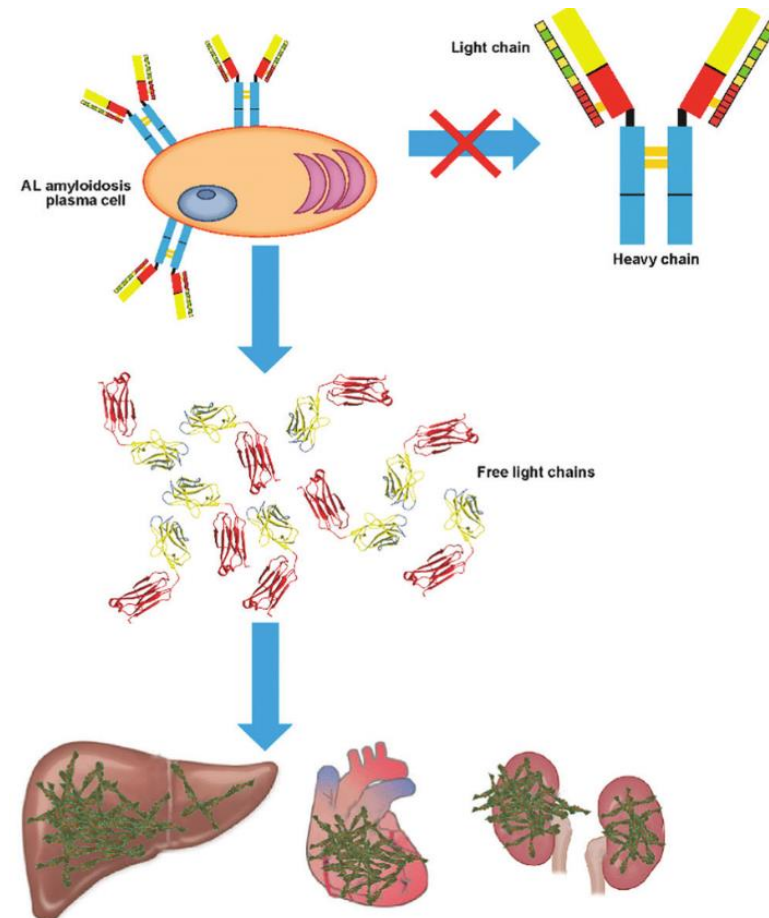


Monoclonal Light Chain Amyloidosis

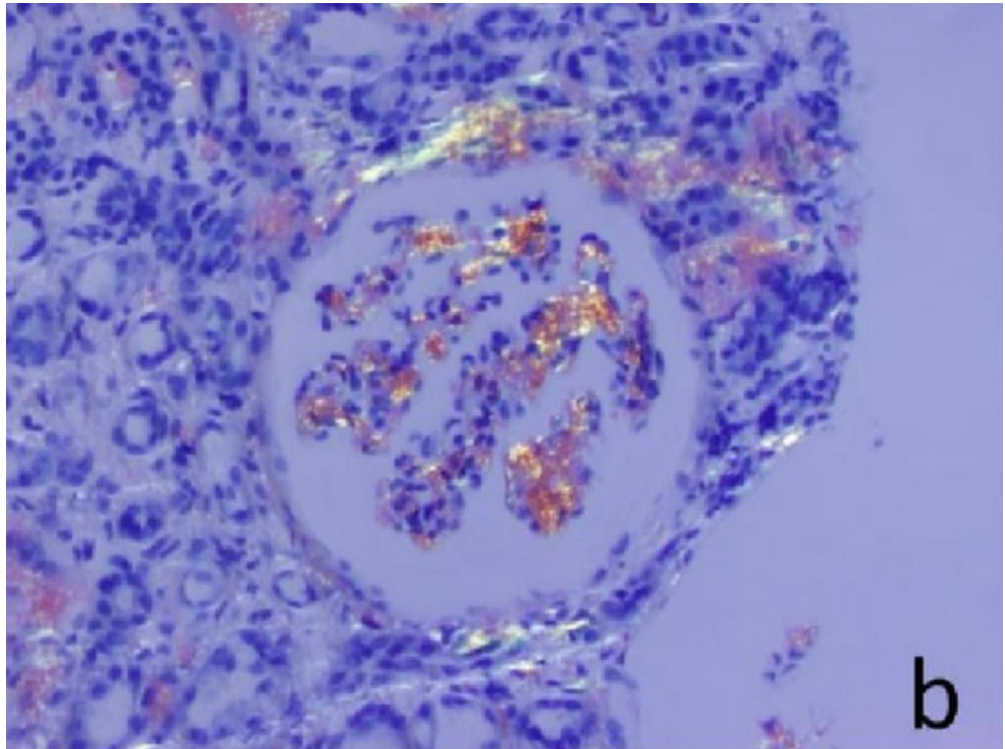
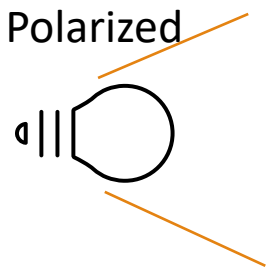
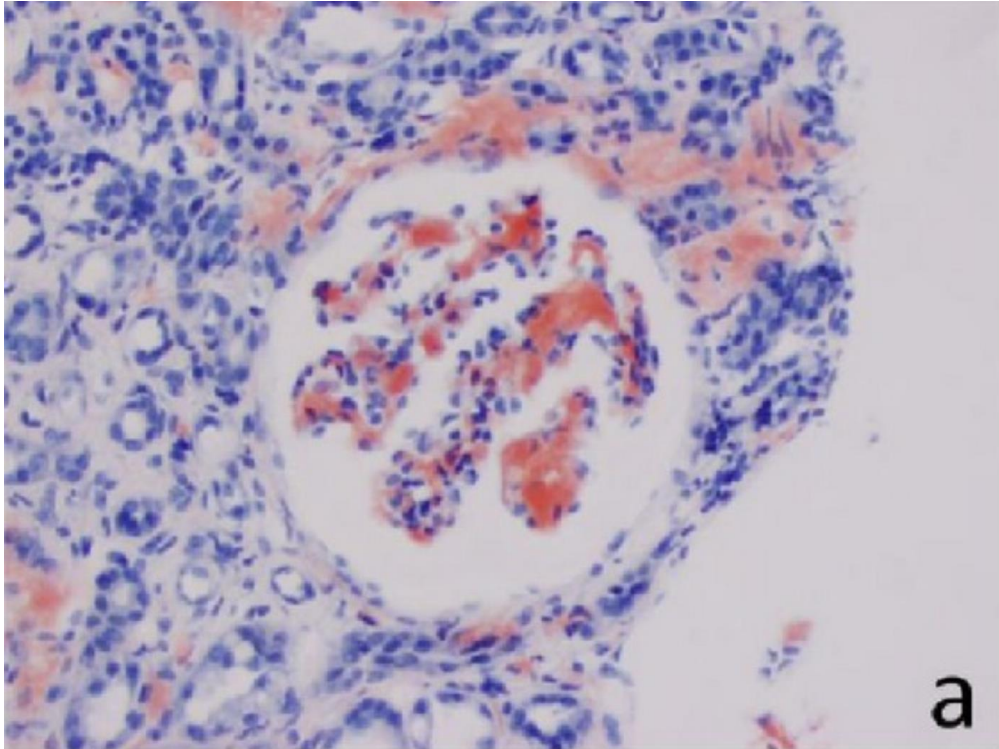
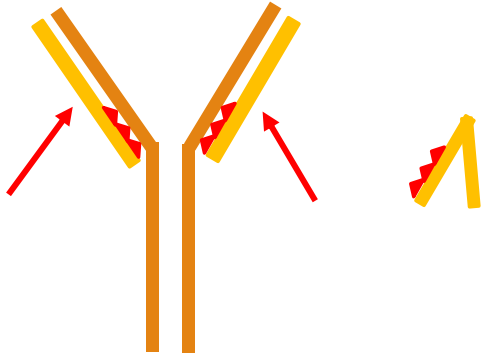
Amyloid fibrils associated with light chains

Diagnostic Criteria:

- M-protein or clonal PC in bone marrow
- Biopsy (affected organ or fat pad)
- Pathogenic immunoglobulin deposition
 - Shortness of breath
 - Cardiomyopathy
 - Nephrotic syndrome
 - Peripheral neuropathy
 - Macroglossia (enlarged tongue)



Monoclonal Light Chain Amyloidosis



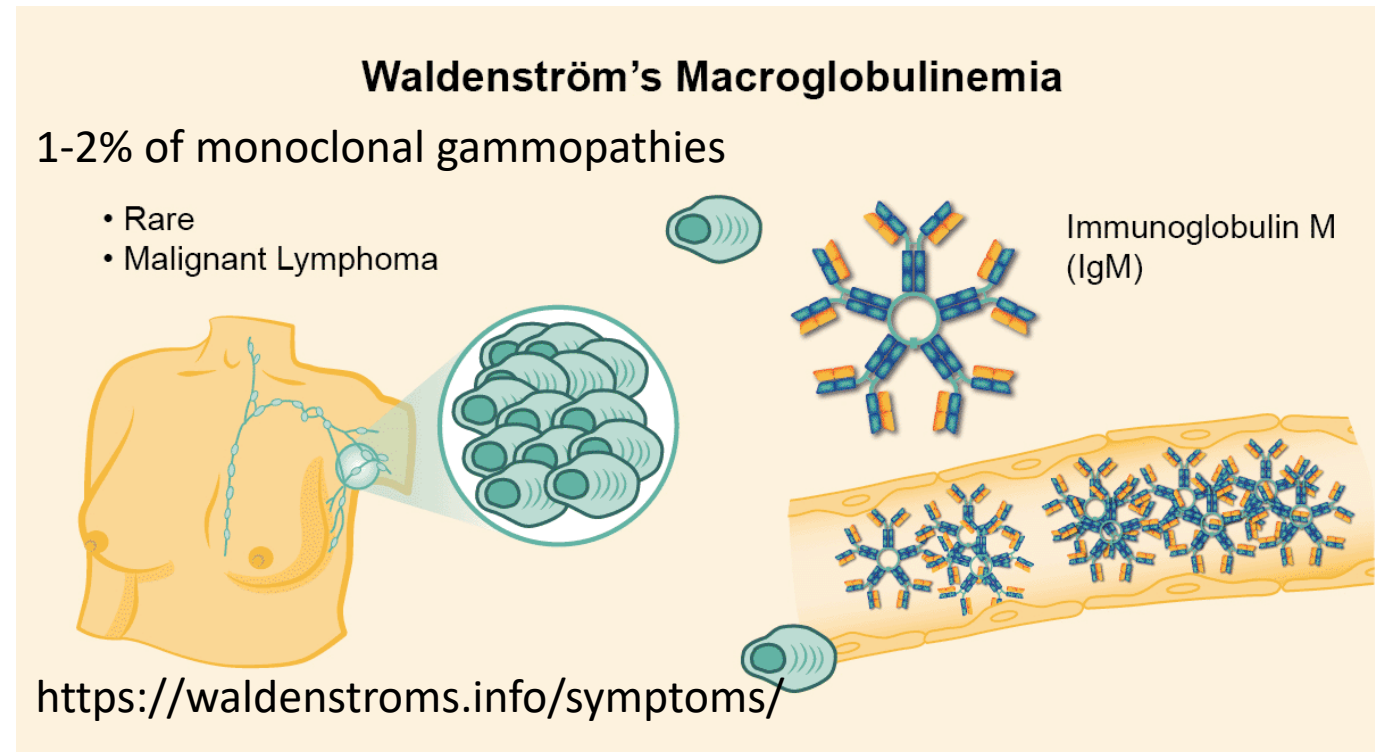
Ref: Bowen K, et al. AL-Amyloidosis Presenting with Negative Congo Red Staining in the Setting of High Clinical Suspicion: A Case Report. Case Rep Nephrol. 2012;2012:593460

Waldenström's Macroglobulinemia

IgM secreting cell accumulation

Diagnostic Criteria:

- Serum IgM M protein (independent of size)
- Bone marrow lymphoplasmacytic infiltration \geq 10%
- End organ damage (not CRAB)
 - Hepatosplenomegaly, lymphadenopathy, anemia; HYPERVISCOCITY
 - Neuropathy



How Can We Assess Monoclonal Gammopathies?

