Serum Protein Electrophoresis: what's behind the lab report?

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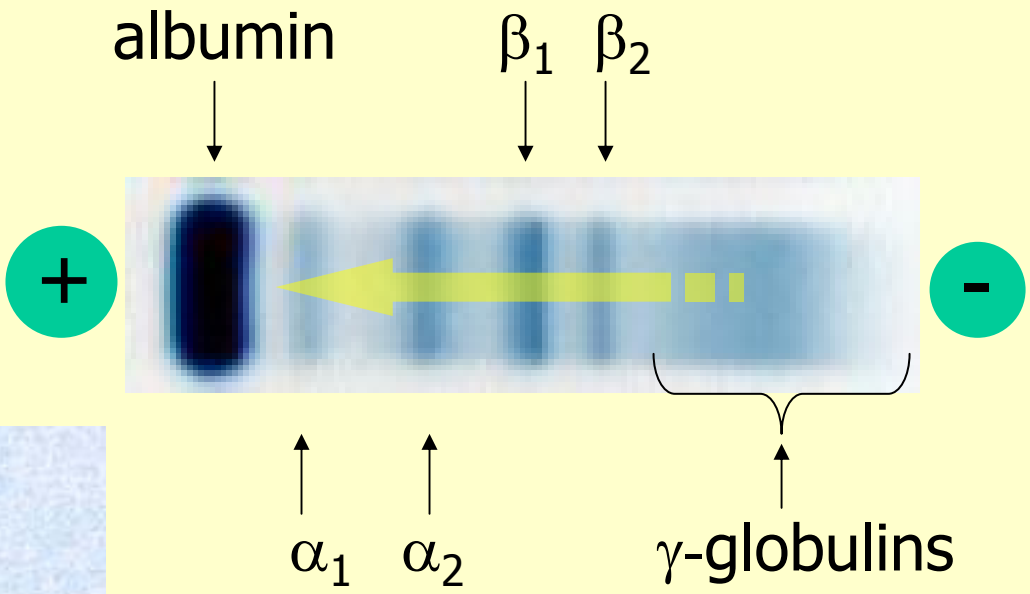
Learning objectives

- Indications for serum protein electrophoresis (SPE)
- Interpretation: common patterns & pitfalls
- Dx & monitoring of monoclonal gammopathy
  - what information is useful to the clinician?
- Adventures in the beta zone
  - signs of a possible underlying paraprotein
  - how to monitor a co-migrating band
- Atypical band descriptions
  - oligoclonal banding & restrictions
  - how to report an ill-defined band without raising undue alarm?
- Urine electrophoresis: utility in hypogammaglobulinemia

Indications for ordering SPE

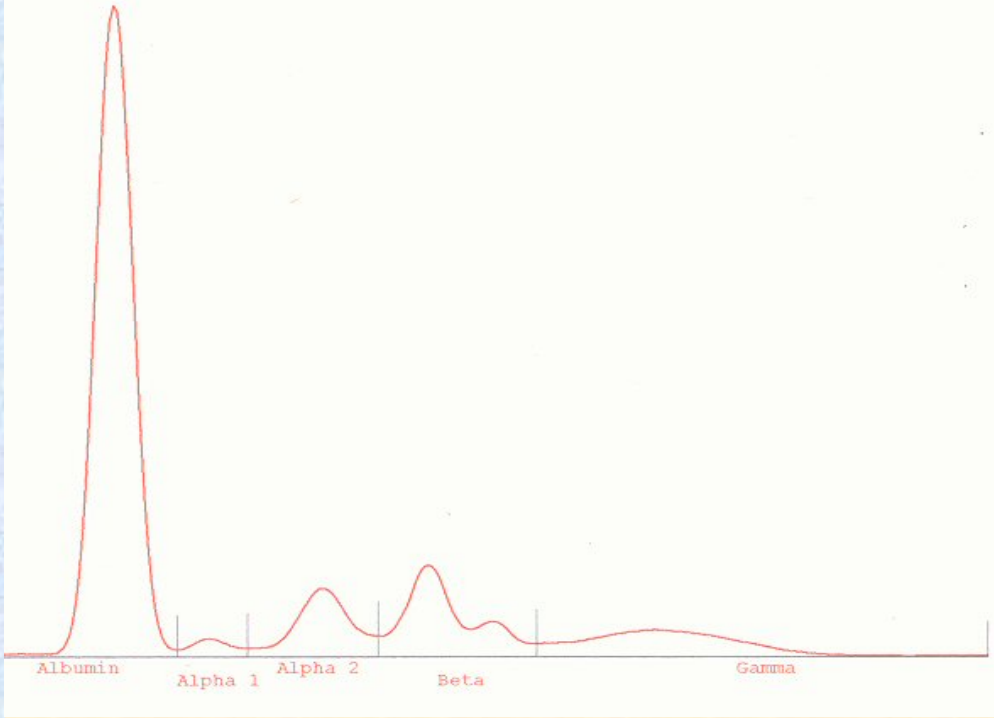
- Unexplained anemia / weakness / fatigue / ↑ ESR
- Unexplained renal insufficiency
- Heavy proteinuria in patient >40yrs
- Bence Jones proteinuria
- Hypercalcaemia
- Hypergammaglobulinemia
- Immunoglobulin deficiency
- Peripheral neuropathy (5% will have MGUS)
- Recurrent infections
- Unexplained bone pain / pathologic fracture / lytic lesion

Serum protein electrophoresis

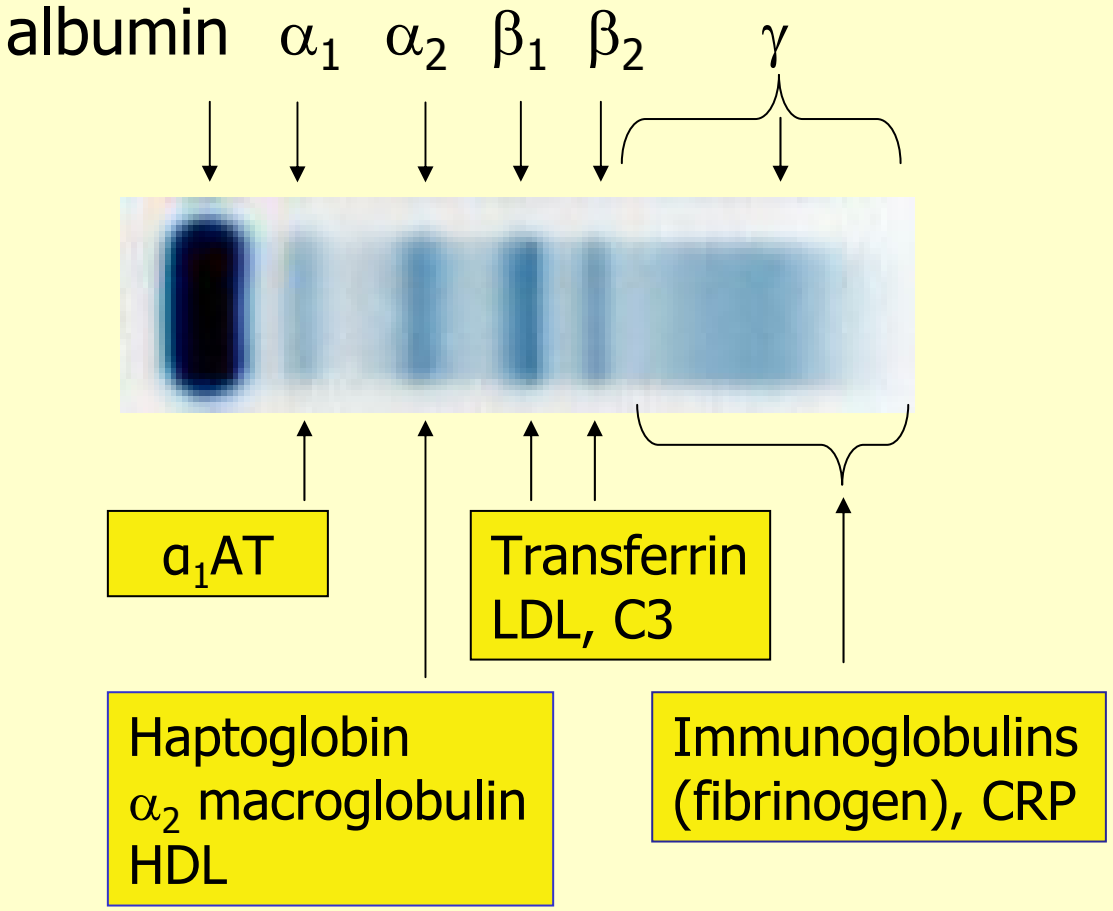


Proteins migrate in the electrical field according to their charge and mass

Densitometric scan of the gel separation

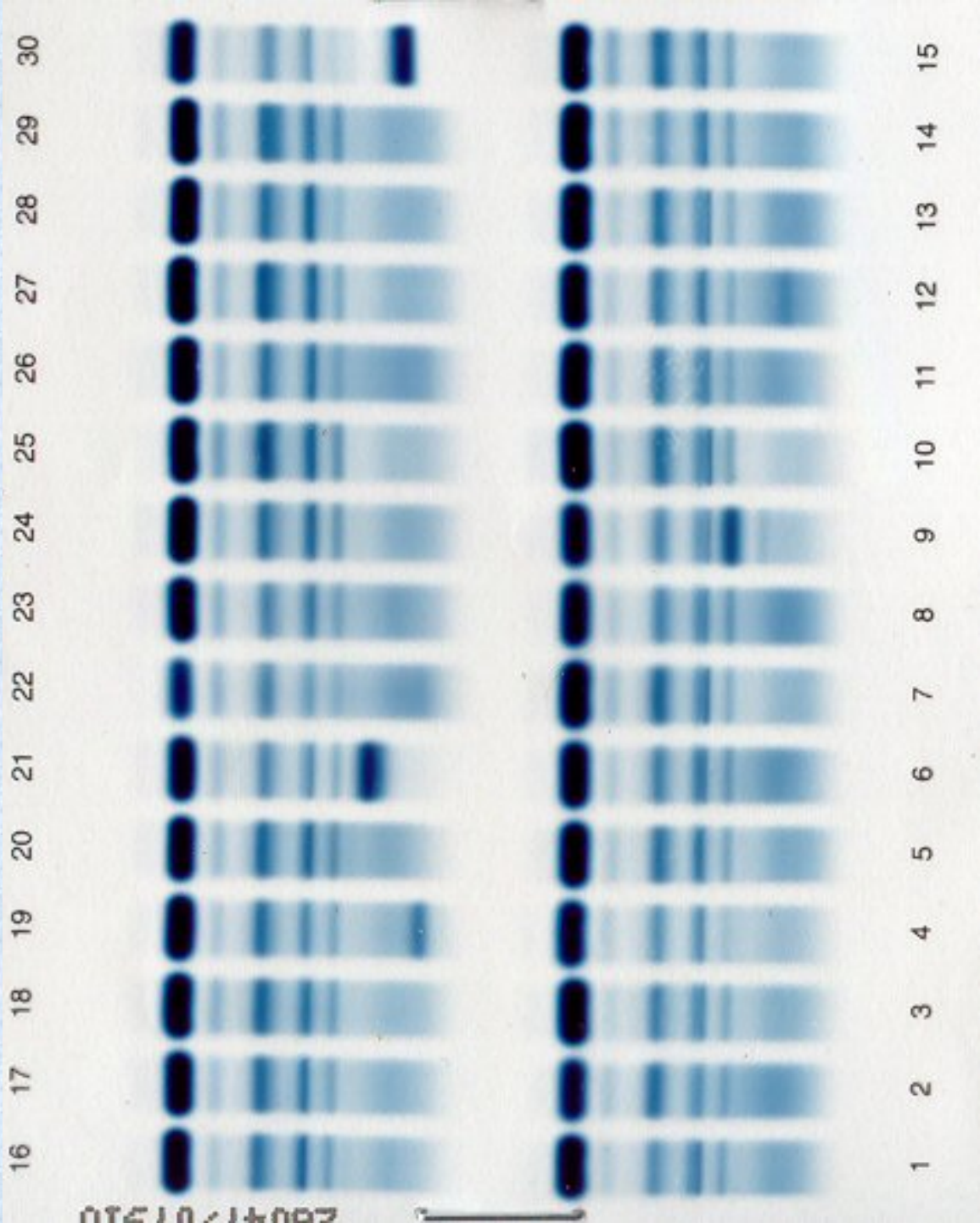


Major components of globulin bands



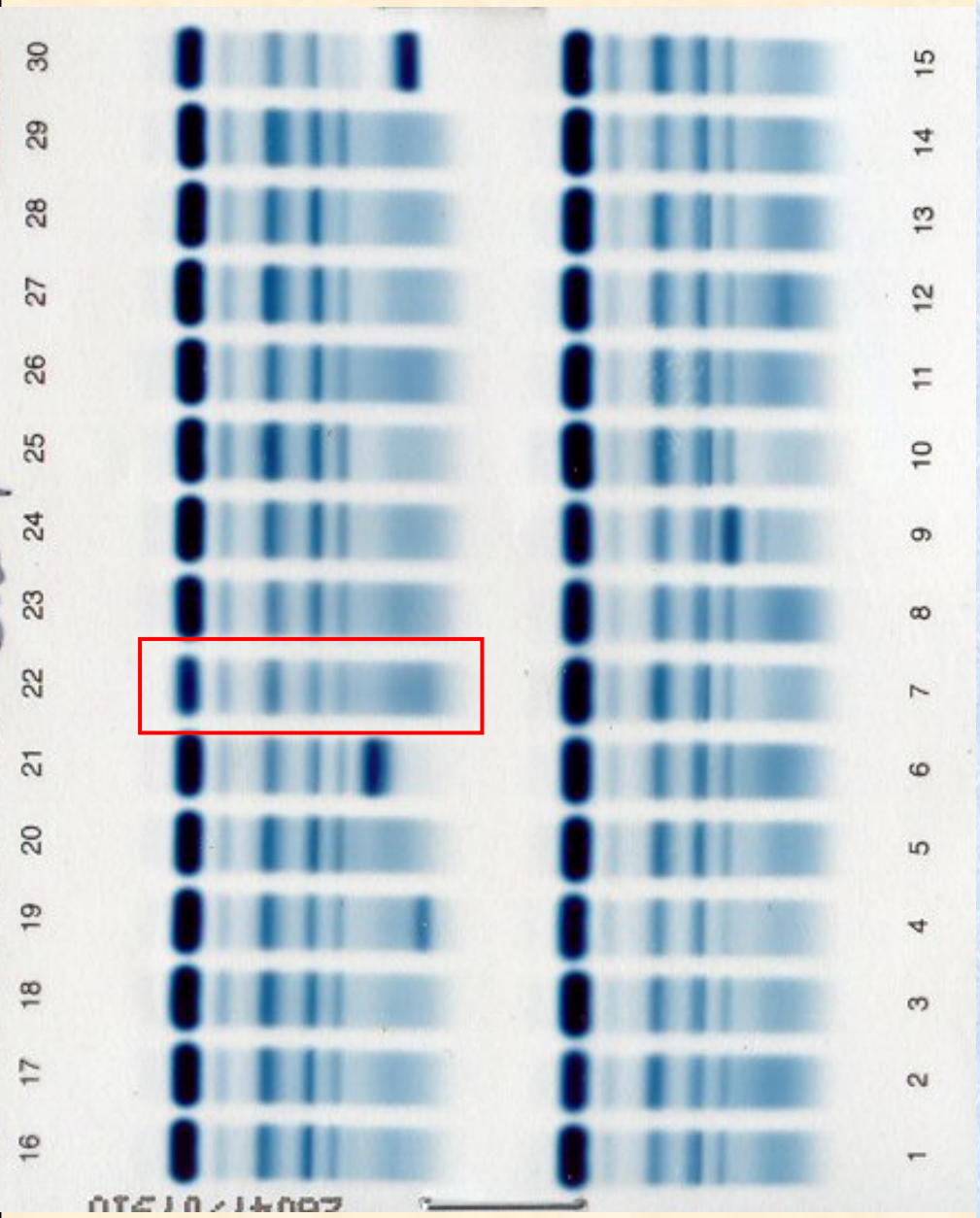
High-resolution serum protein electrophoresis