Serum/urine protein

- Total
- Immunoglobulin specific

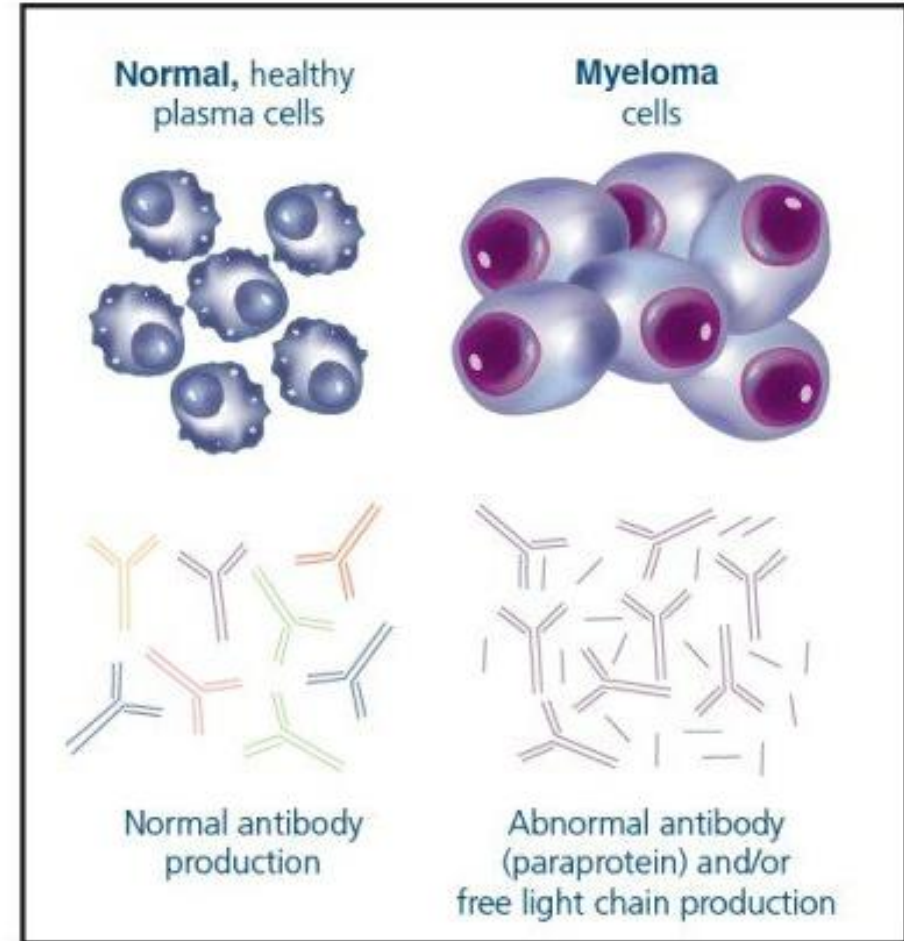
Protein electrophoresis

- Serum and urine
- Immunofixation

Serum/urine free light chain

Mass spectrometry

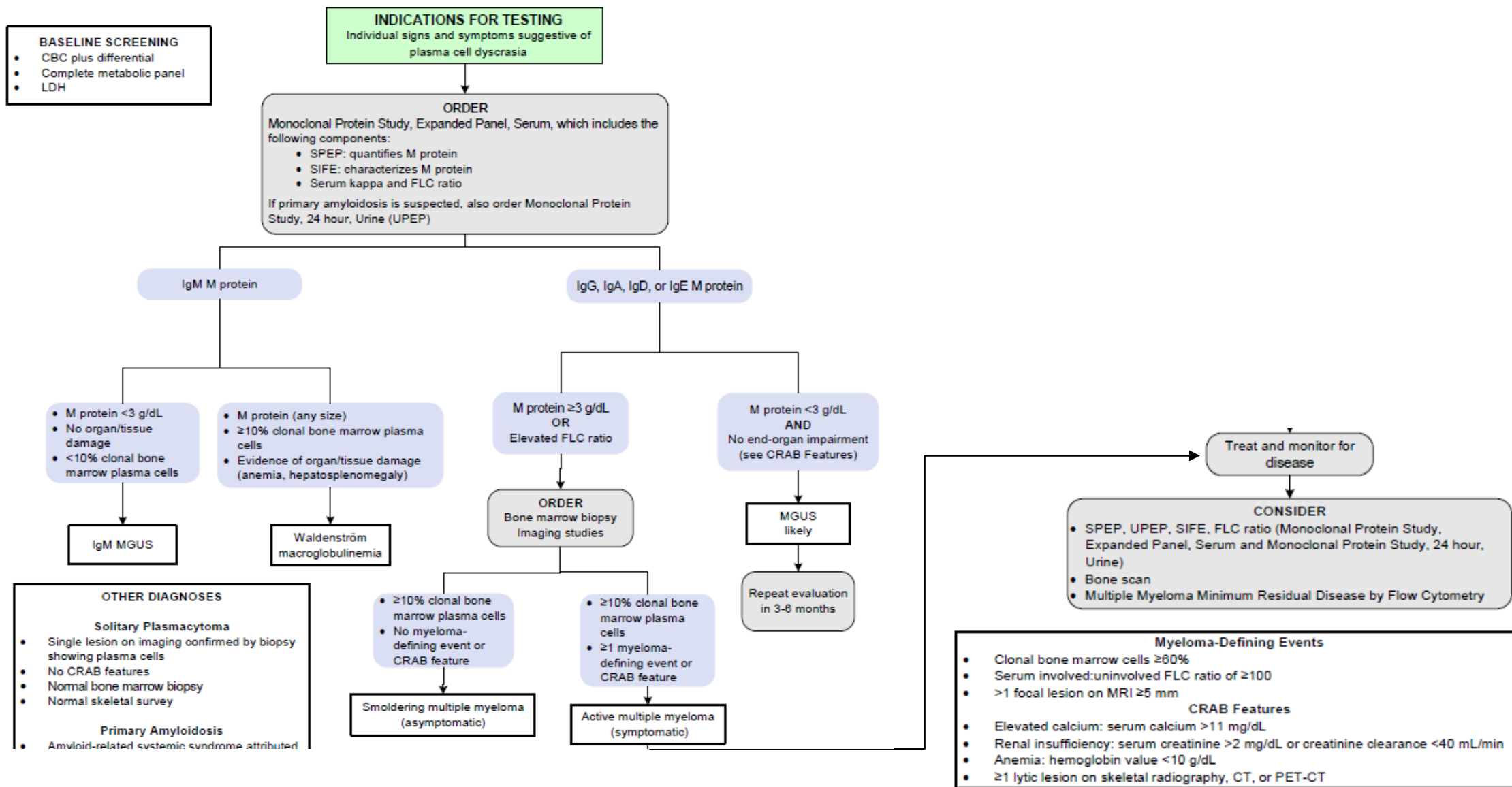
- Increased sensitivity and specificity



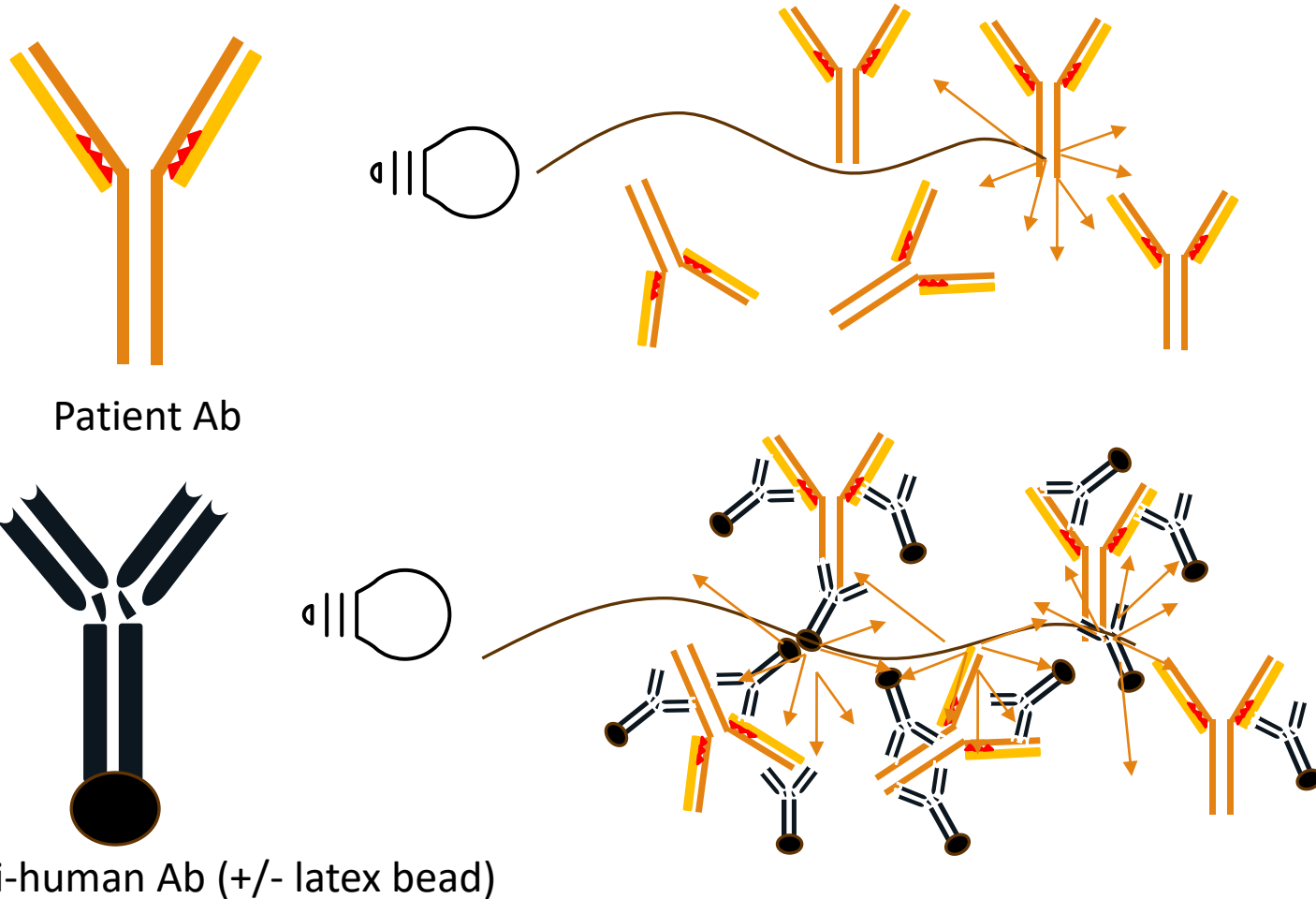
<https://thewaitingroom.karger.com/tell-me-about/what-are-the-types-and-effects-of-myeloma/>

Plasma Cell Dyscrasias

[Click here for topics associated with this algorithm](#)

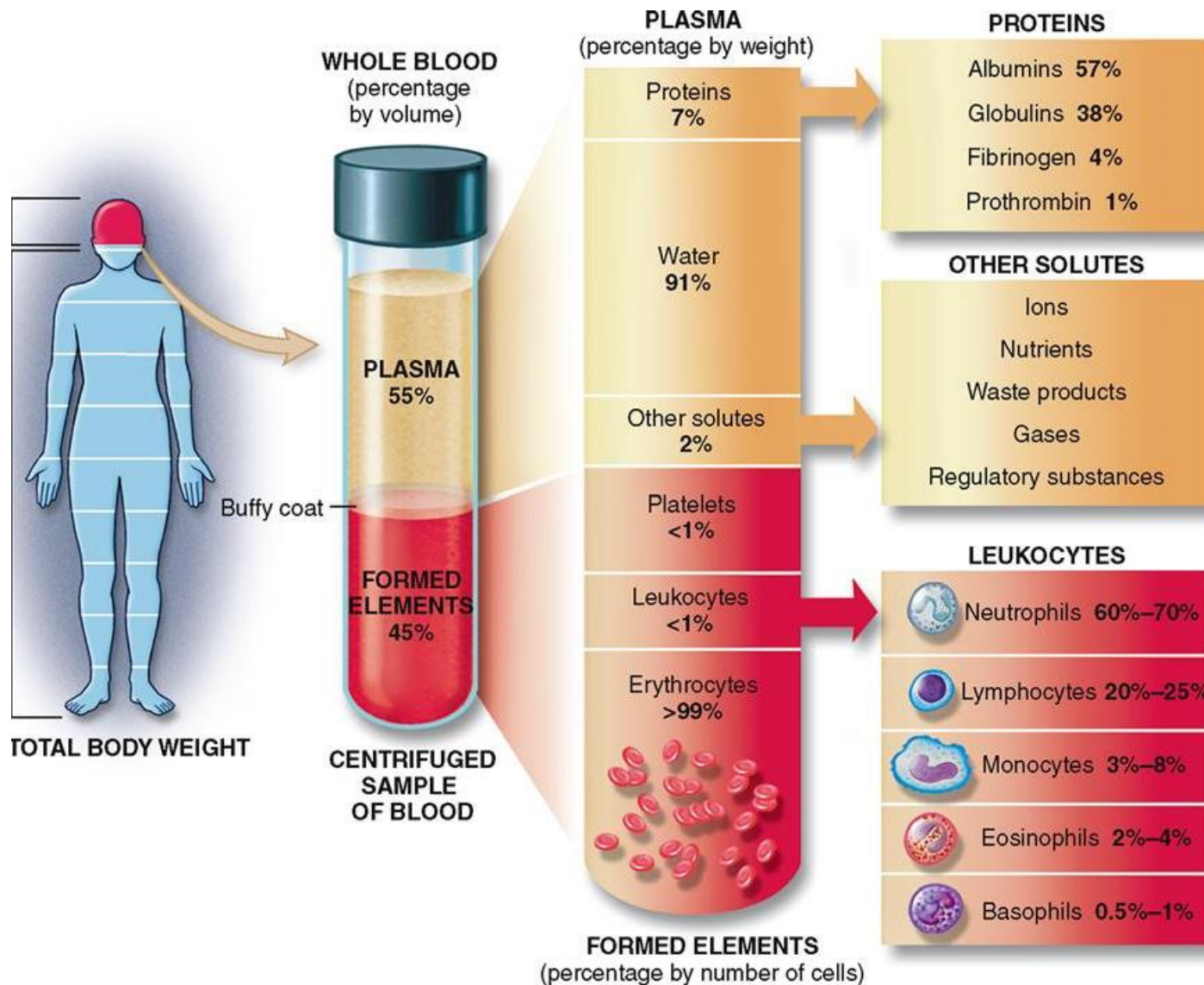


Quantification of Immunoglobulin (Ab)

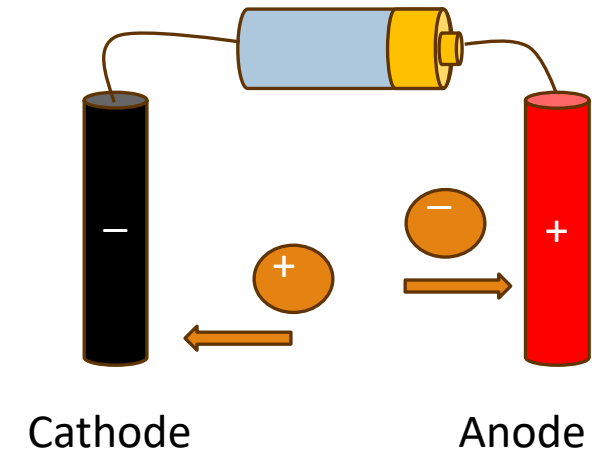


- **Turbidimetric/Nephelometric methods**
- **Measures IgG, IgA, and IgM total**
- **Increase of one class can indicate the presence of monoclonal protein**

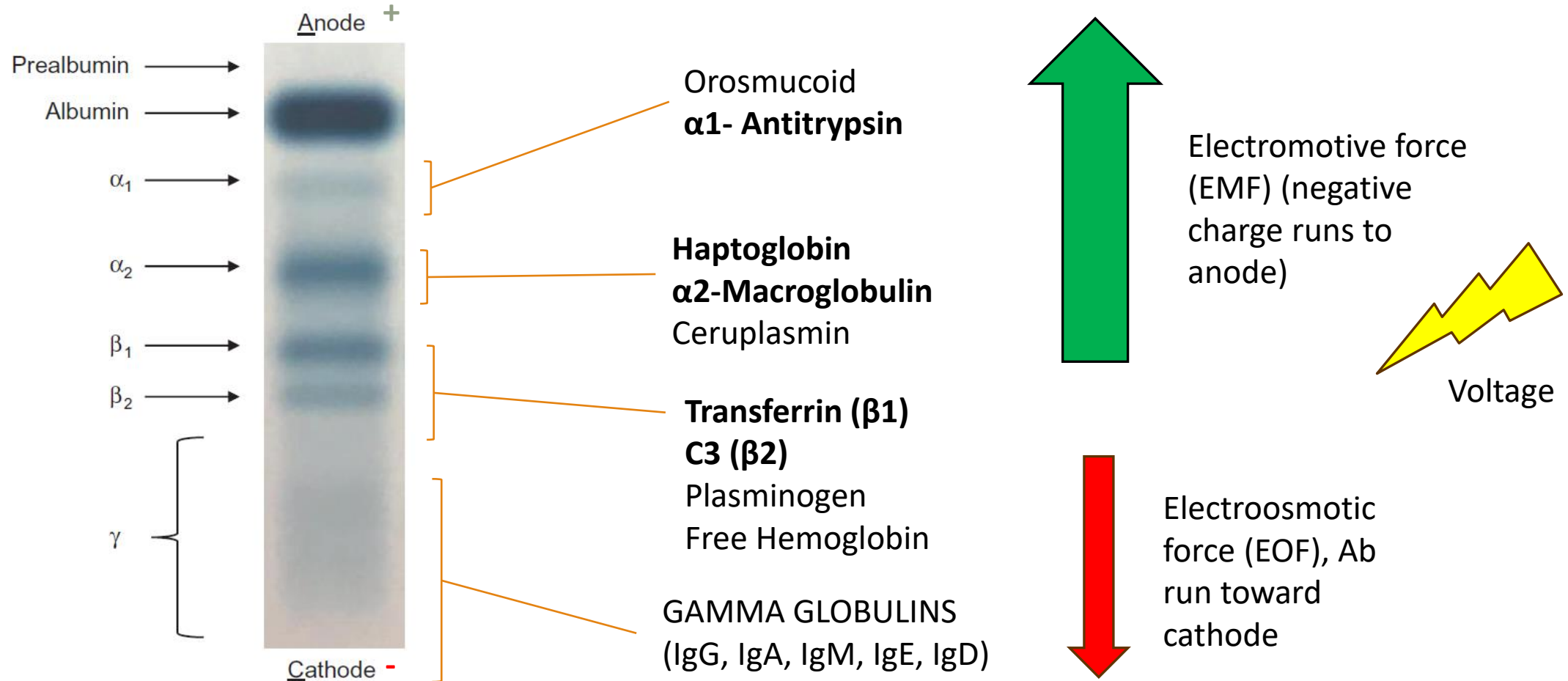
Electrophoresis and protein analysis:



- Albumin (60%)**
Major component of osmotic pressure of plasma
- Globulins (35%)**
Antibodies (immunoglobulin) and transport proteins
- Fibrinogens (4%)**
Functions in blood clotting
- Other (<1%)**
Various roles (α -1-antitrypsin, coagulation factors, etc.)



Predictable Protein Band Migration Pattern After Electrophoresis (S/UPEP)



Gel-Based Electrophoresis

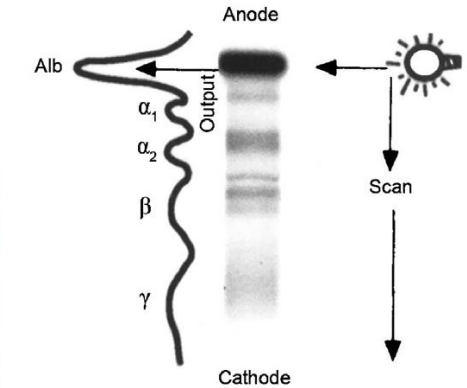
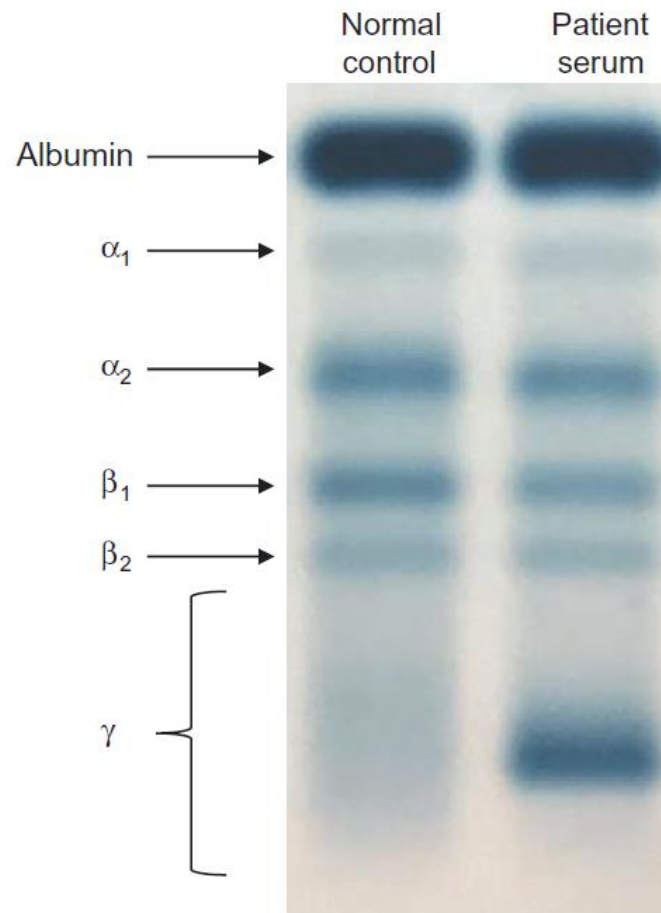
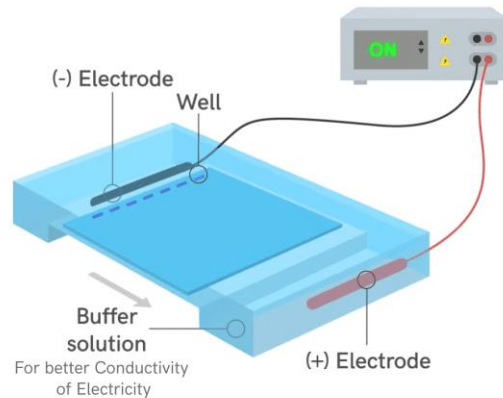
Serum/urine is loaded onto agarose gel
(**agarose**, polyacrylamide etc.)

Current is applied

Predictable pattern

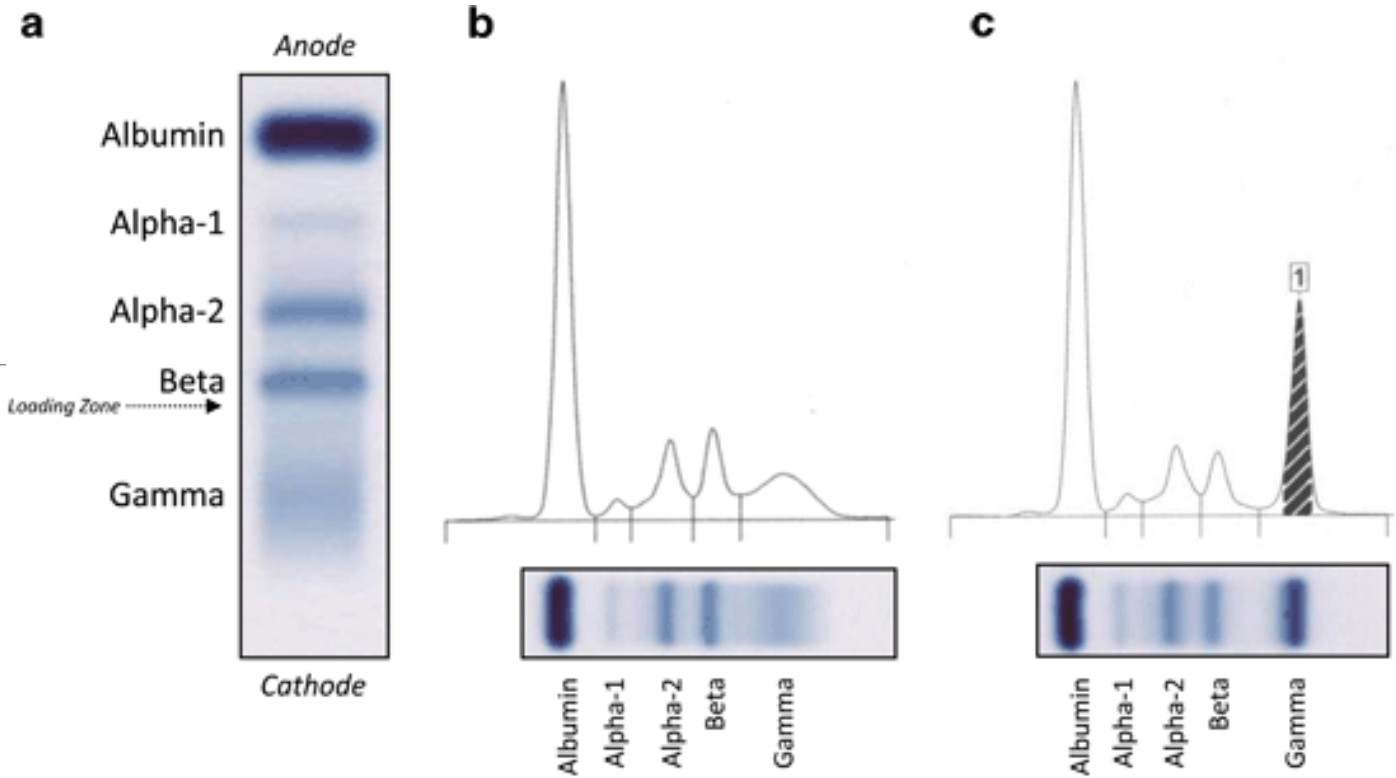
M-spike?

Electropherogram → QUANTIFY protein



Sample Loading Zone
←

← M-protein



Fraction	%	Ref. %	g/dL	Ref. g/dL
Albumin	56.2	47.0-71.0	3.95	2.58-5.68
Alpha 1	2.2	2.0-4.4	0.6	0.11-0.35
Alpha 2	10.5	5.8-13.4	0.81	0.32-1.07
Beta	11.8	4.5-15.7	0.80	0.25-1.26
Gamma	16.1	11.3-24.0	1.07	0.62-.92

TOTAL PROTEIN and gating dependent

Images from: <https://www.medicalexpo.com/prod/sebia/product-69959-442598.html>

Figures adapted from: Genzen, J. (2016). Clinical Protein and Immunofixation Electrophoresis.