A typical gel -
28 patients and
2 controls

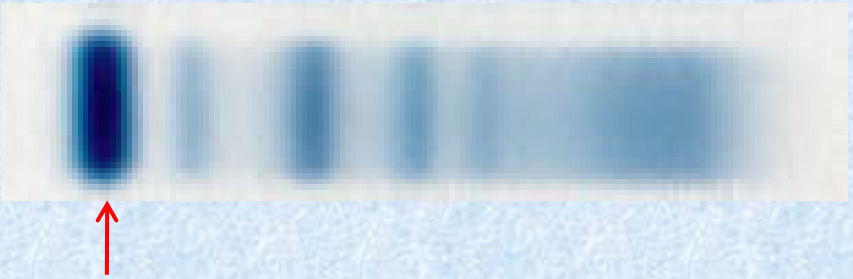


Common band patterns

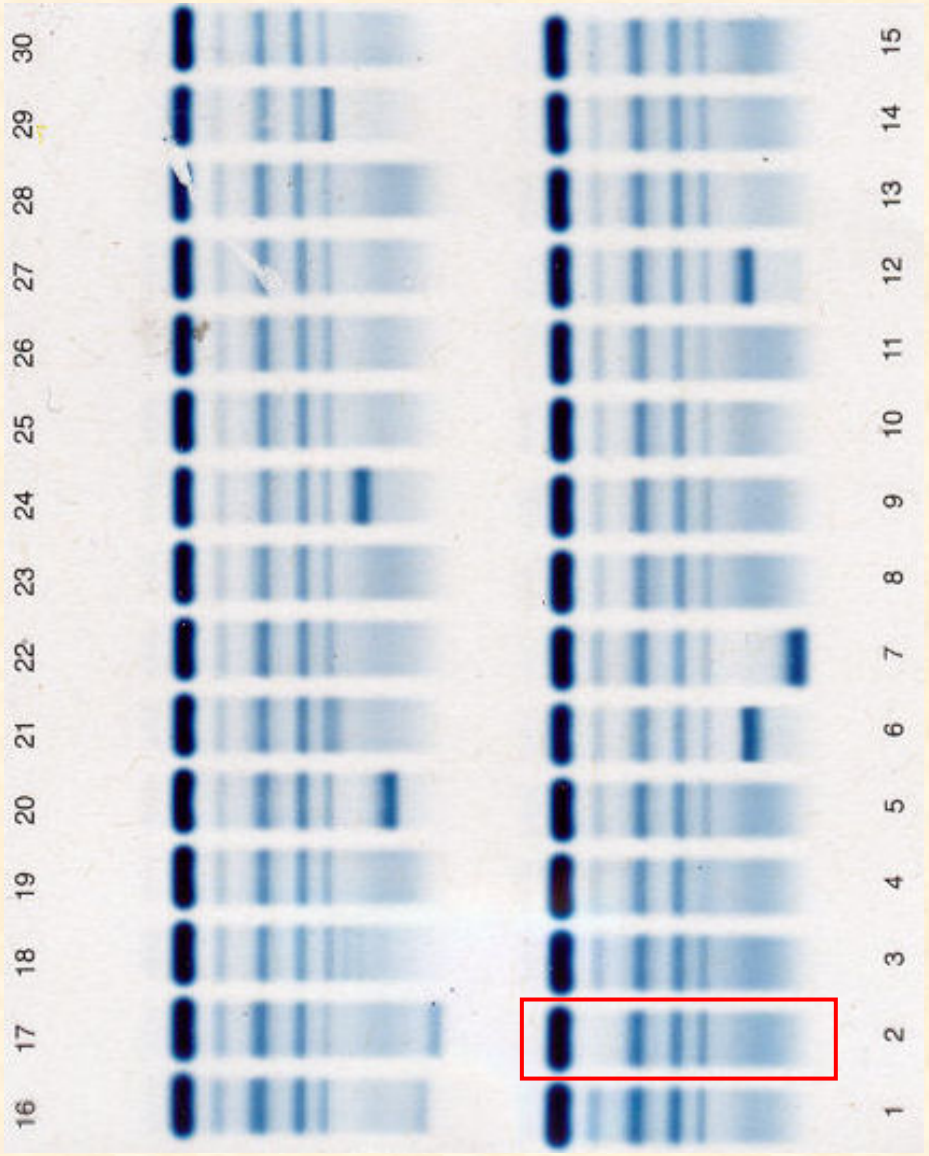
Sorting out the zebras from the tigers



Hypoalbuminemia



- organ losses
  - renal
  - GI
- liver disease
- malnutrition



Markedly decreased alpha-1 globulins

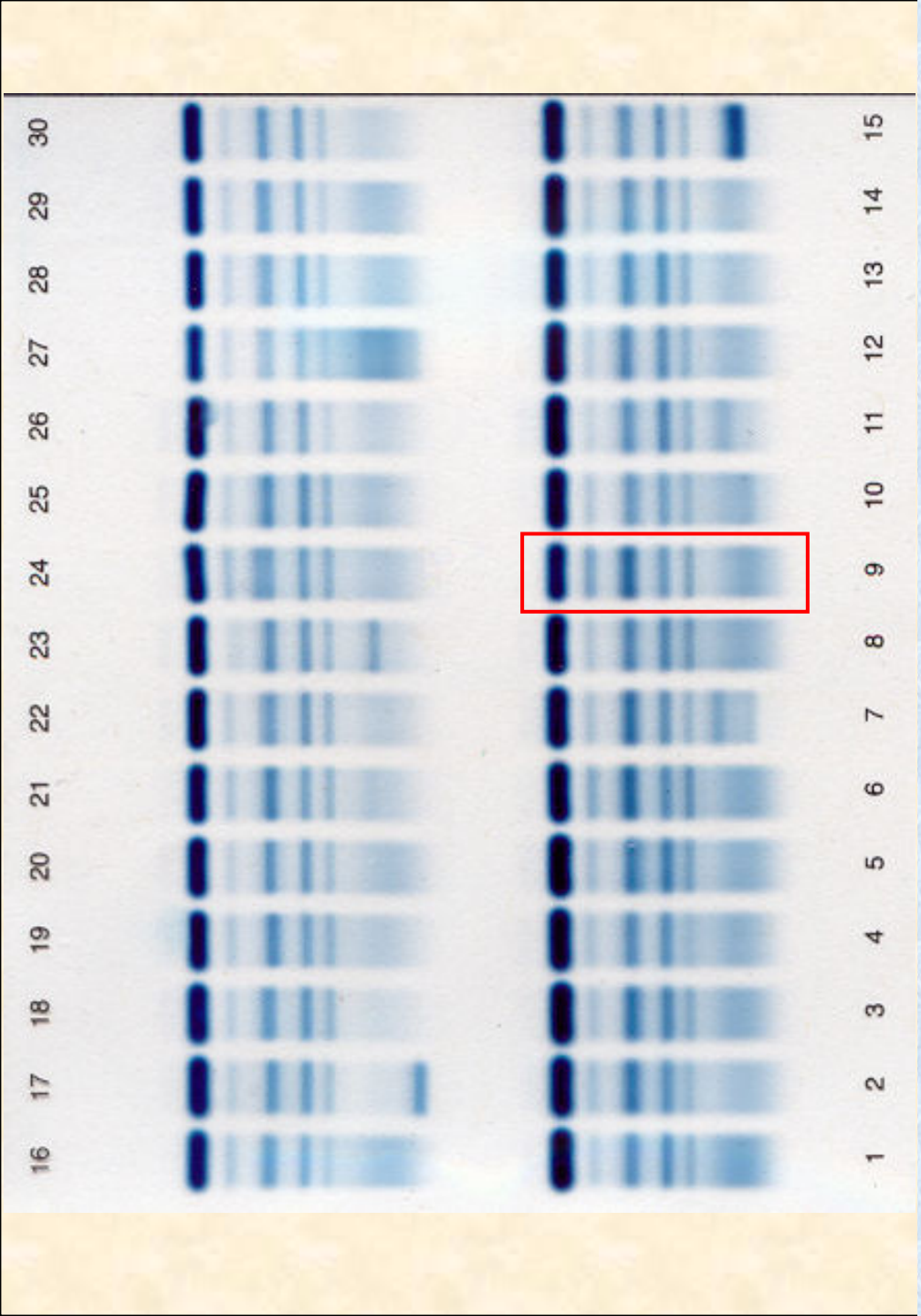
Isolated ↓ α1-AT

α1-antitrypsin deficiency

- LL ref range: 1-3 g/L
- suggest phenotyping if <0.6
- PiZZ genotype: 10% α1-AT

Combined with ↓ albumin

- liver disease
- malnutrition
- protein loss



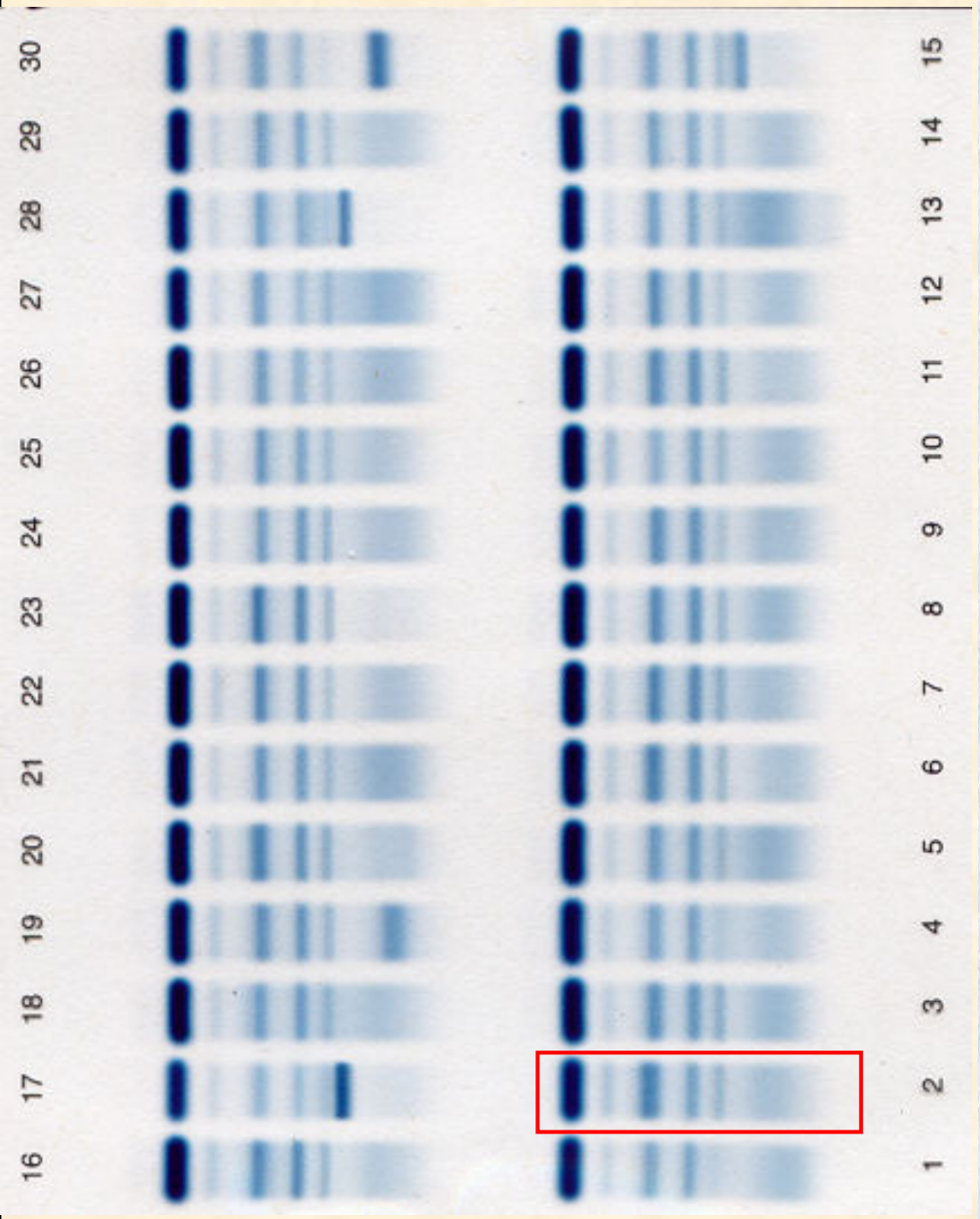
Increased alpha-2 globulins

Isolated

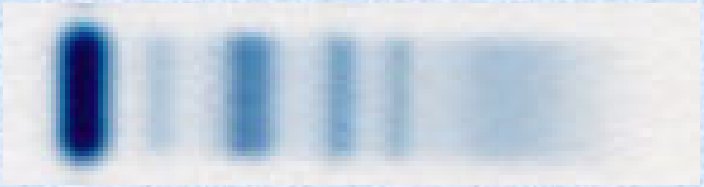
- hemolysis
- ? monoclonal protein

Combined (eg with ↑ α1)

- acute phase response
- selective protein loss



Selective protein loss

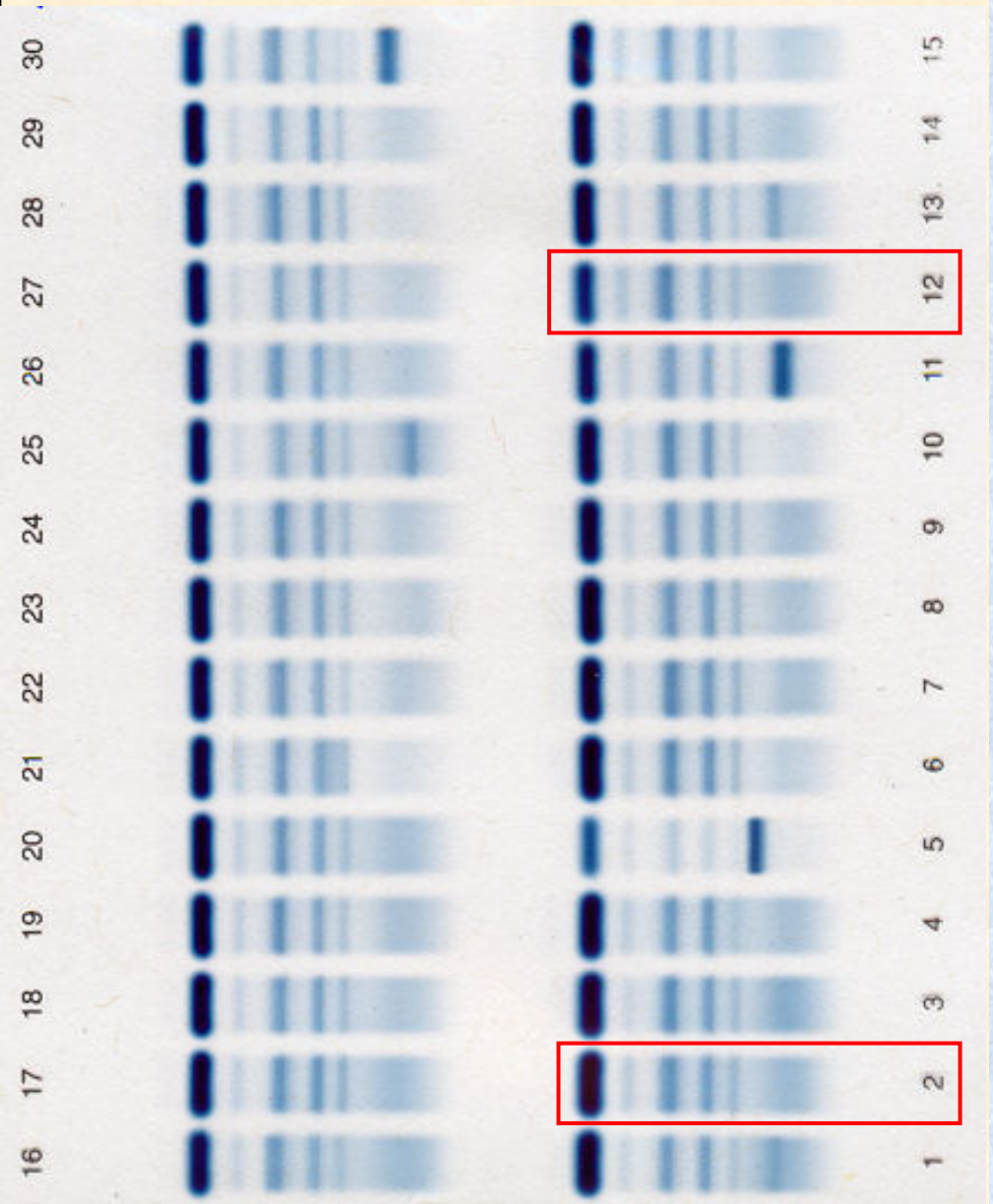


Long-term loss of albumin and IgG in kidney

↓ albumin

↑↑ α_2 & ↑ β globulins

- nephrotic syndrome

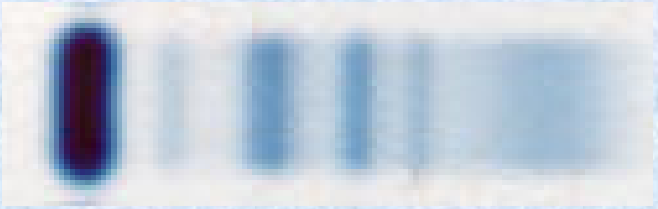


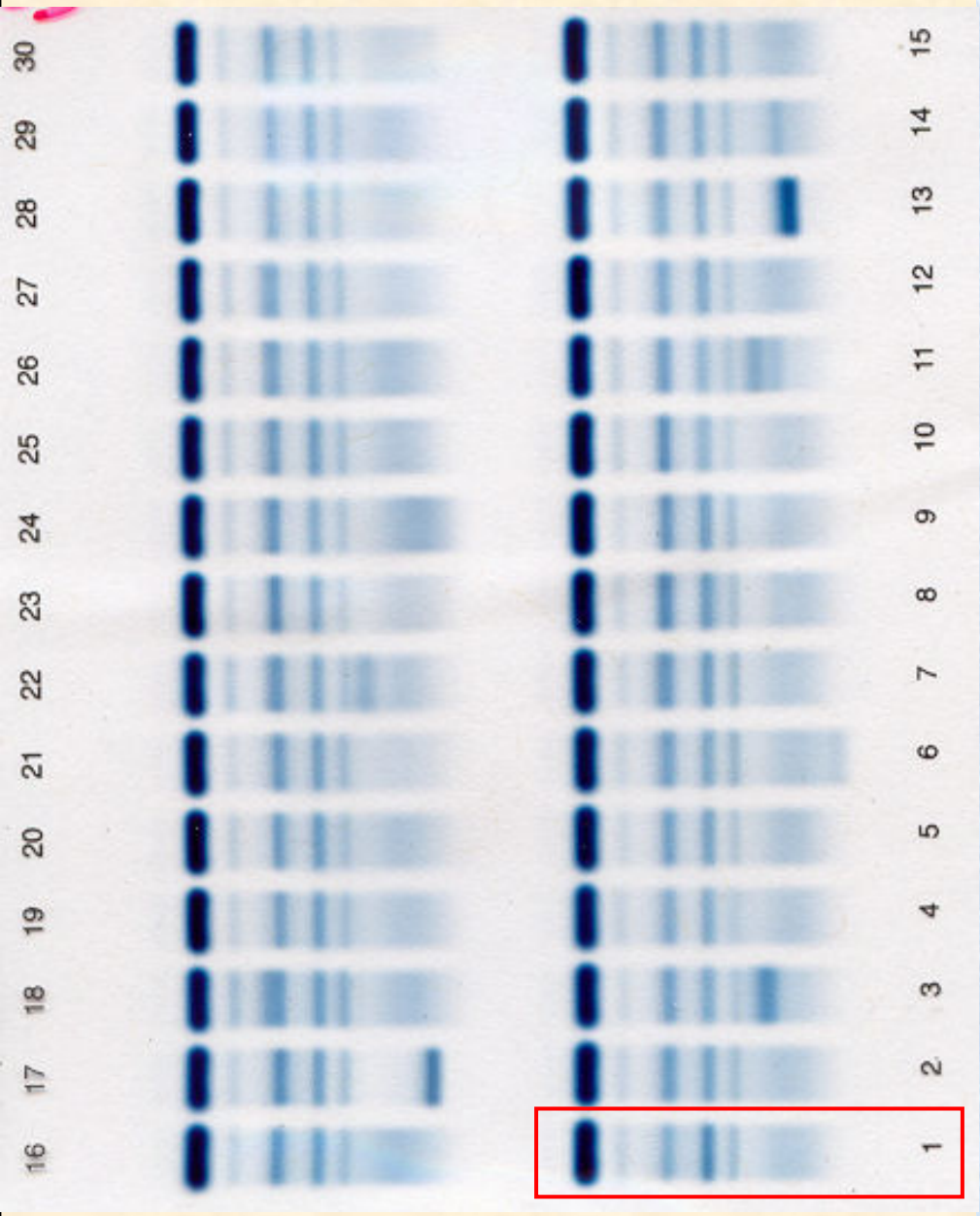
Acute inflammation

↑ α1- and ↑ α2-globulins

Often with decreased albumin, as shown in #12

- infection
- injury
- surgical trauma





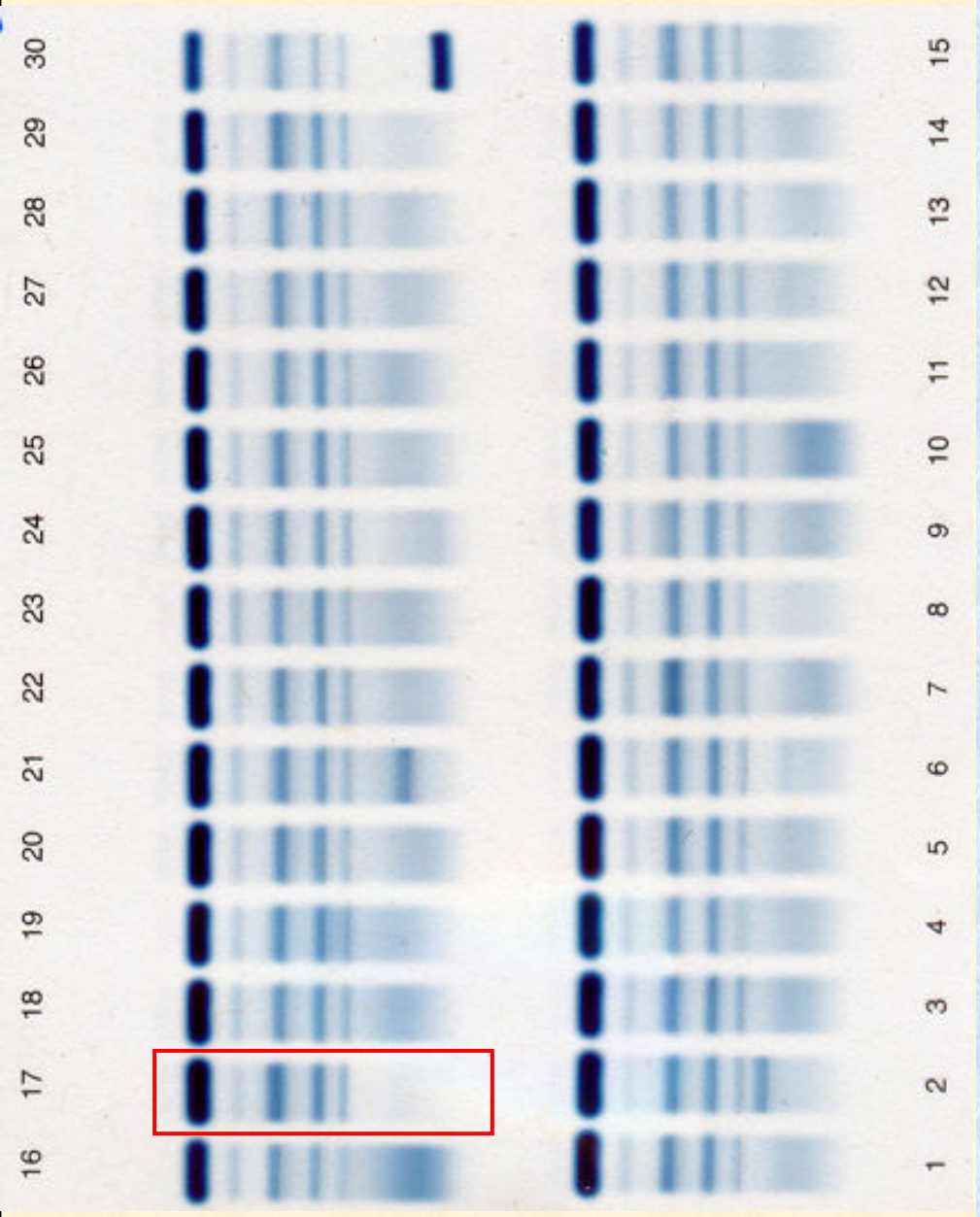
Increased beta-1 globulins (isolated)

normal: beta-1 > beta 2
here: beta-1 >> beta-2

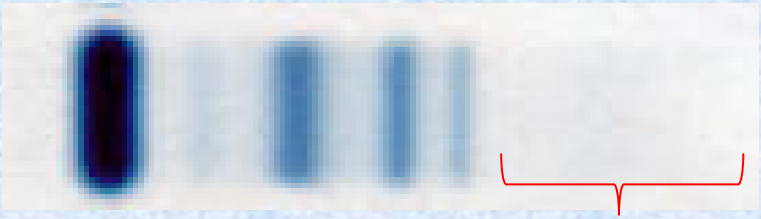
compare with neighbours

- iron deficiency anemia (↑ transferrin)
- β lipoproteins
- ? monoclonal protein

this case: immunofixation negative;
- iron deficiency anemia

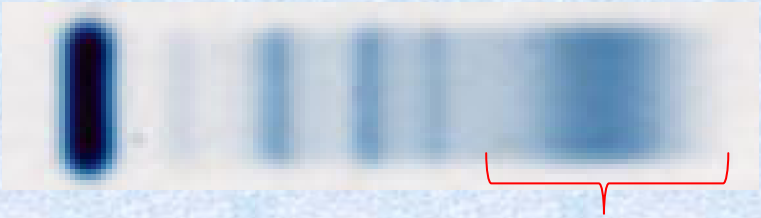


Hypogammaglobulinemia



- congenital:
  - combined immunodeficiency
- acquired:
  - multiple myeloma
  - primary amyloidosis
  - CLL
  - lymphoma
  - nephrotic syndrome

Polyclonal gammopathy



diffuse ↑↑ γ-globulins

± ↓ albumin

- liver disease
- connective tissue disease
- chronic infection
- hematological disorders
- malignancy