In: Linden, M., McKenna, R. (eds) Plasma Cell Neoplasms. Springer, Cham.

https://doi.org/10.1007/978-3-319-10918-3_1

Capillary Zone Electrophoresis

Gel-less system

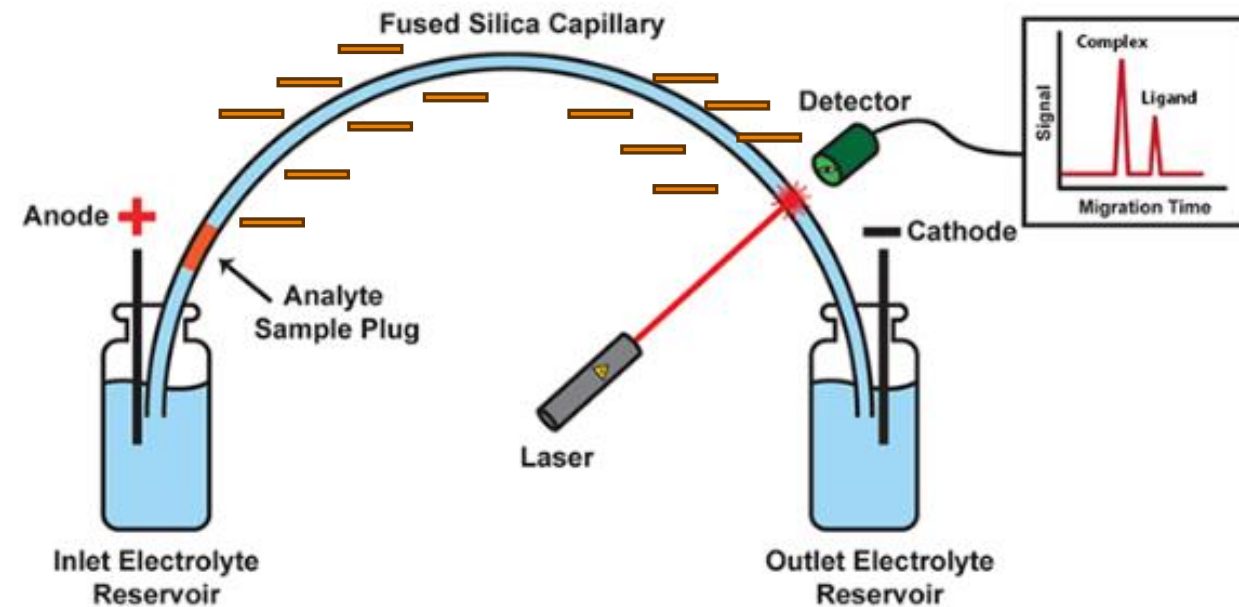
- Protein migration occurs in solution in a fused silica capillary
- Proteins move with flow of the buffer due to electroosmotic force

Pros:

- Fast
- Automated
- No stain required

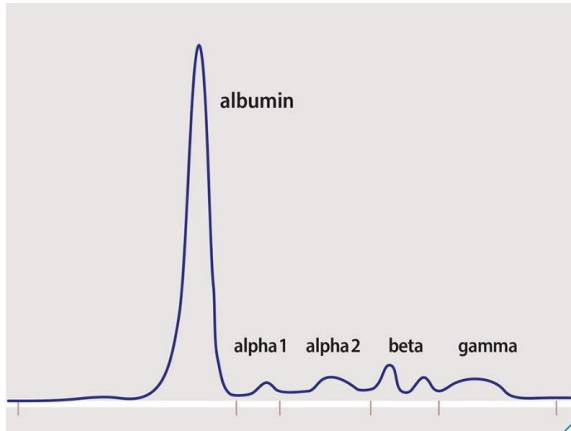
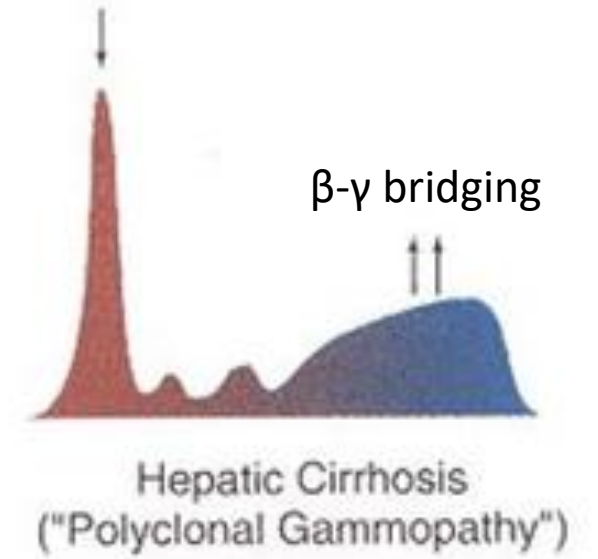
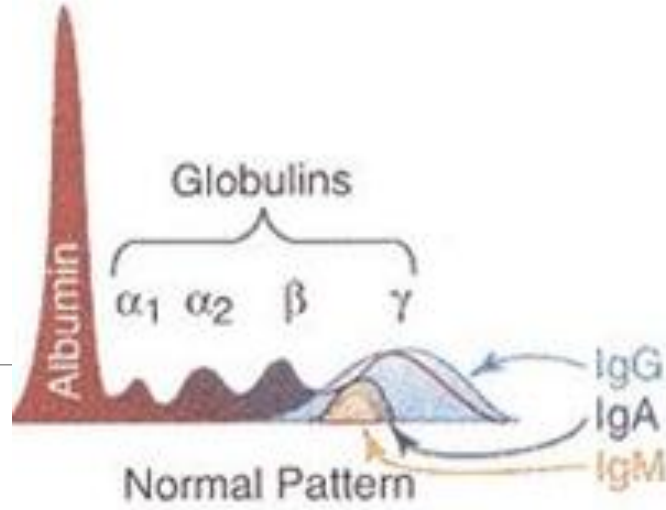
Cons:

- No gel* (digital gel)
- Contrast dye peak can cause interference
- Difficulty with small peaks

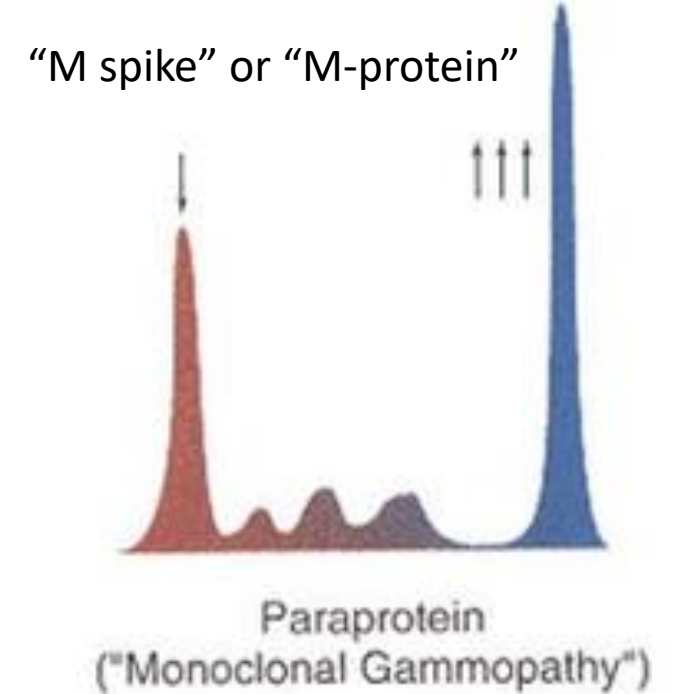
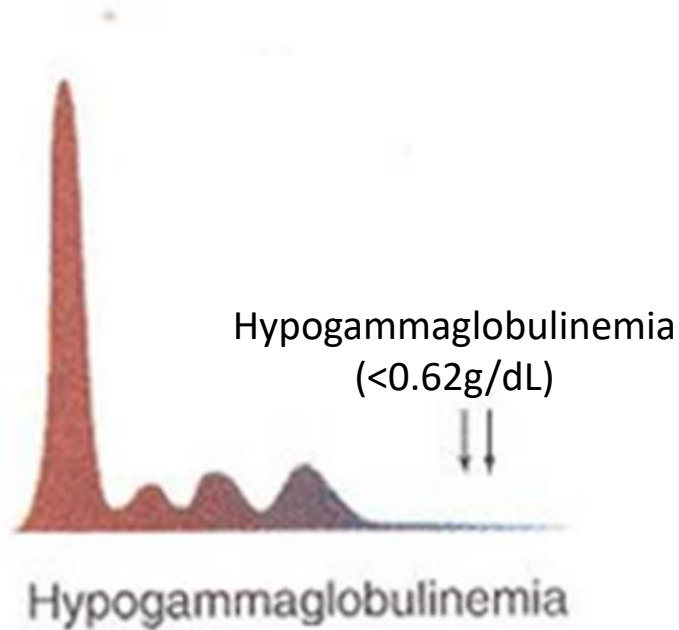


<https://www.labgene.ch/qsep-instruments/622-qsep100.html>

Interpretation of U/SPEP Patterns



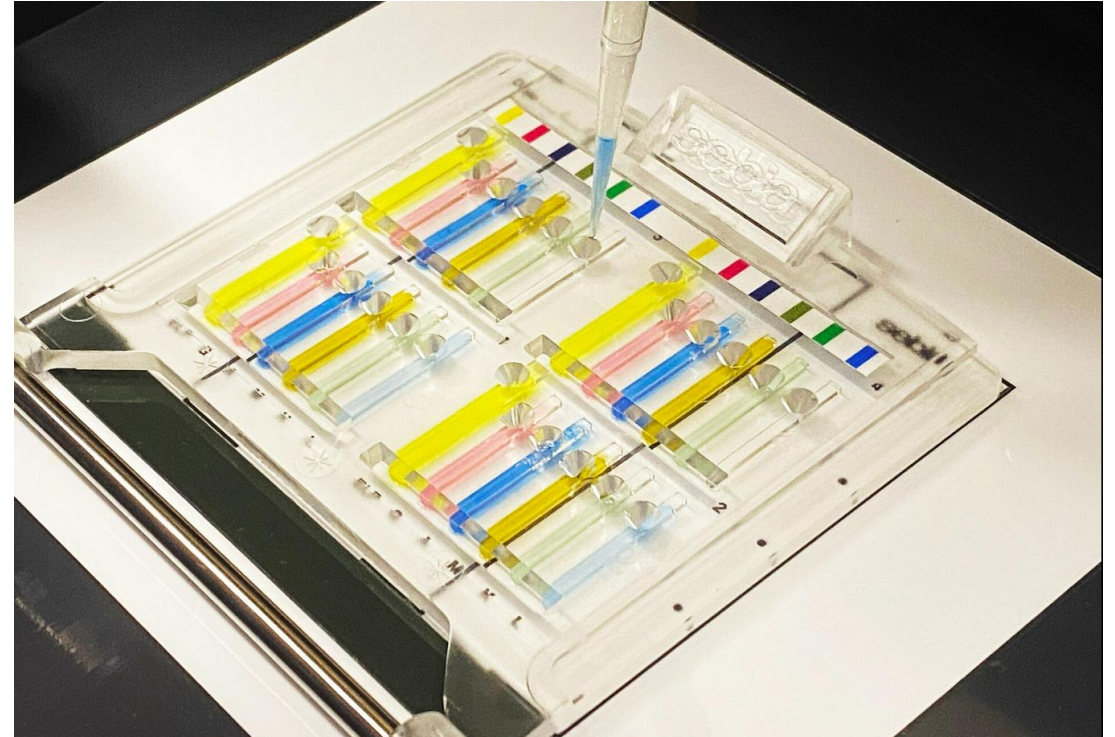
"Text book" normal pattern



How Do We Attribute a Peak to an M-Spike?