Chronic inflammation

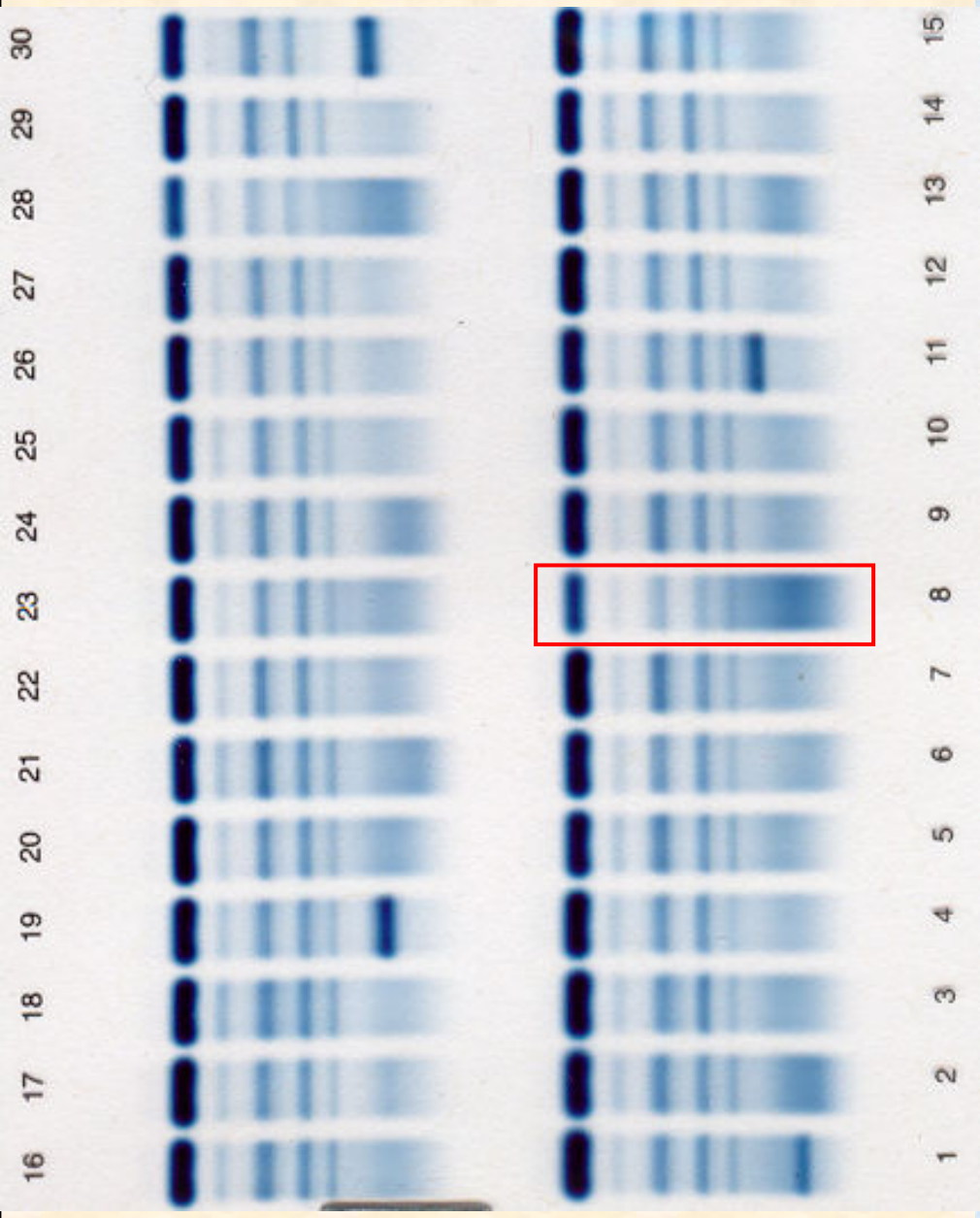
↑↑ γ-globulins

↑ α_2-globulins

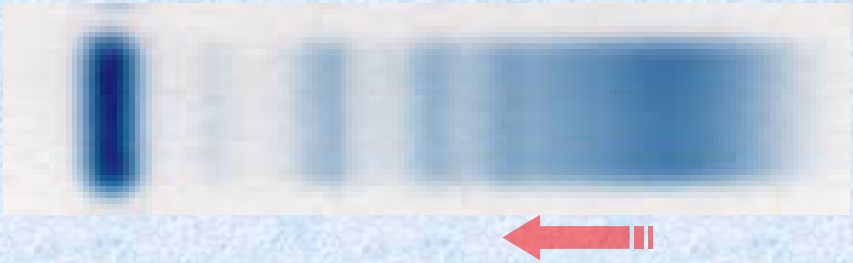
± ↑ α_1-globulins

± ↓ albumin

- autoimmune disease
- chronic liver disease
- chronic infection
- malignancy



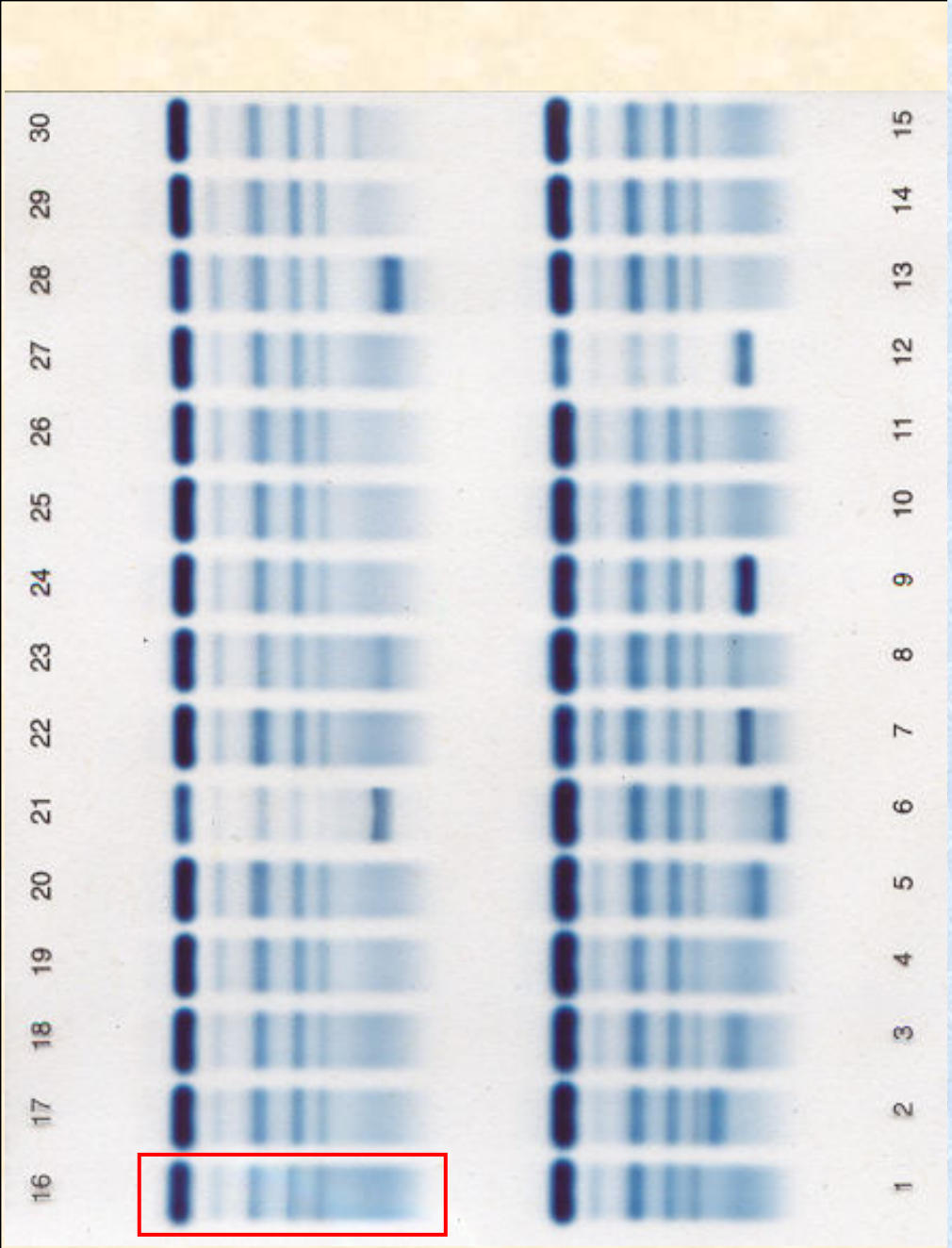
β-γ bridging



Polyclonal increase in IgA extending into beta region

This patient also shows decreased albumin

- cirrhotic liver disease
- malignancy
- inflammatory disease



Liver damage - cirrhosis

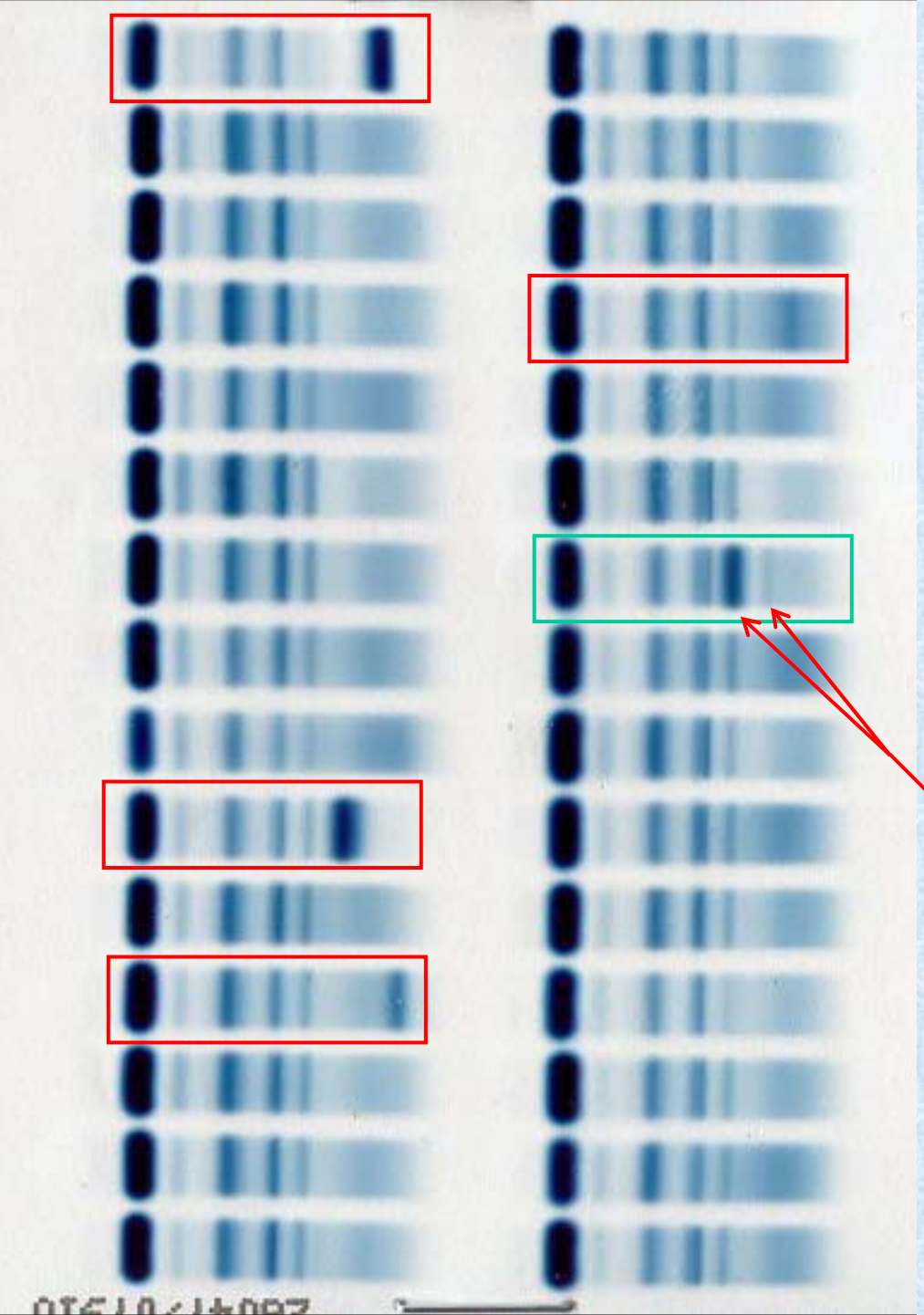
↓ albumin

↓ α_1, α_2 and β-globulins

↑ IgA in γ-fraction

β-γ bridging

- cirrhotic liver disease
- viral hepatitis
- chronic alcohol abuse



Monoclonal Gammopathy

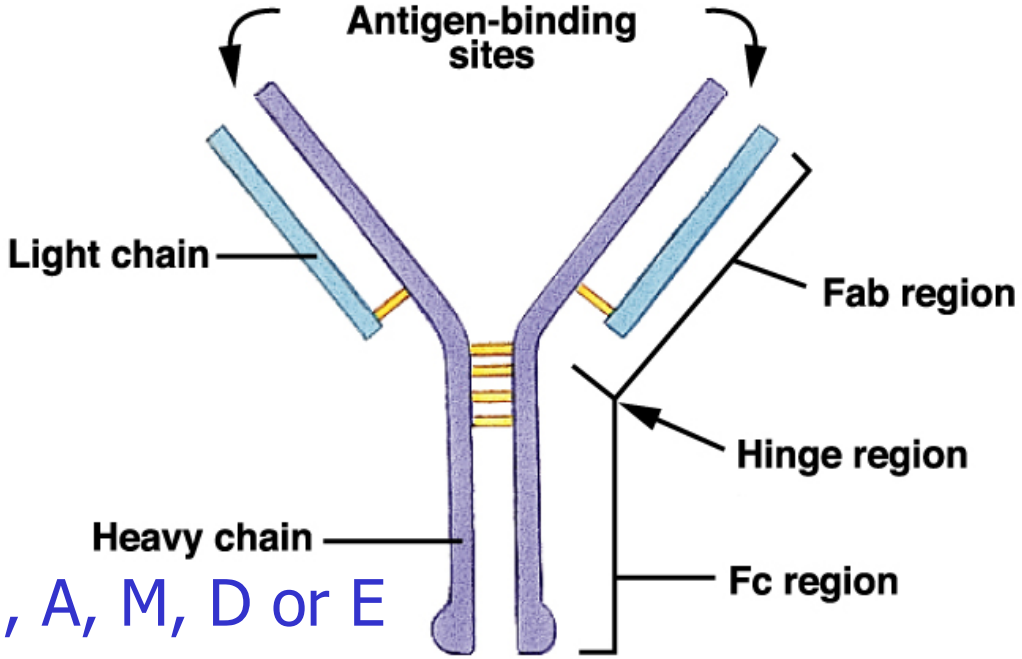
Monoclonal proliferation of β-lymphocytes, producing an abnormal immunoglobulin paraprotein

Discrete band, typically within β - γ region

Monoclonal IgA and free light chains may migrate as far as α_2 region

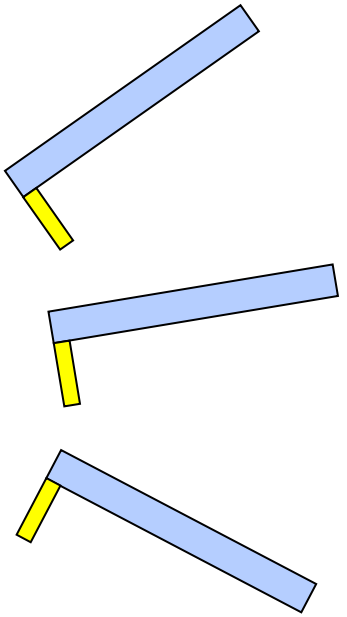
example of a biclonal gammopathy

Immunoglobulin: heavy and light chains



G, A, M, D or E

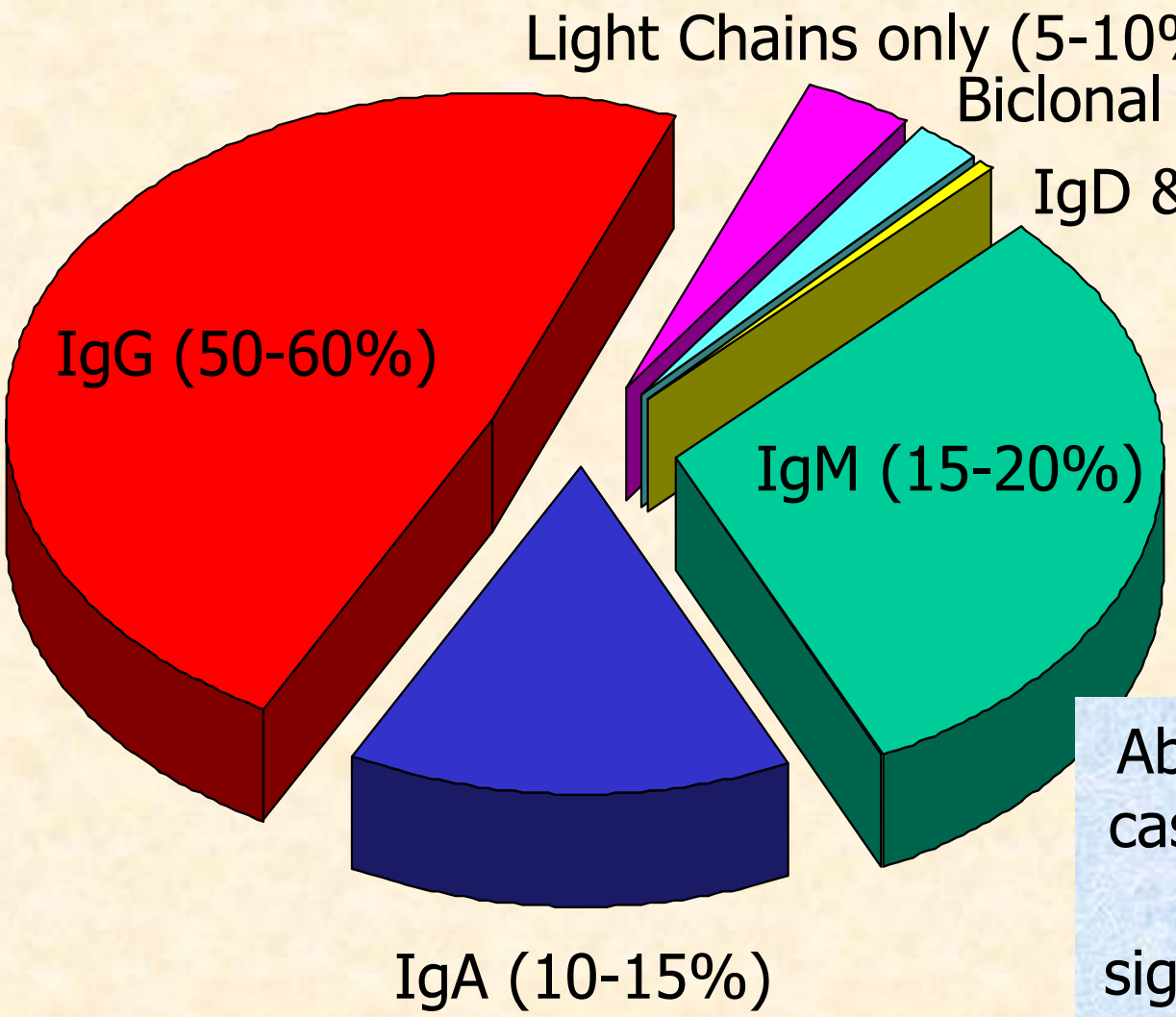
Intact immunoglobulin



Free light chains (FLC)

κ (kappa) or λ (lambda)

Monoclonal Gammopathies



About half of these cases are initially of undetermined significance (MGUS)

Monoclonal Gammopathy of Uncertain Significance (MGUS): Diagnostic Criteria

1. Monoclonal protein <30g/L
2. No substantial amount of light chain in urine
3. Bone marrow <10% plasma cells
4. No osteolytic lesions, anemia, hypercalcemia, or renal insufficiency

The key issue in following these patients is to detect any sign of progression to a more serious related disorder, such as myeloma

MGUS

- these patients are generally asymptomatic
- condition is usually discovered on routine testing

Clinical evidence suggesting disease progression:

- anemia-related symptoms
- neurological manifestations
- wt loss, bone tenderness
- soft tissue mass

} suspect myeloma

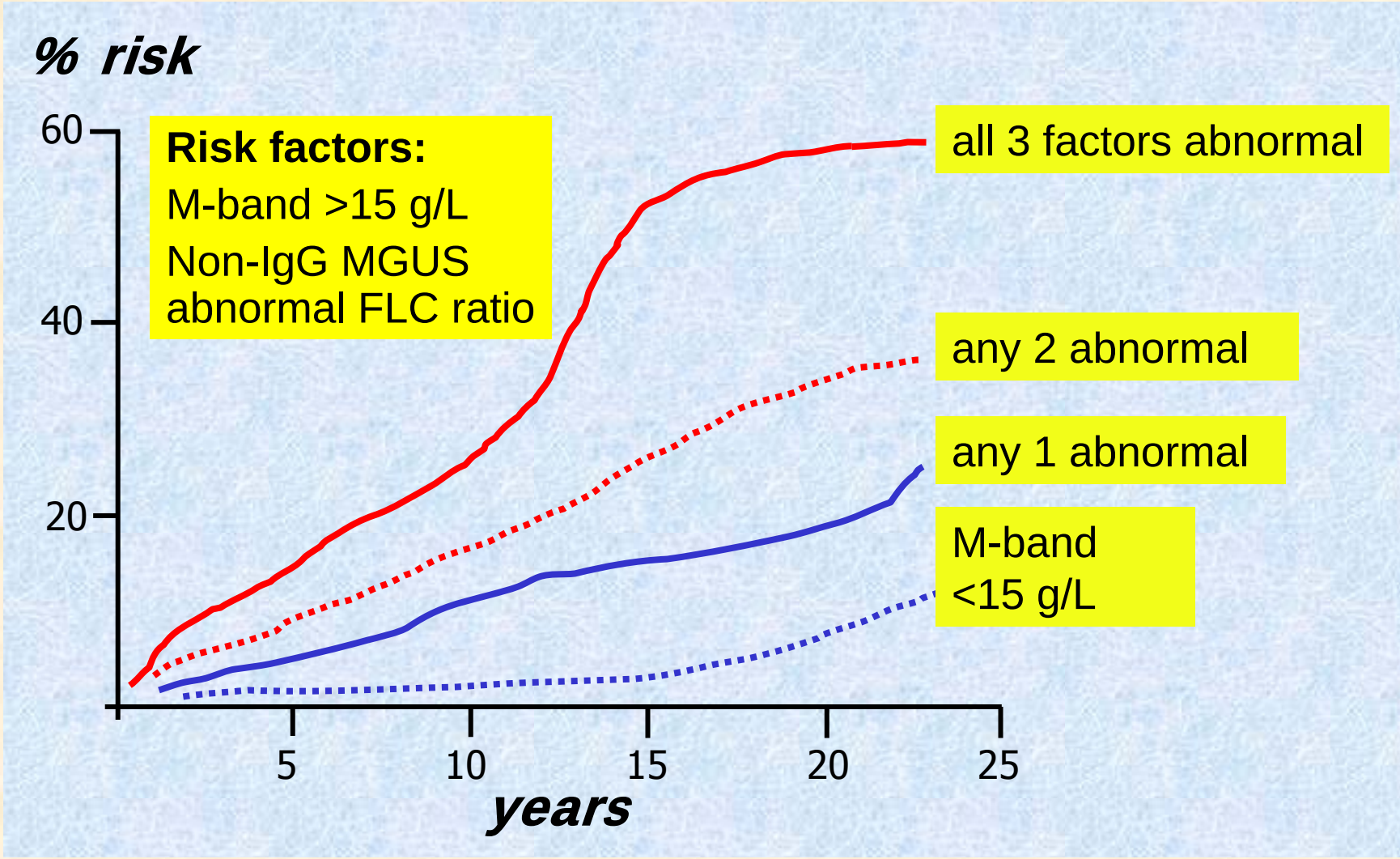
- hepatosplenomegaly
- purpura
- edema

} suspect Waldenström's macroglobulinemia, or amyloidosis

Laboratory Predictors of MGUS Progression

- size of serum M-protein
- type of M-protein
- bone marrow: % plasma cells
- abnormal serum FLC ratio

Risk of MGUS Progression to Multiple Myeloma or a related disorder



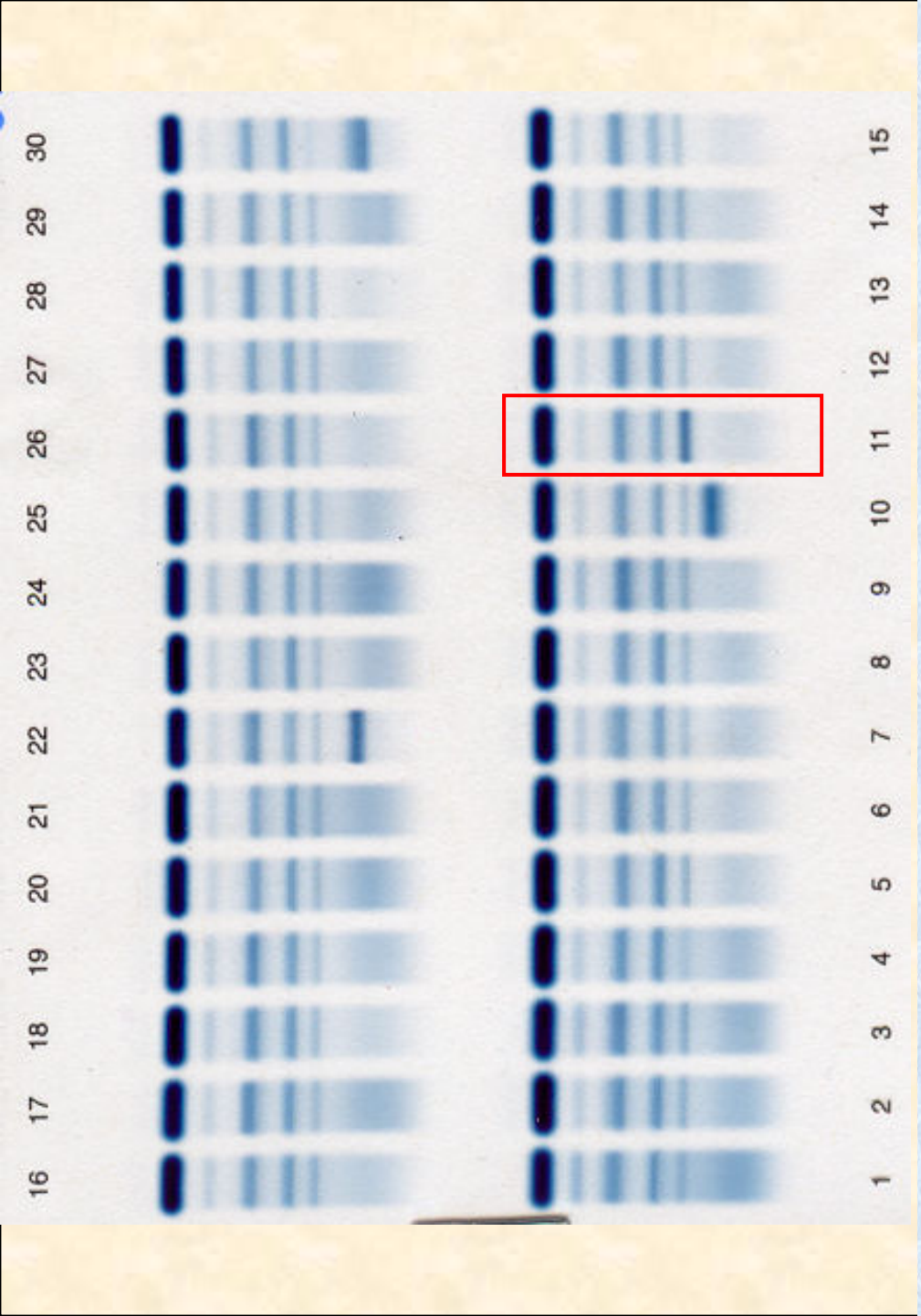
SPE & Monoclonal Gammopathy: what's helpful to the clinician?

- Detect a monoclonal gammopathy, if present
  - SPE: preferred method of detecting an M-protein
- If M-protein found: document prognostic parameters
  - number of bands and band size
  - immunofixation: classify heavy chain type (*ie* IgG, IgA, IgM, *etc*) and light chain type (*ie* kappa or lambda)
  - 24h urine protein & UPE / immunofixation: detect and quantify any monoclonal protein in urine
- Long-term monitoring for evidence of progression
  - any change in amount of M-protein (band size)
  - degree of suppression of normal gamma globulins

Adventures in the beta zone: signs of a possible hidden paraprotein

- Either beta band appears atypically dense
  - comparison with neighbouring separations is often helpful
- Abnormal beta-1/beta-2 ratio (normal: beta-1 > beta-2)
  - be very suspicious if beta-2 > beta-1
  - but also be suspicious if beta-1 >> beta-2
- If a beta band is smudged
  - be suspicious
- If the region between beta-1 and beta-2 is "smeared"
  - be suspicious, even if the bands themselves look normal

Examples…

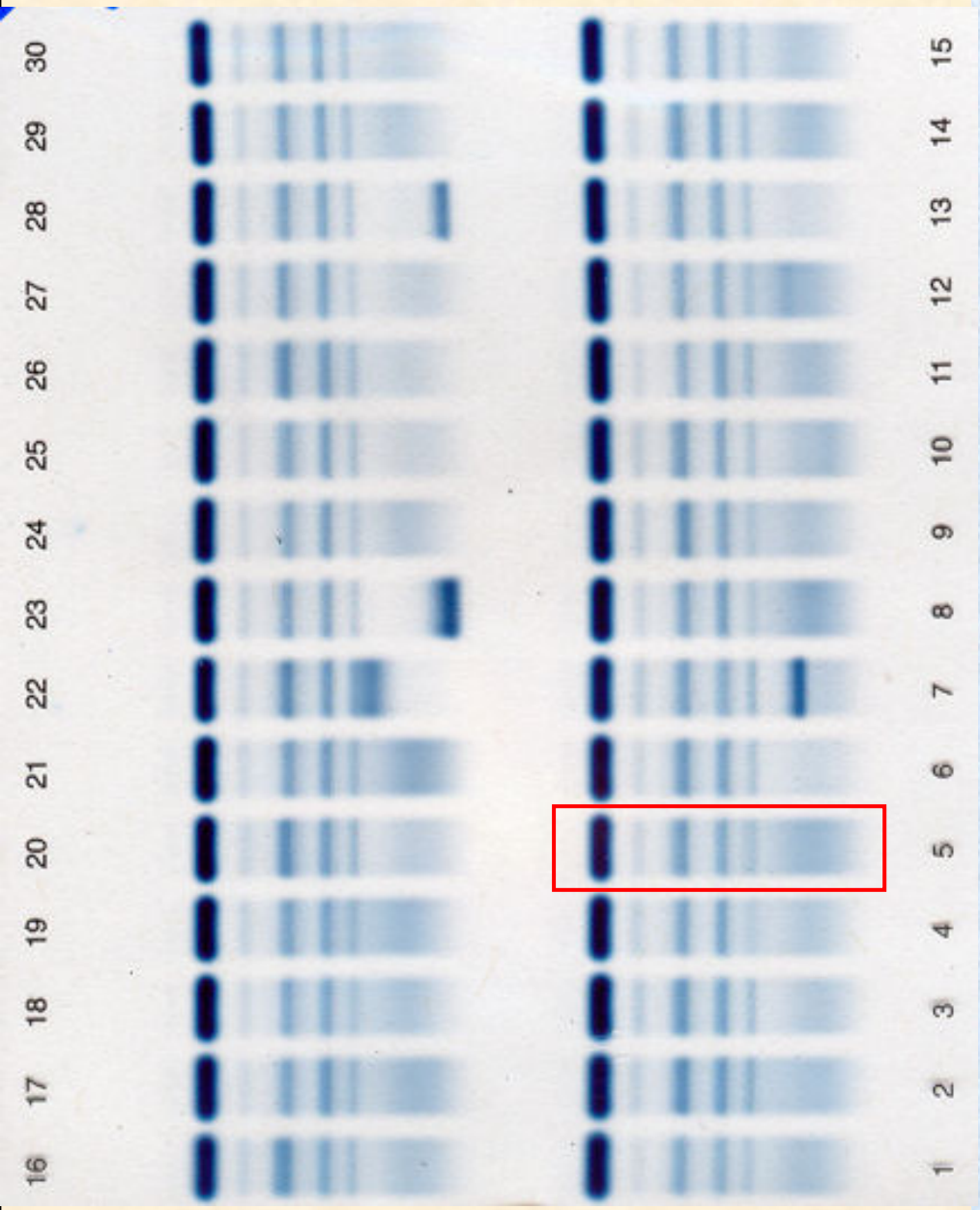


Gammopathy in beta zone?