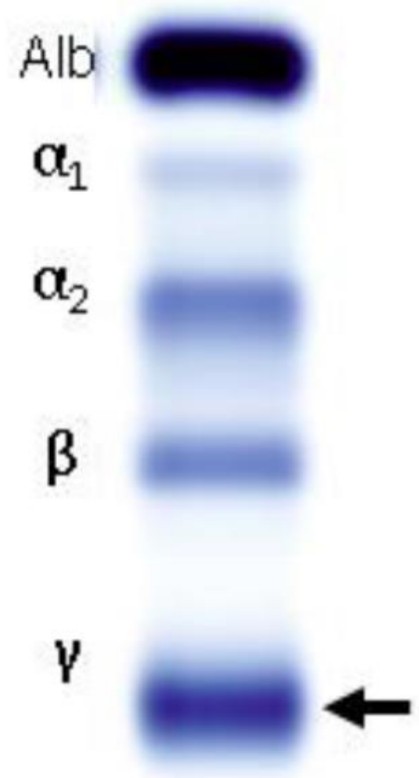
Immunofixation Electrophoresis (IFE)

- Based on the principle that antibody will precipitate with antigen (fix) in a gel matrix
- Allows identification of the clone type (heavy and light chain)
- Hypogammaglobulinemia → “hidden” spike
 - 5-10 fold greater analytical sensitivity than PEP for M-spike detection
- Can differentiate free and bound light chains

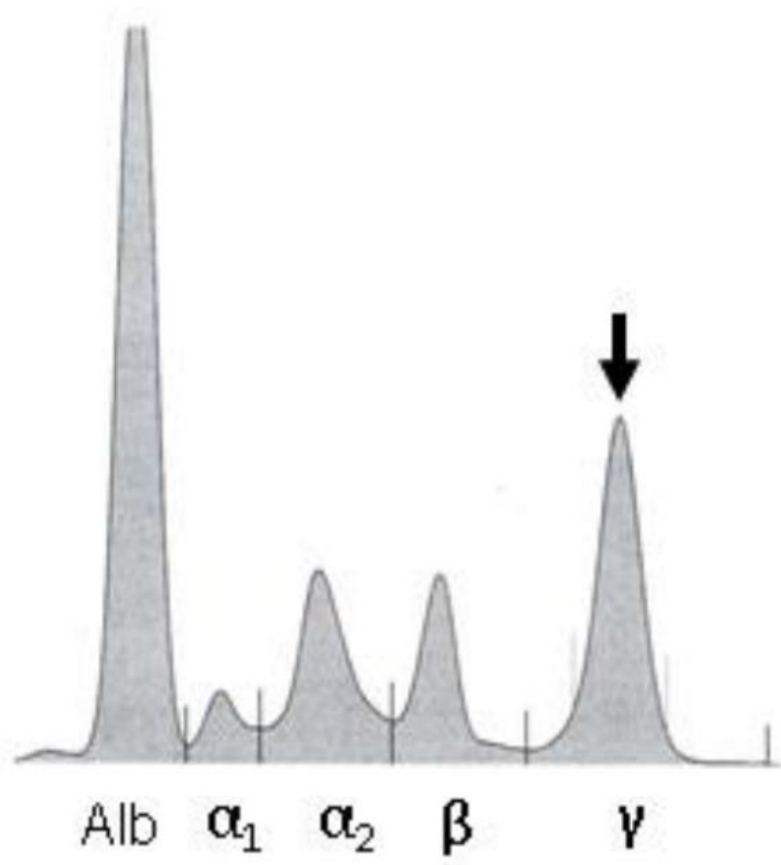


https://en.wikipedia.org/wiki/Immunofixation#/media/File:Pipetting_anti-immunoglobulins_to_immunofixation_panel.jpg

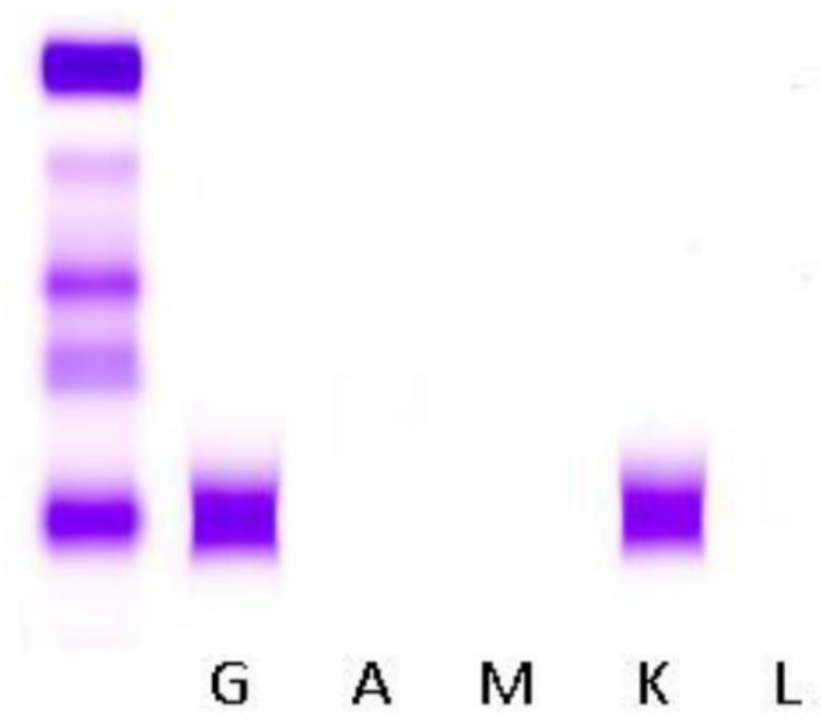
(A)



(B)

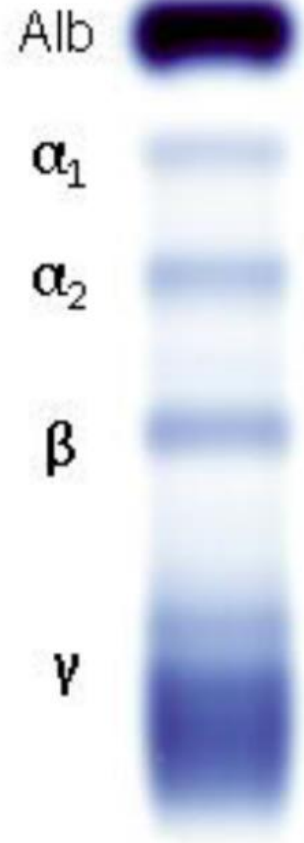


(C)

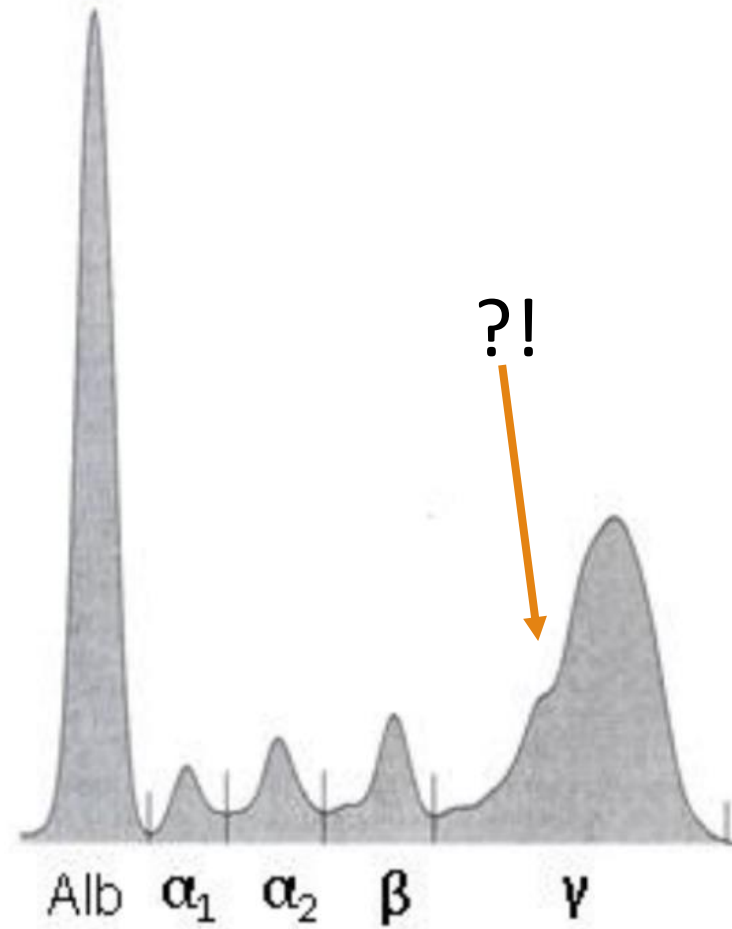


Majority of monoclonal gammopathies IgG, IgA, or IgM

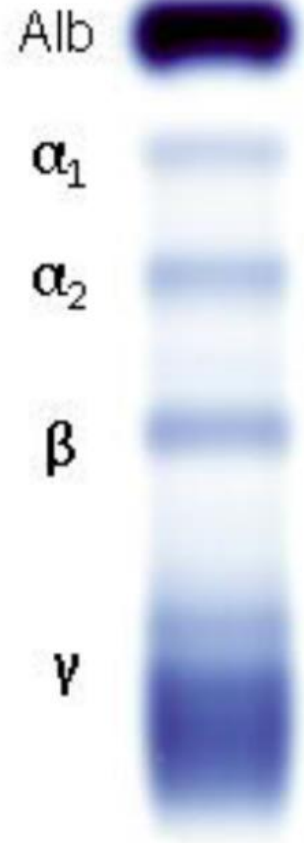
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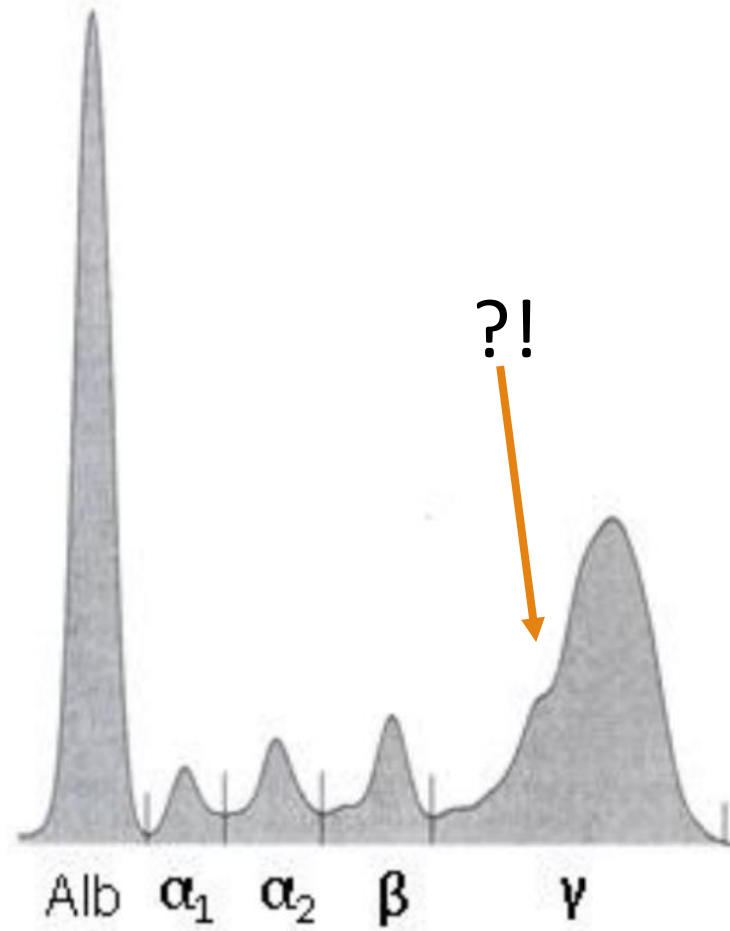
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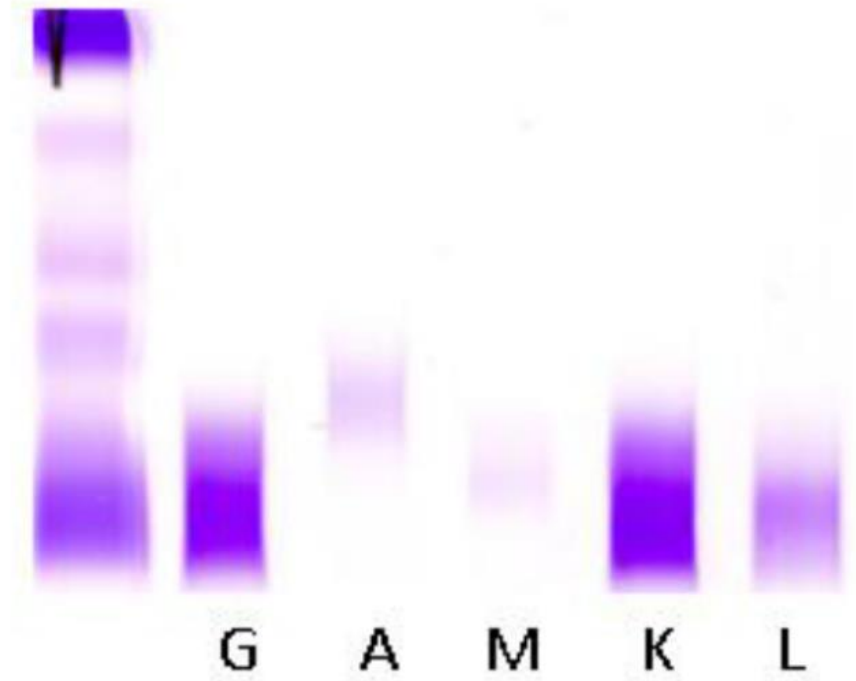
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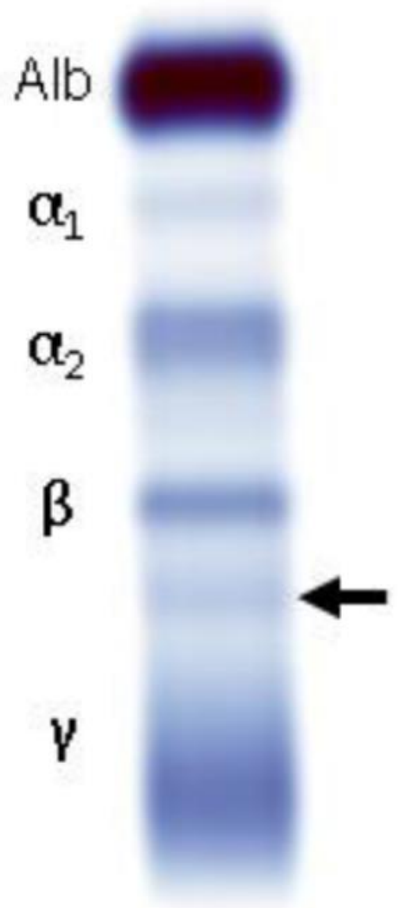
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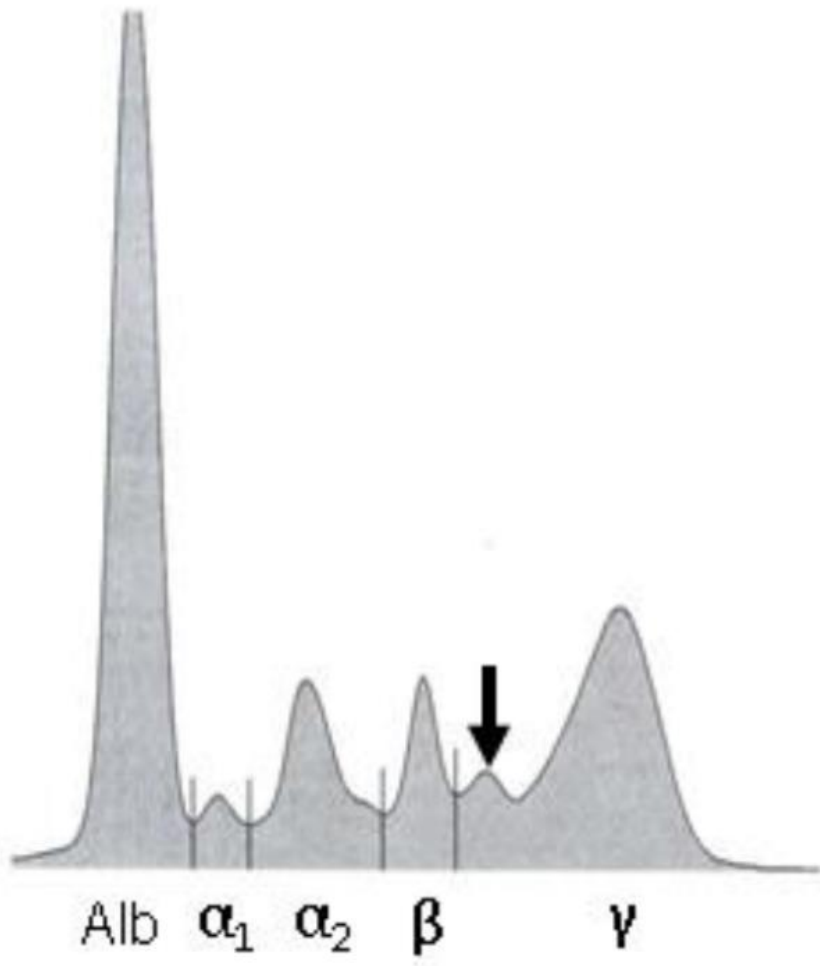
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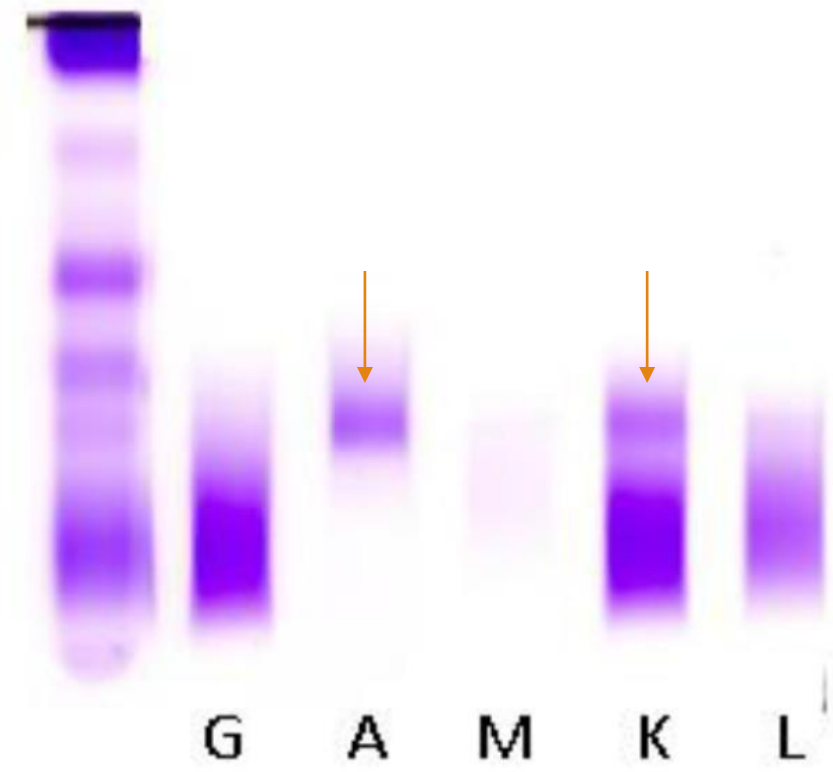
(A)