Abnormal beta-2 band

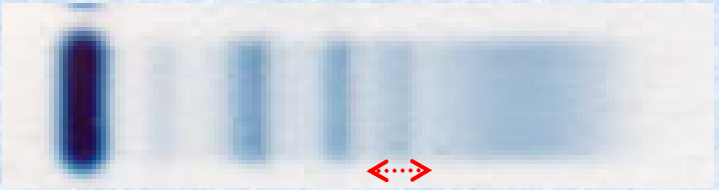
- beta-2 band appears dense
- beta-2 > beta-1 (atypical)

Immunofixation confirmed an underlying paraprotein, co-migrating with normal beta-2 proteins



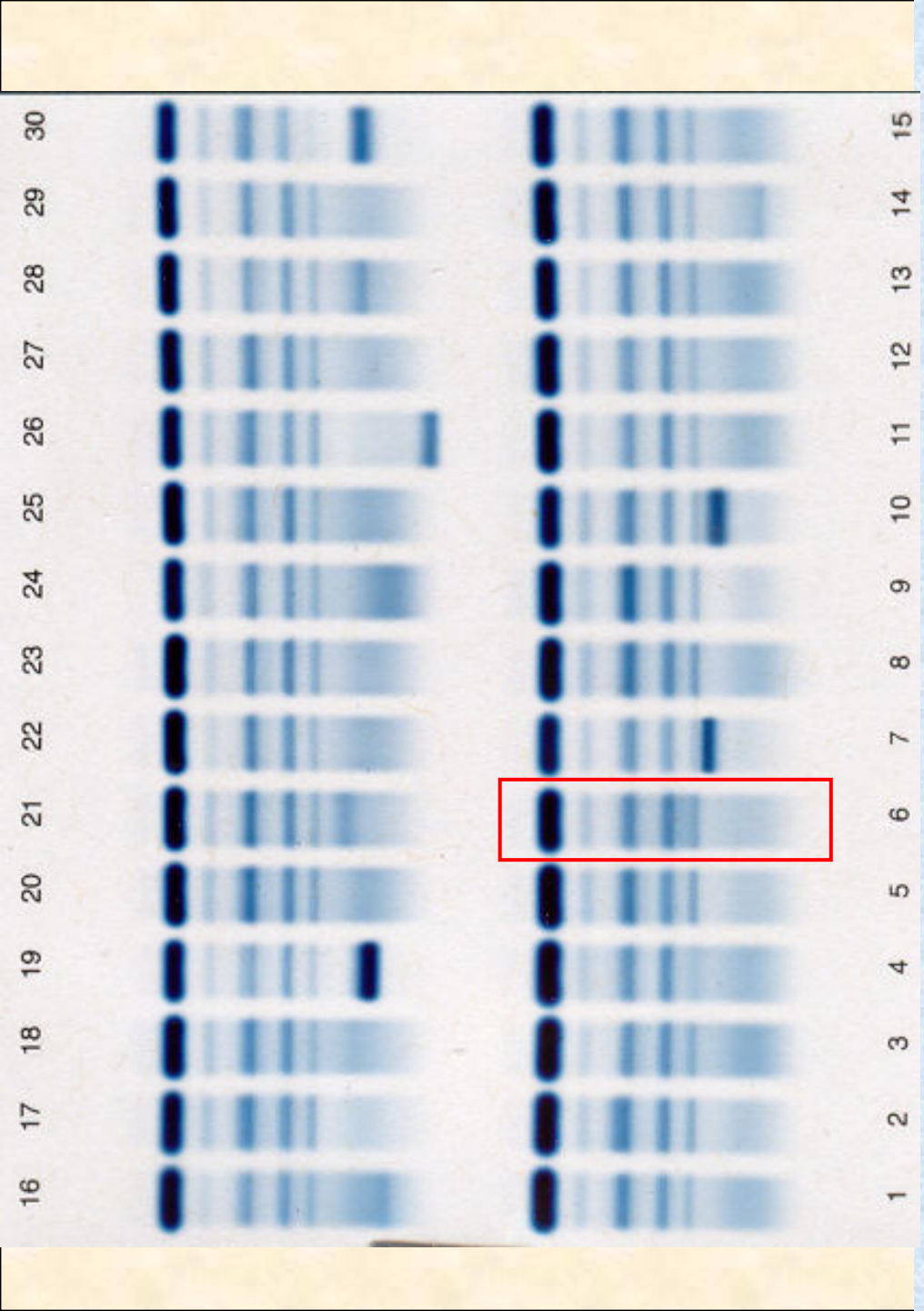
Gammopathy in beta zone?

Smudged beta band?



Beta-2 band "smudged" (compare to neighbours)

Immunofixation confirmed an underlying paraprotein, co-migrating with normal beta-2 proteins

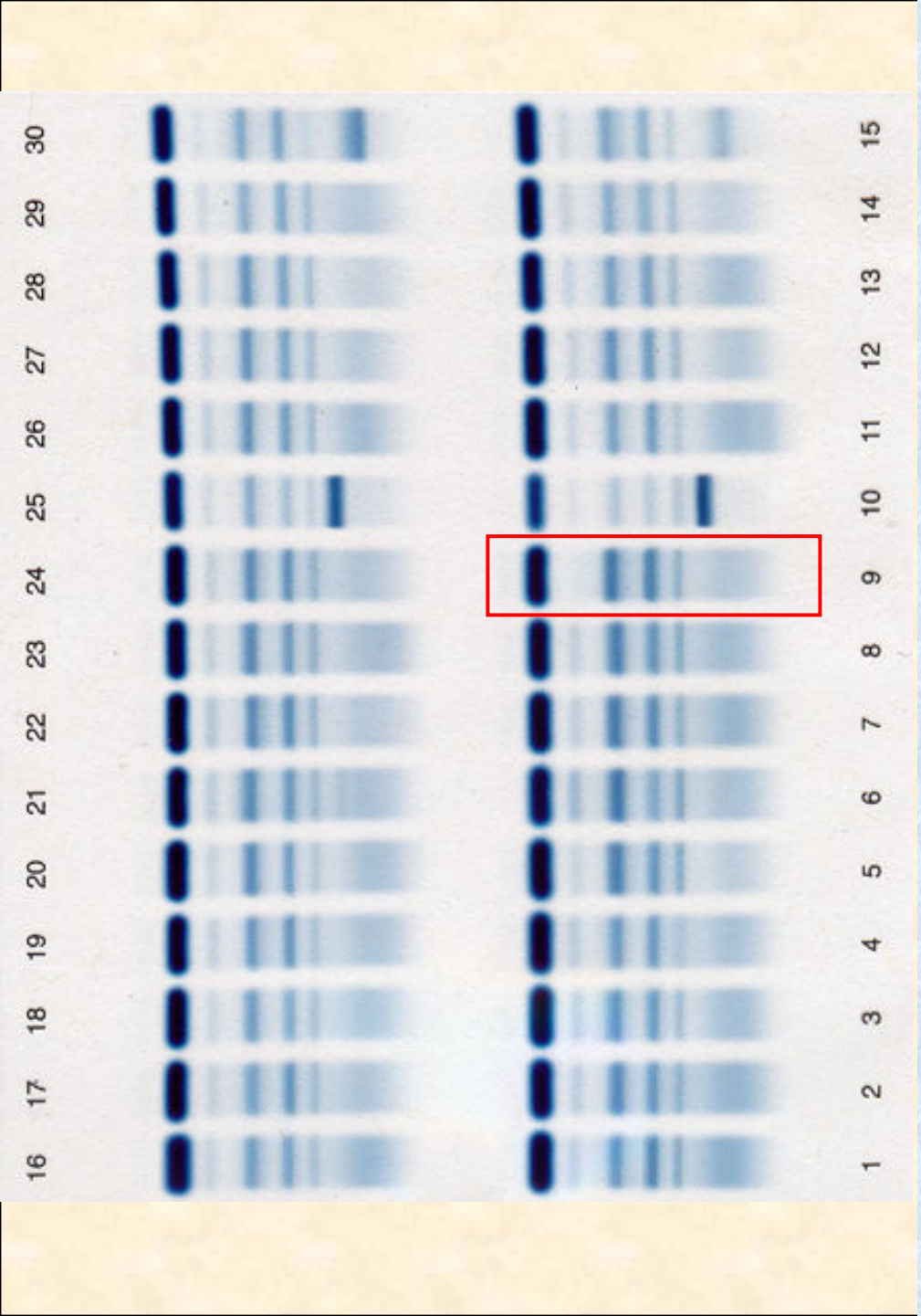


Gammopathy in beta zone?

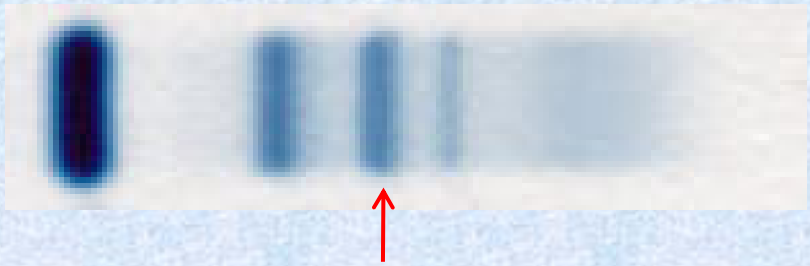
Smear in beta region?

No obvious paraprotein band, but region between beta-1 and beta-2 is smeared

Immunofixation showed an underlying IgA lambda monoclonal protein band



Trust your instinct…



29 yr old woman:
Beta-1 is slightly dense but still well within ref range

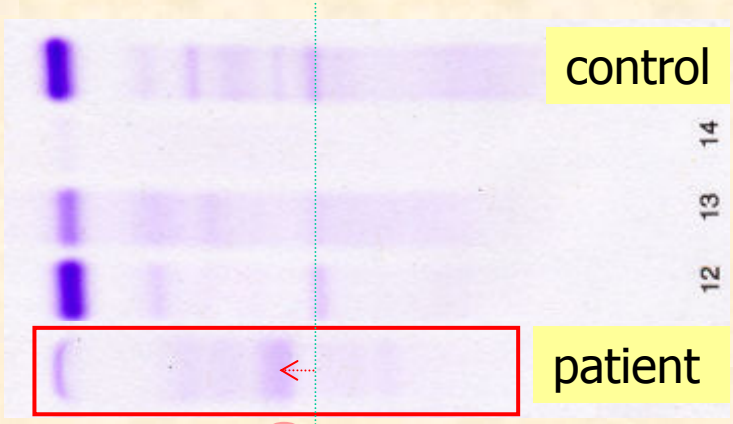
Most likely cause?
- transferrin (anemia)?

BUT - Dx: "R/O myeloma" !

Trust your instinct (cont.)

Serum protein immunofixation

"...possible but very faint IgM lambda monoclonal protein band"



Urine protein electrophoresis

"...major urine protein constituent is an abnormally migrating beta band"

lambda light chains?

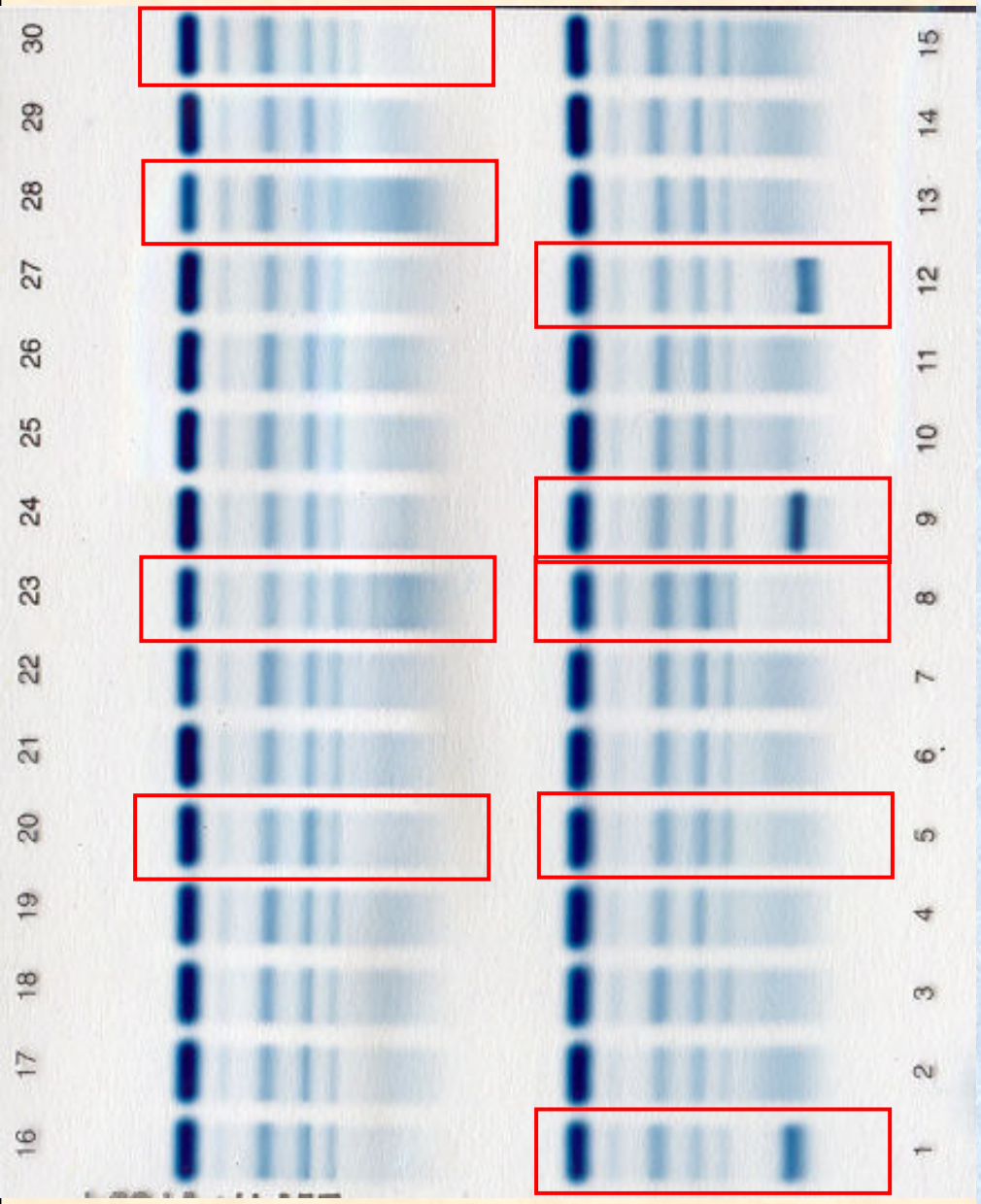
Subsequent urine protein immunofixation was indeterminate; suggest follow-up with repeat SPE and UPE in 3-6 months

Ordering physician can help the lab (and the patient)…

*A diagnostic comment can be **so** helpful…*

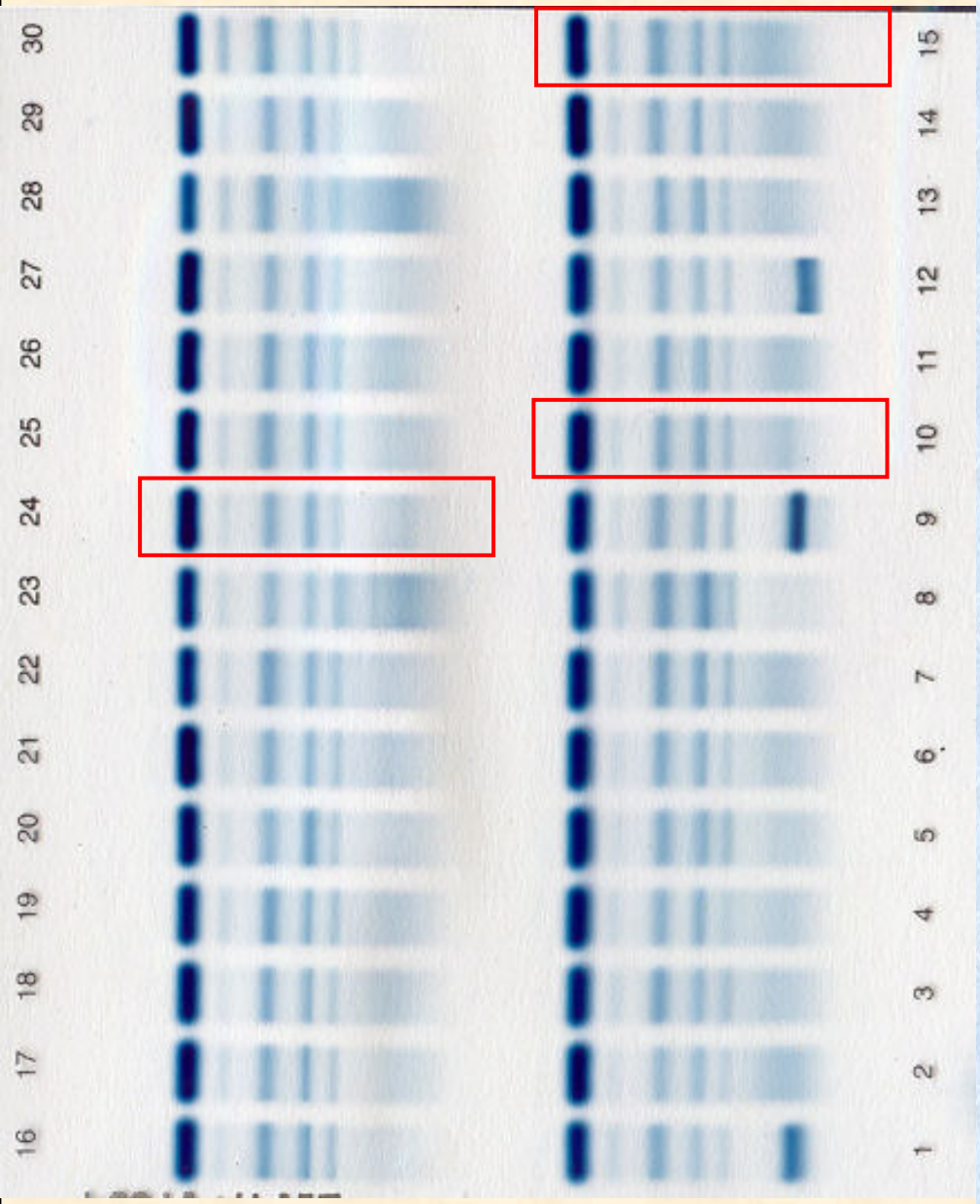
- peripheral neuropathy
- osteoporosis
- back pain
- unexplained anemia
- R/O myeloma
- unexplained renal failure

Any of these *raises the pre-test probability* and may influence our decision to add an immunofixation



Obvious abnormals…

See any more?



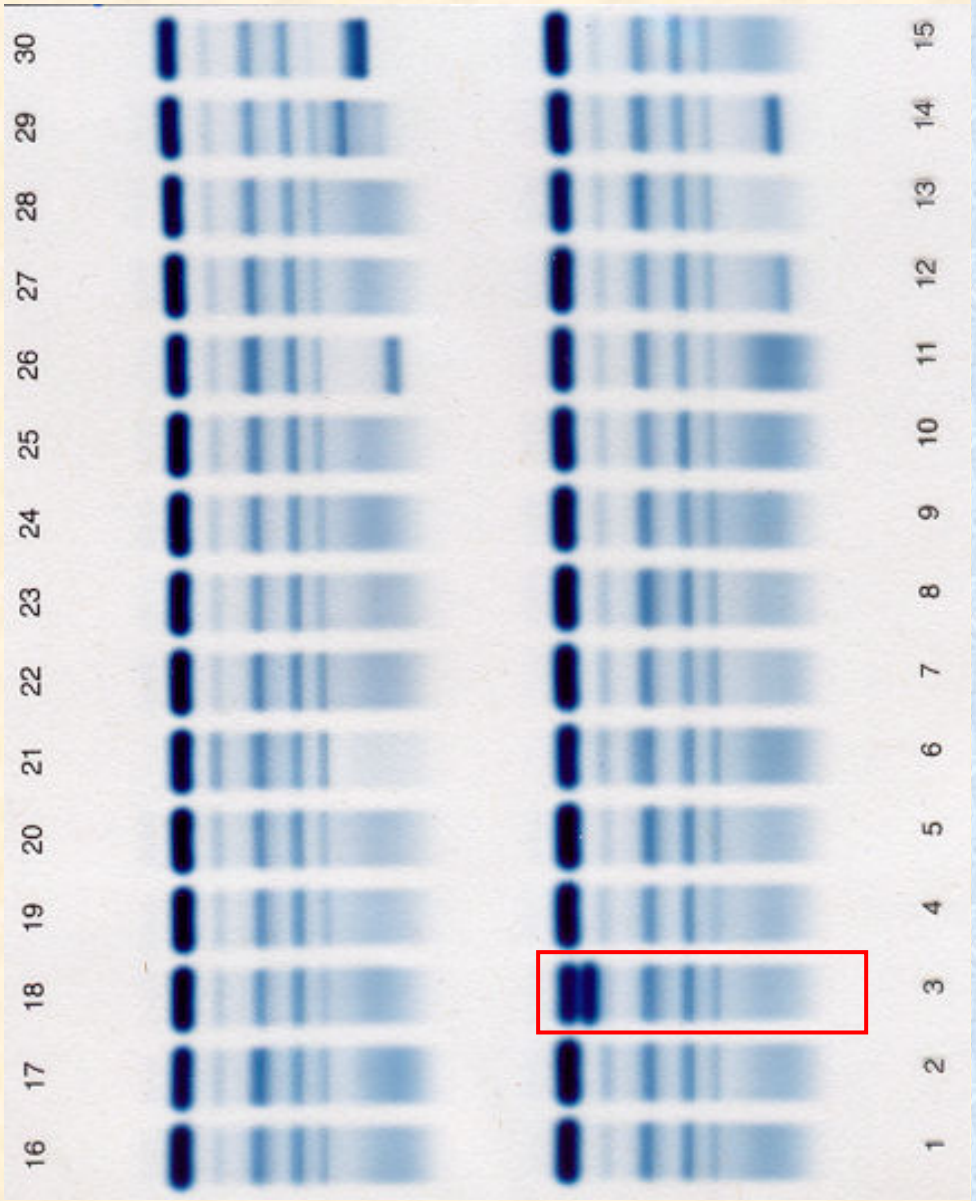
Less obvious abnormals…

…these ones are positive, too

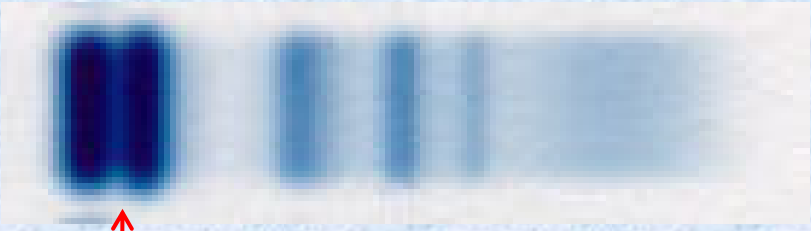
Some underlying bands can easily be missed

Other atypical bands

extra bands can cause anxiety but are not *necessarily* pathological…



Bisalbuminemia



Double albumin band

Hereditary mutation:

- no associated pathology
- fast & slow variants
  (N. American First Nations & European ancestry)

Acquired:

- transient
- pancreatitis; penicillin Rx

Heterozygous alpha-1

Double α1 band

α1-antitrypsin phenotypes

eg PiMS instead of PiMM

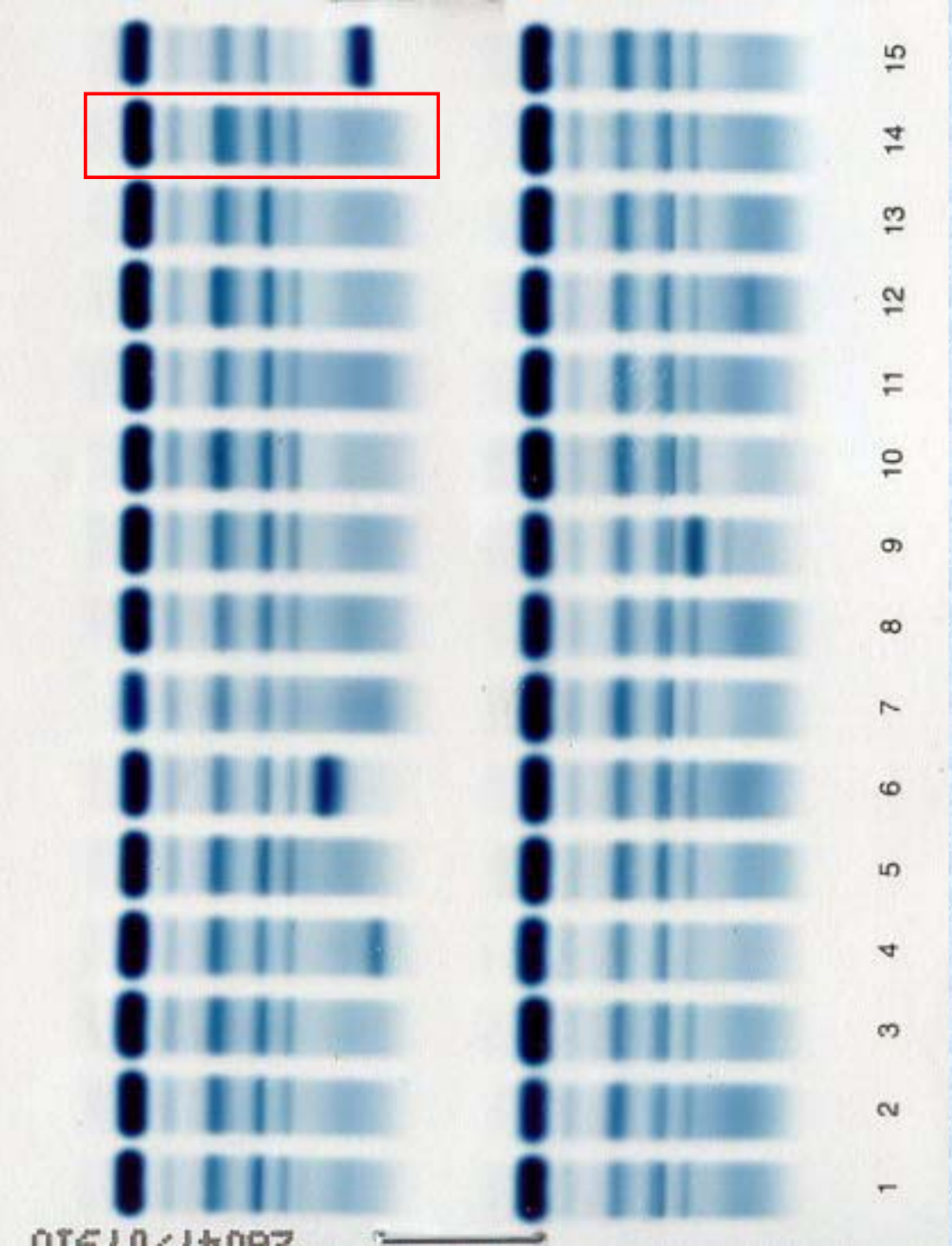
Split α_2 -globulin band

Hemolysis

- (haptoglobin + hemoglobin)
- confirm that specimen **is** hemolysed!

Other causes

- unusual haptoglobin phenotype (of no pathological significance)
- lipoprotein with α_2- mobility
- monoclonal band

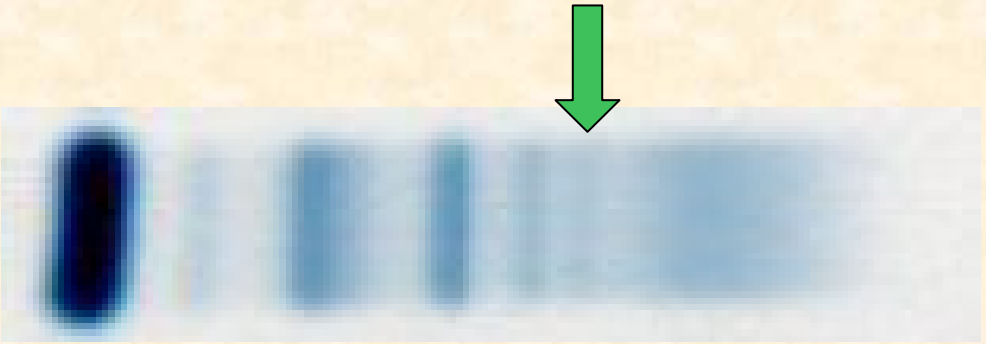


Hemolysis

α_2-globulin "smeared"

If specimen is NOT hemolysed, consider possibility of an underlying paraprotein band

Fibrinogen?



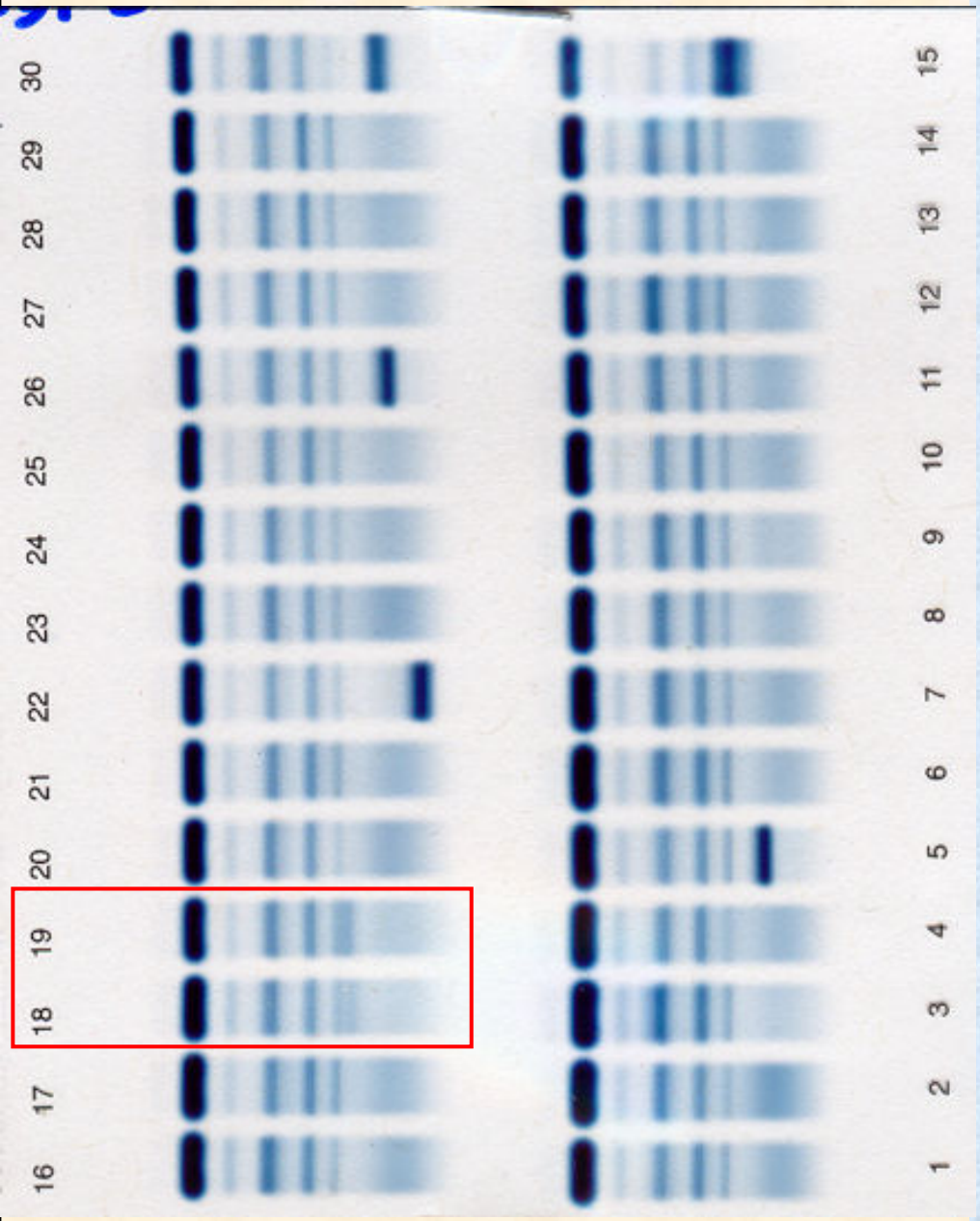
Fibrinogen is a normal component of *plasma*

It is usually consumed during clotting as it converts to fibrin

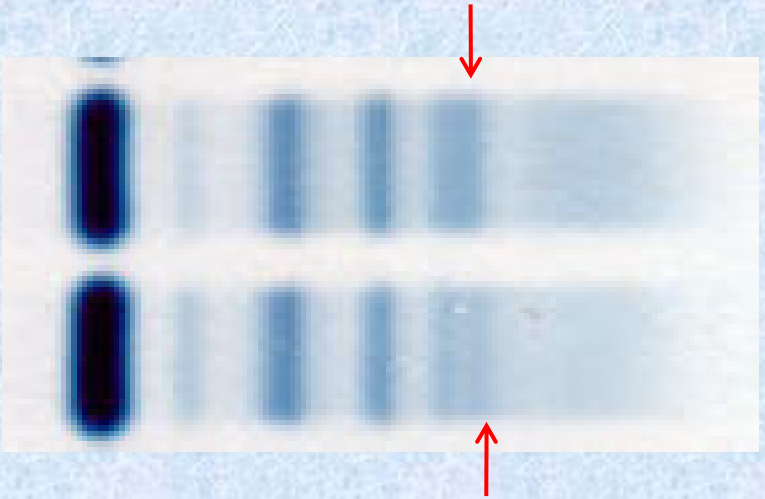
Therefore, fibrinogen is *not* usually present in serum

If present, it migrates in beta-gamma region, and may appear visually identical to a tiny monoclonal band

Immunofixation will distinguish between them



Is it fibrinogen, or not?