(B)



(C)



(A)

Alb



(B)

Alb



(A)

Alb



(B)



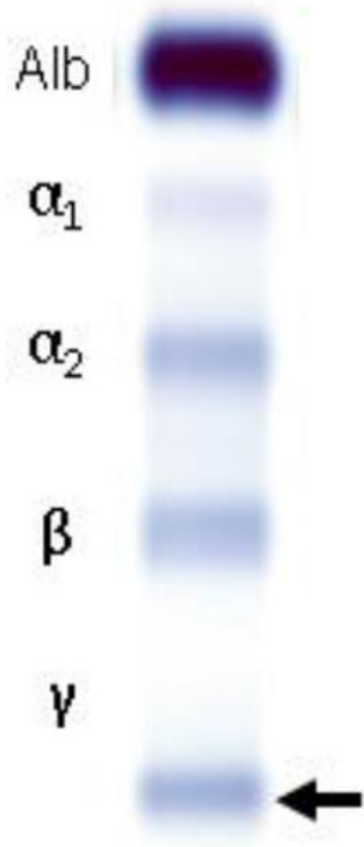
Alb

(C)

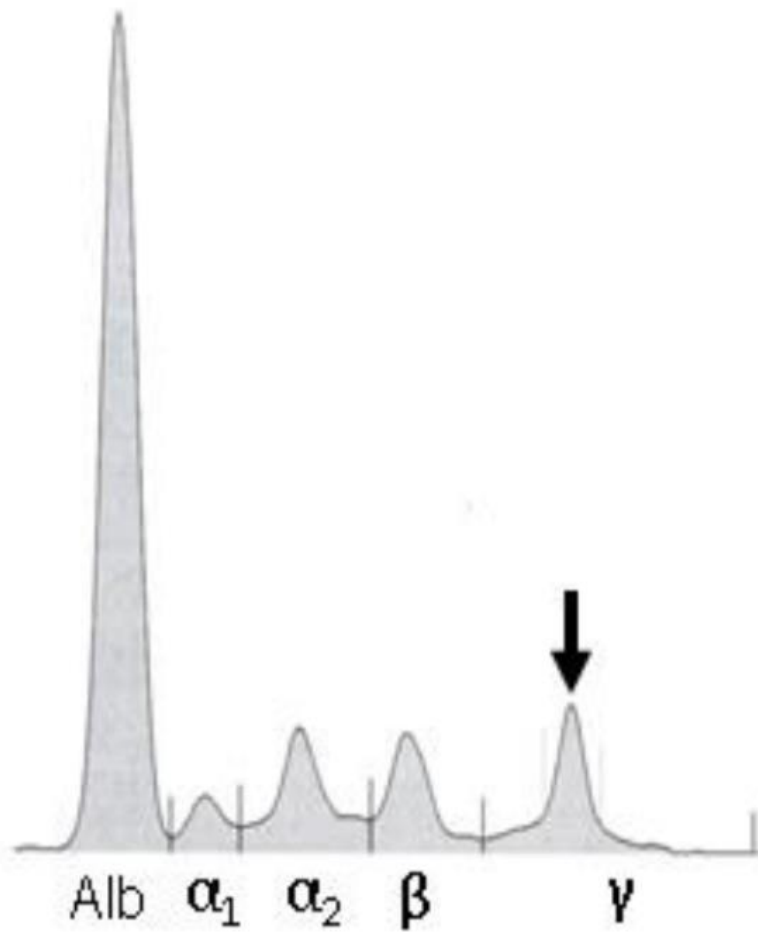


G A M K L

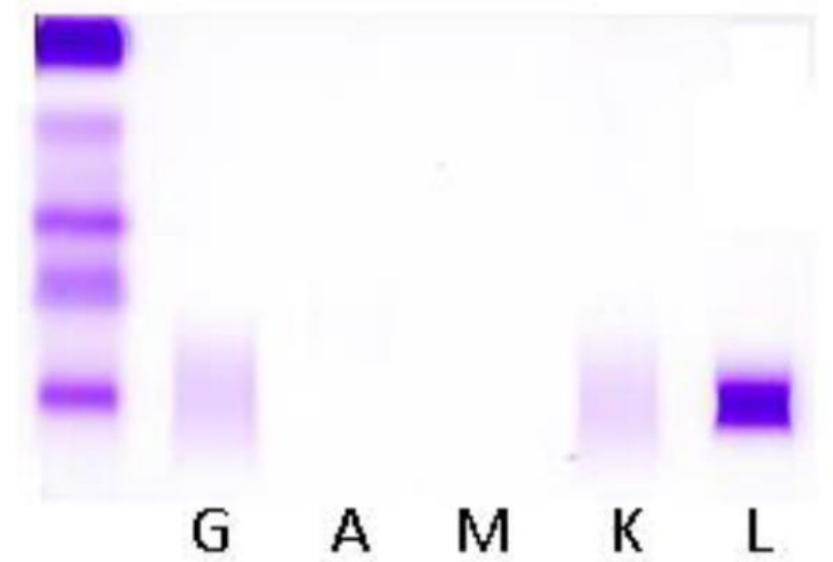
(A)



(B)

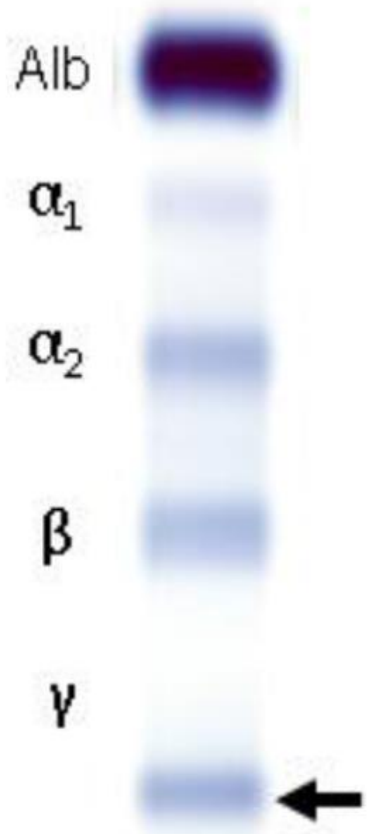


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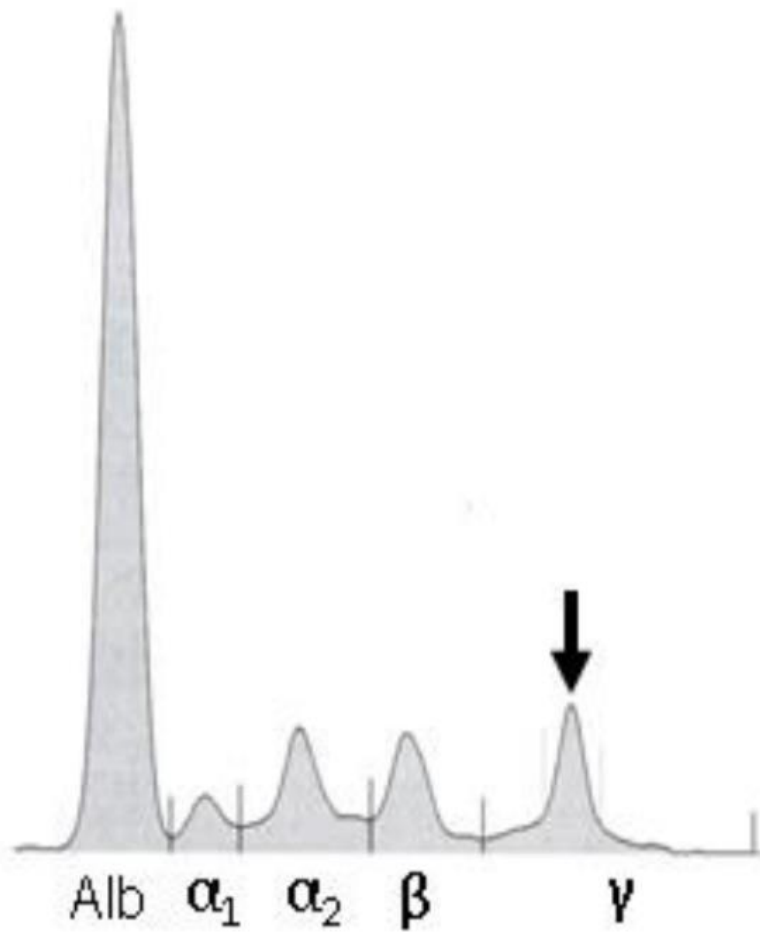


D or E???

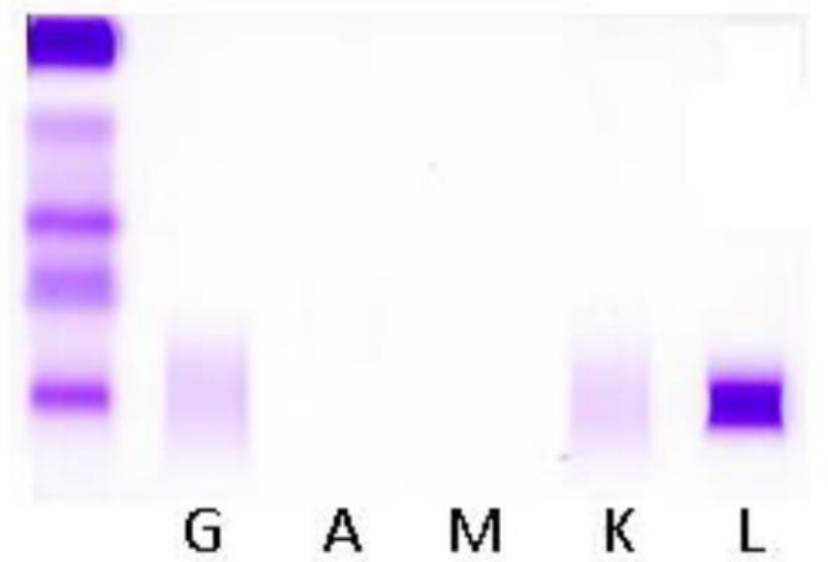
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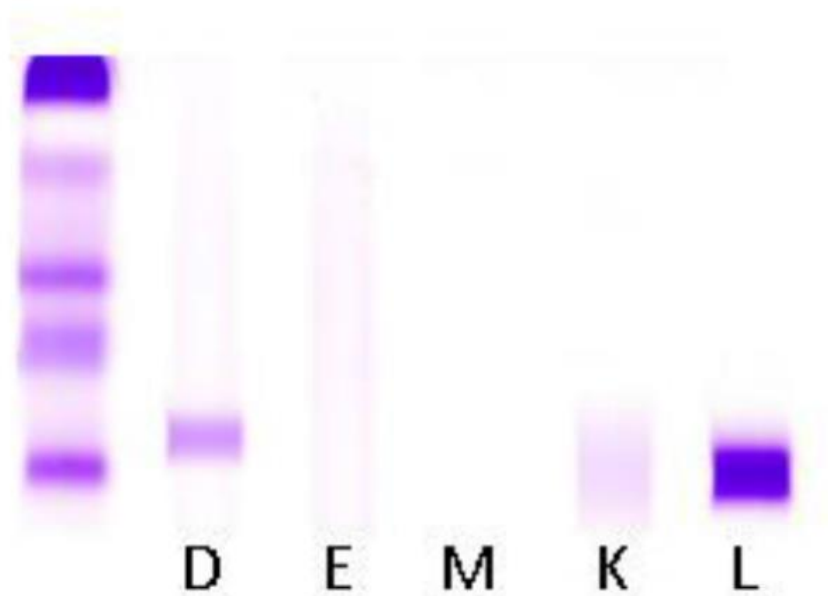
(B)



(C)



(D)

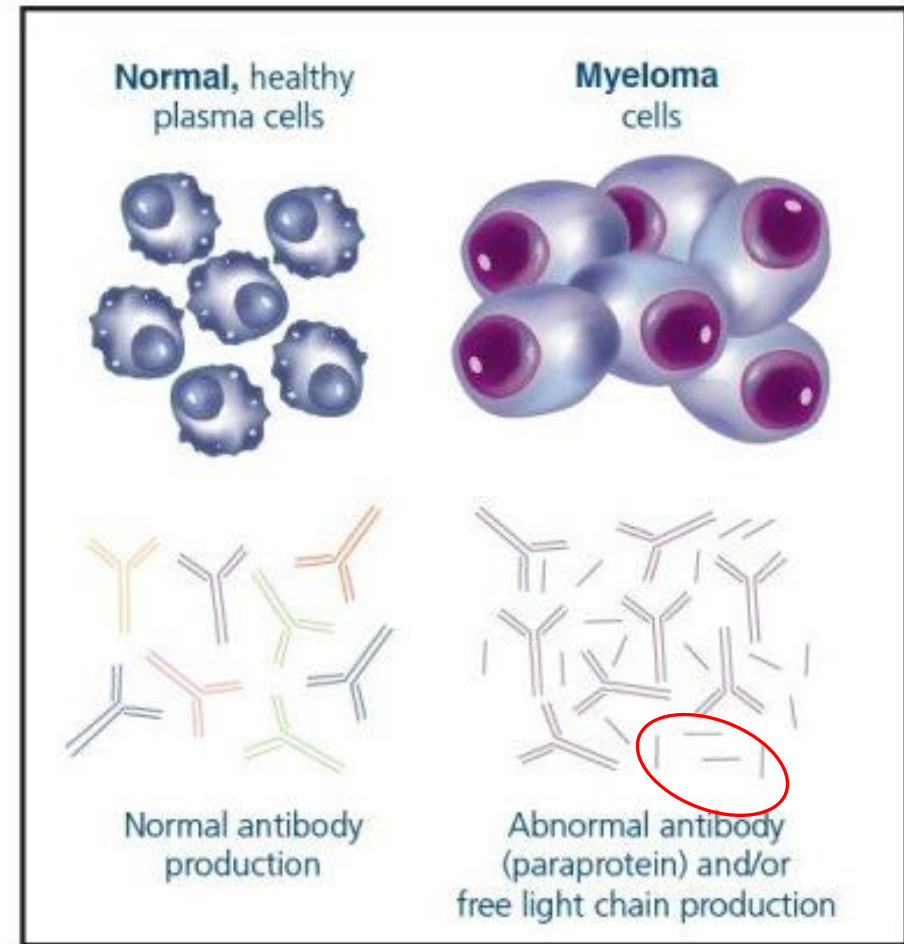


Free Light Chains (FLC) and Monoclonal Gammopathies

Under normal conditions, very little free light chains present in serum (sFLC)

Malignant plasma cells secrete excess FLC, raising serum concentration

- Renal impairment → not clonal
- κ/λ ratio of 0.26-1.65 considered normal



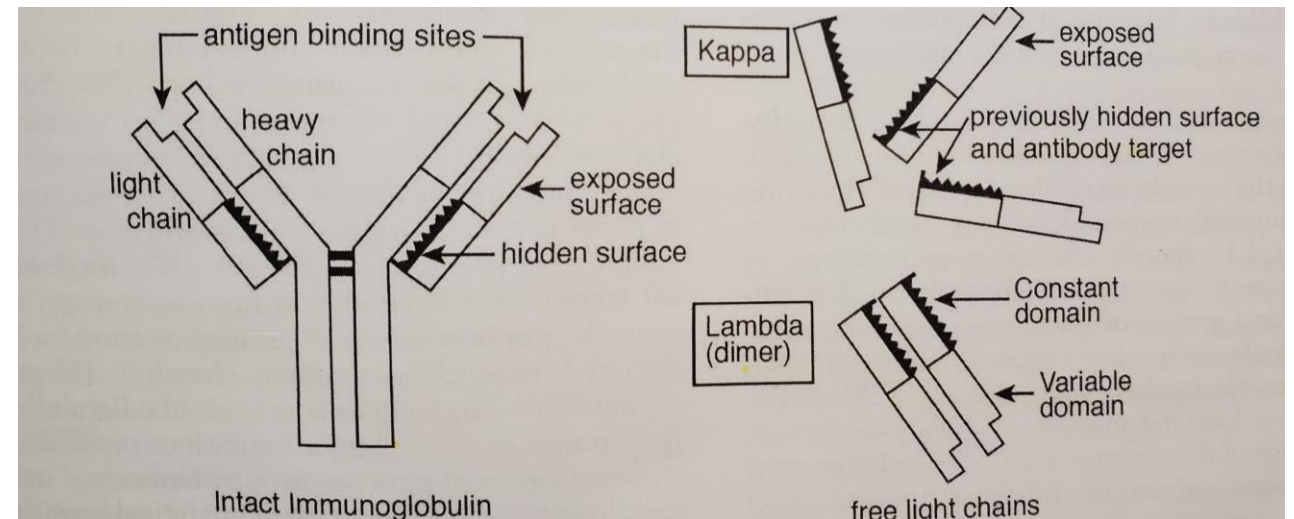
<https://thewaitingroom.karger.com/tell-me-about/what-are-the-types-and-effects-of-myeloma/>

Detection of Free Versus Bound Light Chains:

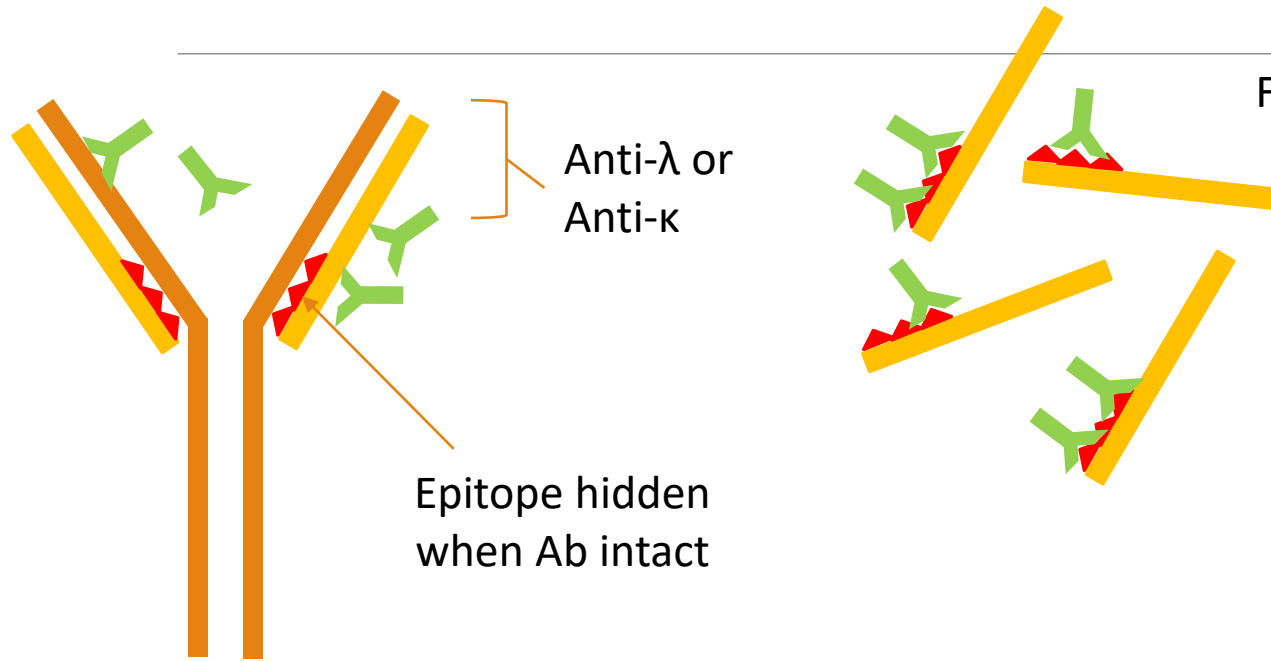
Quantifies κ -FLC and λ -FLC

Useful for monitoring disease progression (increase)

Can be used to diagnose Myeloma



Quantification of sFLC and uFLC



Free Light Chains (serum or urine)

- Measure with turbidimetry or nephelometry
- Can detect LOW concentrations (< 1mg/dL LOD)
- Can use to monitor disease progression

Normal Adult serum	Mean Conc.	Median Conc.	95 percentile range
Free Kappa	8.36 mg/L	7.30 mg/L	3.30-19.40 mg/L
Free Lambda	13.43 mg/L	12.40 mg/L	5.71-26.30 mg/L
	Mean	Median	Total Range
Kappa/Lambda Ratio	0.63	0.60	0.26-1.65

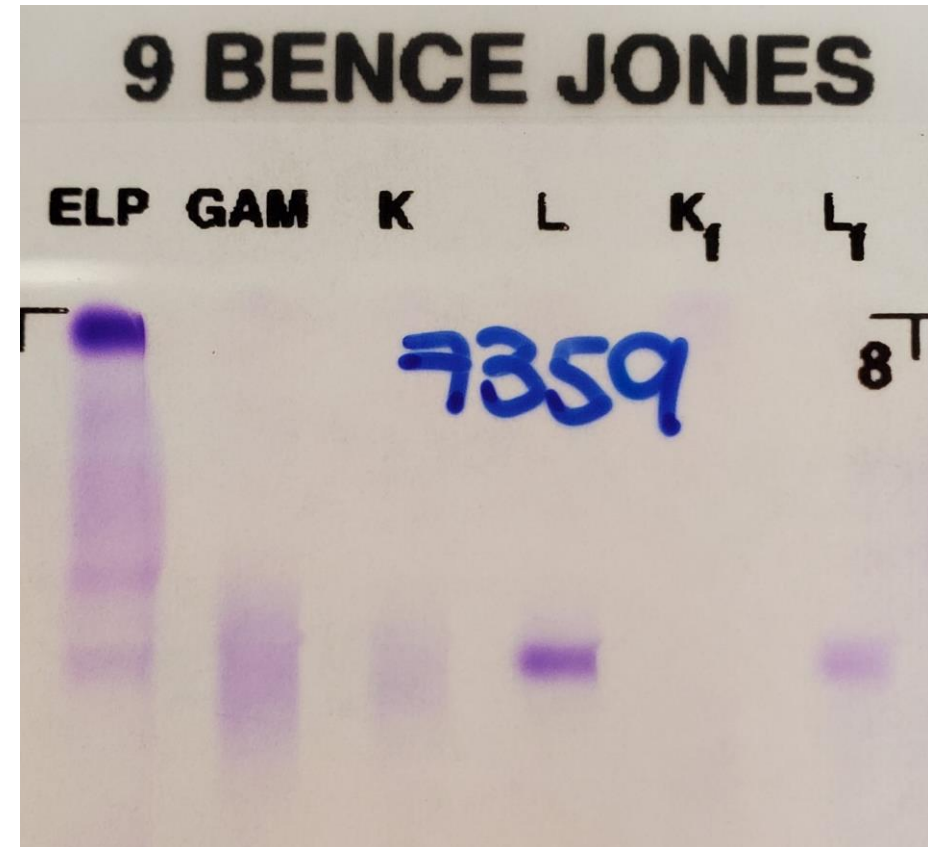
Urine IFE (uIFE) with Free Light Chains

sFLC is recommended for initial detection in patients suspected of monoclonal gammopathy, BUT

- Kidney function-dependent
- FLC may only be detectable in urine (Bence Jones)

Suspected AL amyloidosis → sFLC AND uIFE recommended

Proteinuria due to renal disease or Bence Jones proteins



Utility of Testing in the Clinic:

Evaluation of M-spike size and character aids in diagnosis/monitoring:

Worse Prognosis associated with:

- Hypogammaglobulinemia → immune deficiency
- Size of M protein
 - $>1.5\text{g/dL}$ increased risk (MGUS)
- Type of Antibody
 - IgA or IgM

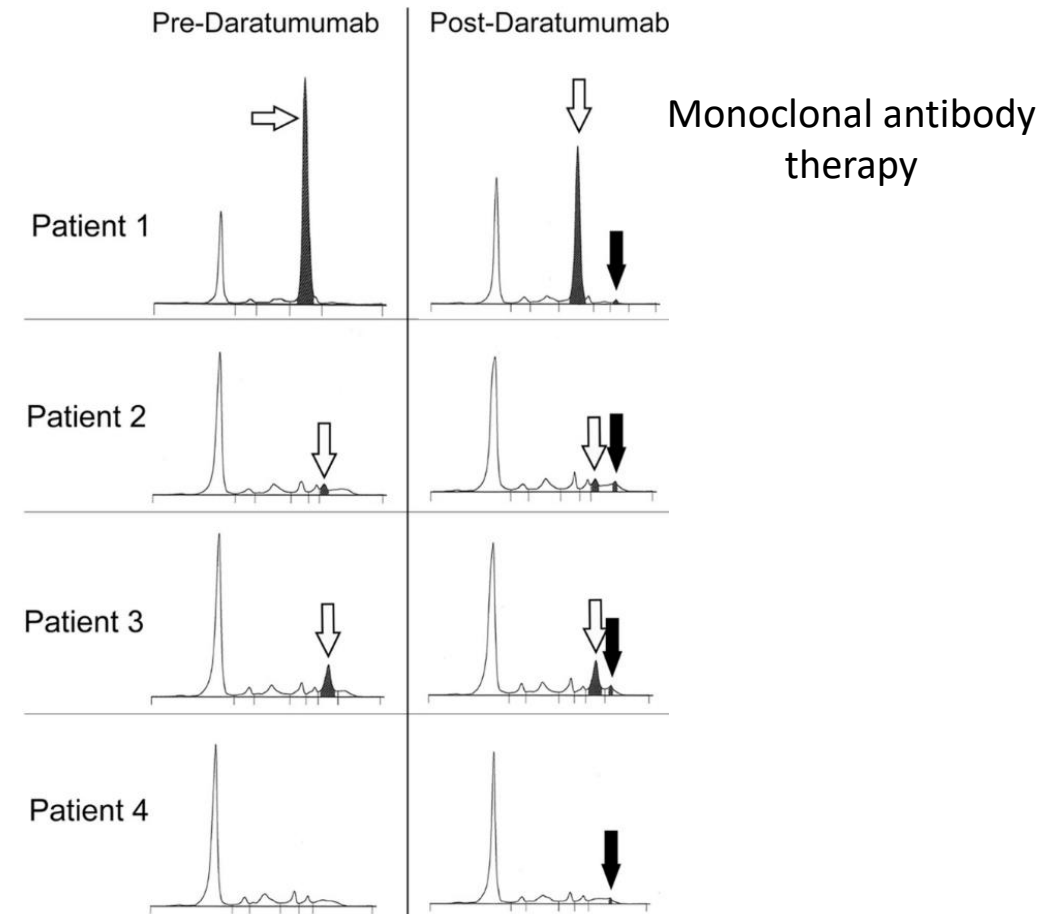
Increased or abnormal sFLC

- ≥ 100 kappa or ≤ 0.01 lambda—Myeloma defining event
- Increase in sFLC can indicate progression



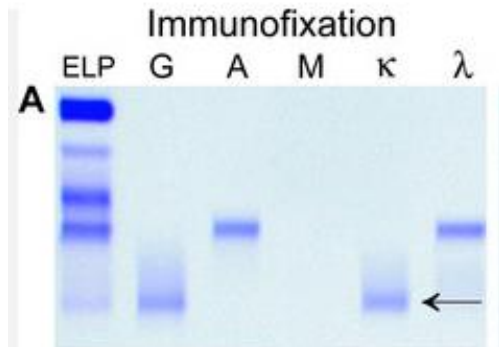
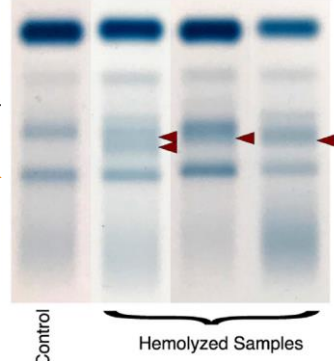
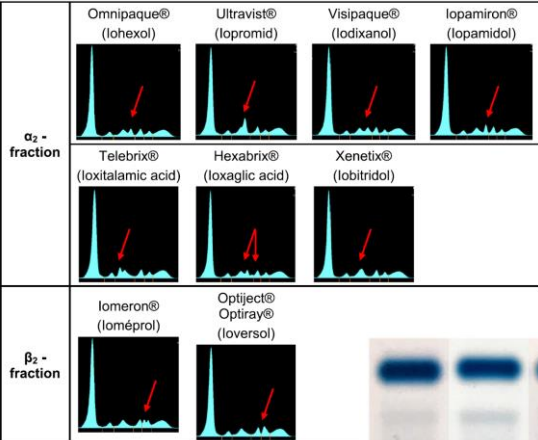
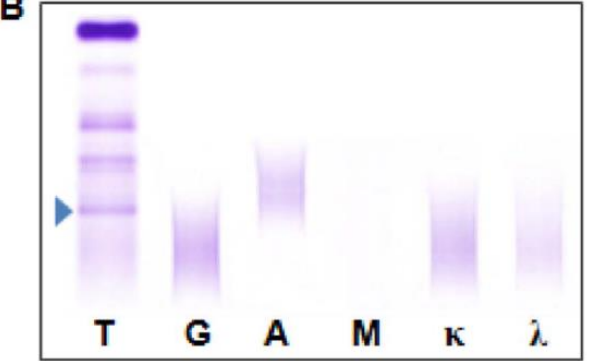
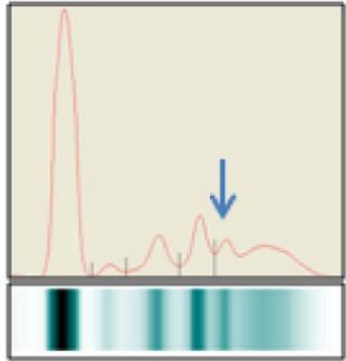
Utility of Testing in the Clinic: MONITORING

- Changes in testing results can gauge response to treatment and/or progression of disease
 - Increases in:
 - sFLC
 - SPEP (M-spike)
 - UPEP (M-spike)
 - Quantitative Ab
 - Less invasive compared to other methods (e.g. bone marrow aspirate)



Notable Interferences:

Interference	Method affected	Possible resolution
Fibrinogen (PLASMA)	Both gel and capillary electrophoresis	Thrombin treatment, ethanol precipitation to remove fibrinogen or IFE
Contrast dyes/antibiotics (β region)	Capillary electrophoresis	Gel electrophoresis or IFE
Hemolysis	Both gel and capillary electrophoresis	IFE
Monoclonal therapies	General, capillary electrophoresis, and IFE	Typically IgG- κ , Mass spectrometry, Patient history



Ref: Thoren KL, et al. Distinguishing drug from disease by use of the Hydrashift 2/4 daratumumab assay. *J App Lab Med.* 2018; 3(5): 857-863.
 McCudden CR, et al. Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. *Clin Biochem.* 2018; 51: 71-79.

Treatment Regimen: War of Attrition

Induction:

- Typically 3-4 drug regimen
- Proteasome inhibitor
- Immunomodulator
- Steroids
- ± monoclonal antibody therapy

Maintenance:

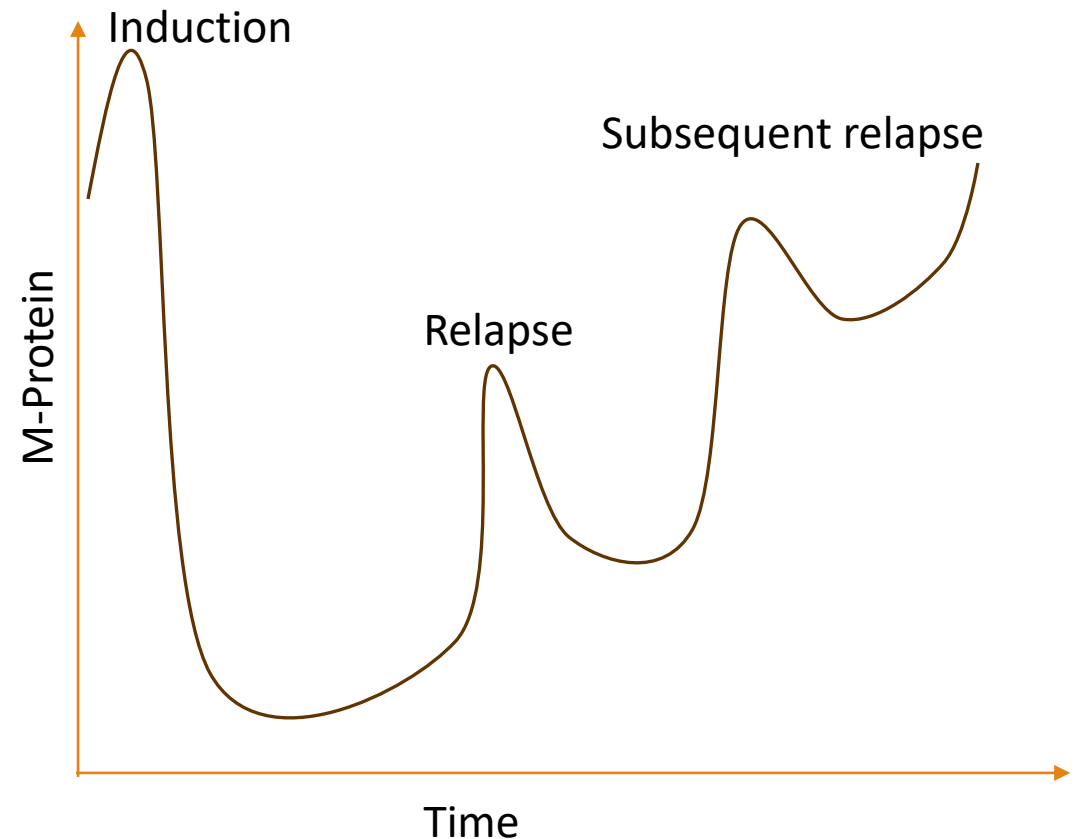
- De-escalate to 1-2 drugs (type of dyscrasia, risk factors, tolerability)

Relapse(s) → treatment intensification/alteration

IVIg/antibiotics as needed

Autologous stem cell transfer

CAR T-cell therapy



Thank you!