In these cases, BOTH were paraprotein bands

18: IgA kappa

19: IgG lambda

It's a paraprotein until proven otherwise…

Ill-defined bands…

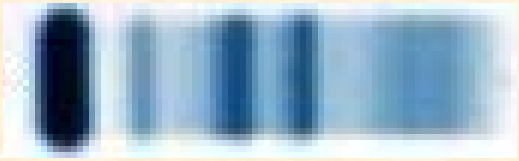
…is there a hidden tiger, or not?

A centuries-old seadog's trick

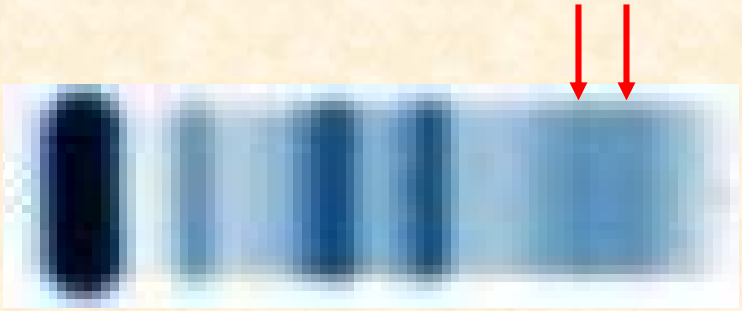
To gain the most acute ability in differentiating shades (*eg* icebergs), shift your vision slightly to one side of your target (about 4-12 degrees)

Fovea in retina has only ones, but rods are better at discerning differences in shades

This can be a useful technique when reading electrophoresis gels!



Serum oligoclonal banding



Typically, two or more small ill-defined bands, against a polyclonal background

- with hypergammaglobulinemia ± beta-gamma bridging?

  chronic antigenic stimulation: viral & bacterial infections (*eg* Hep C), vaccines; autoimmune diseases and angioimmunoblastic lymphadenopathy

- with hypogammaglobulinemia?

  CLL; post heart- and BM- transplants (associated with immunosuppressive Rx); acquired immunodeficiency

Serum oligoclonal banding: recommended follow-up

Repeat serum PRE in 2-3 months (resolves or evolves?)

If banding persists without known cause, order serum immunofixation and recommend urine protein electrophoresis

Restriction

"A tiny restriction is noted in the late gamma region. Suggest repeat serum electrophoresis in 3 months, to see if process resolves"

It is not our standard practice to attempt to classify such bands by immunofixation if they are <1.0 g/L

Clues that a restriction is **unlikely** to be due to a malignant clonal expansion

- Acute phase pattern is also present
- Transient restriction, evolving into an oligoclonal pattern
- All immunoglobulin classes are elevated
- kappa:lambda ratio is only slightly abnormal
- No Bence Jones protein detected
- Band is less intense than the alpha-1 band

Suggestions: how to describe a suspected underlying band without causing undue alarm

"Gamma region…

-shows asymmetry"

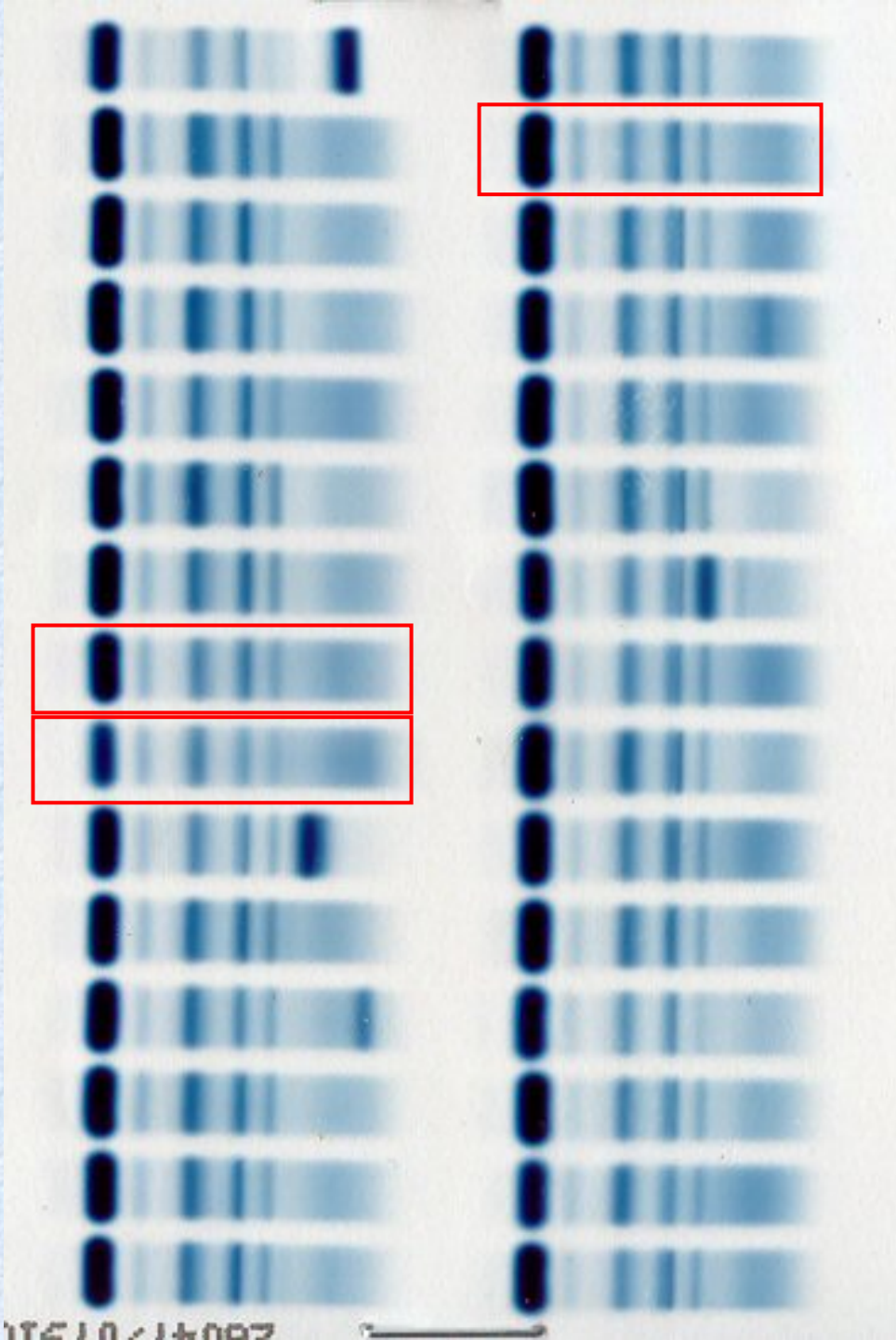
-shows increased central density"

-suggests a possible ill-defined underlying band"

Immunofixation of vague bands is *not* informative

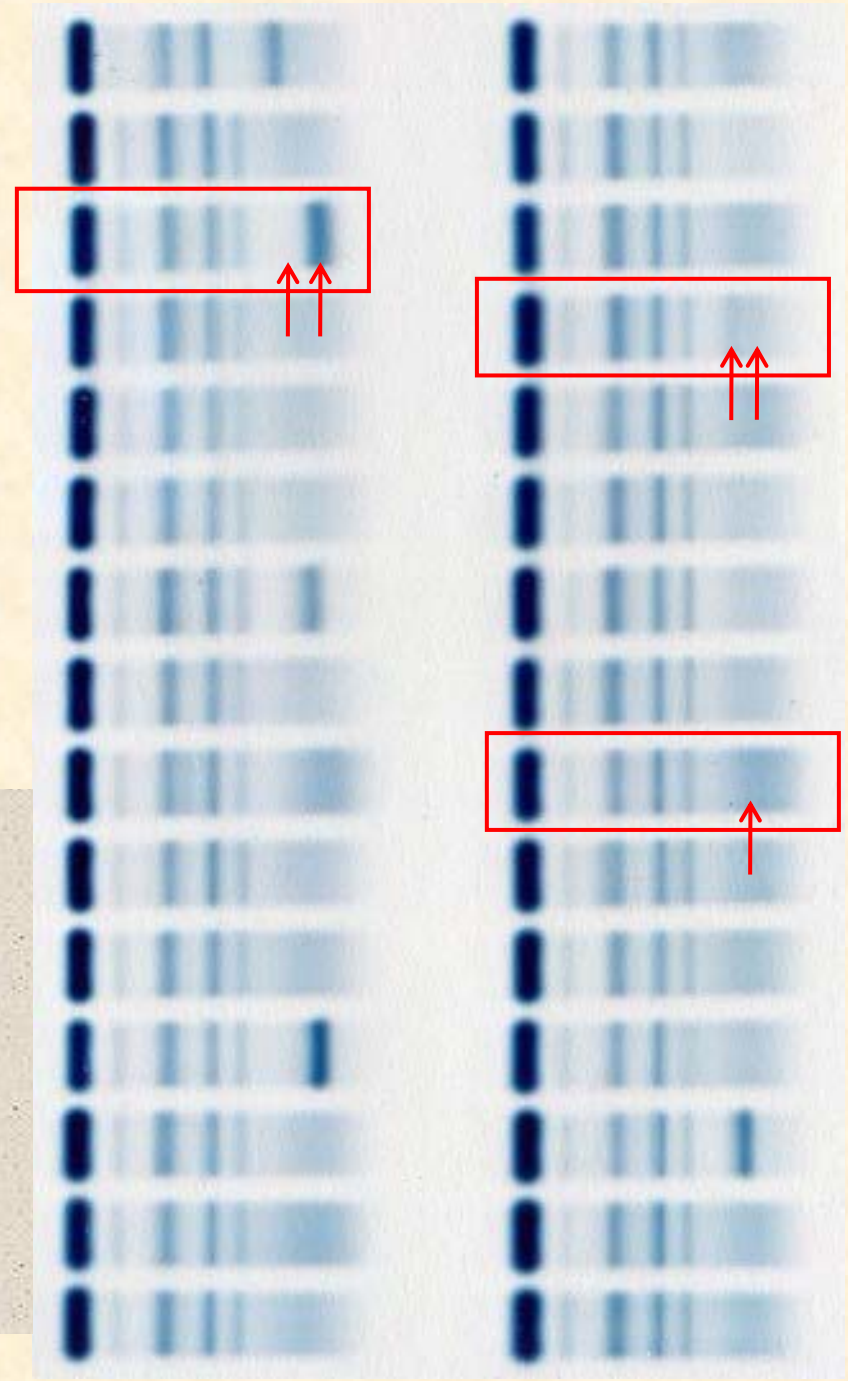
"Suggest repeat serum electrophoresis in 2-3 months"

(see if process resolves or evolves)



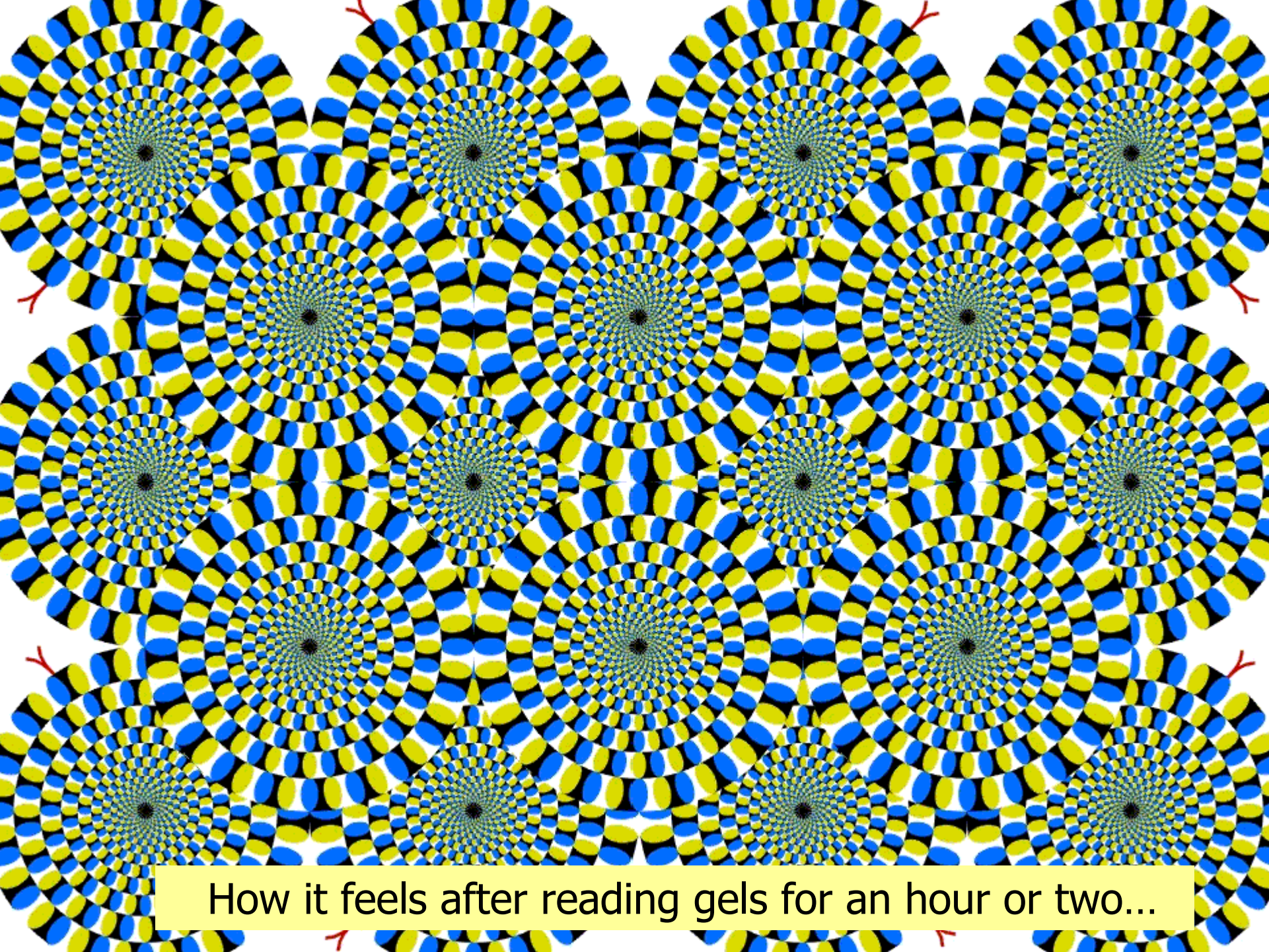
Examples of various band descriptions

discrete band in gamma region; suppression of normal gamma globulins is also noted



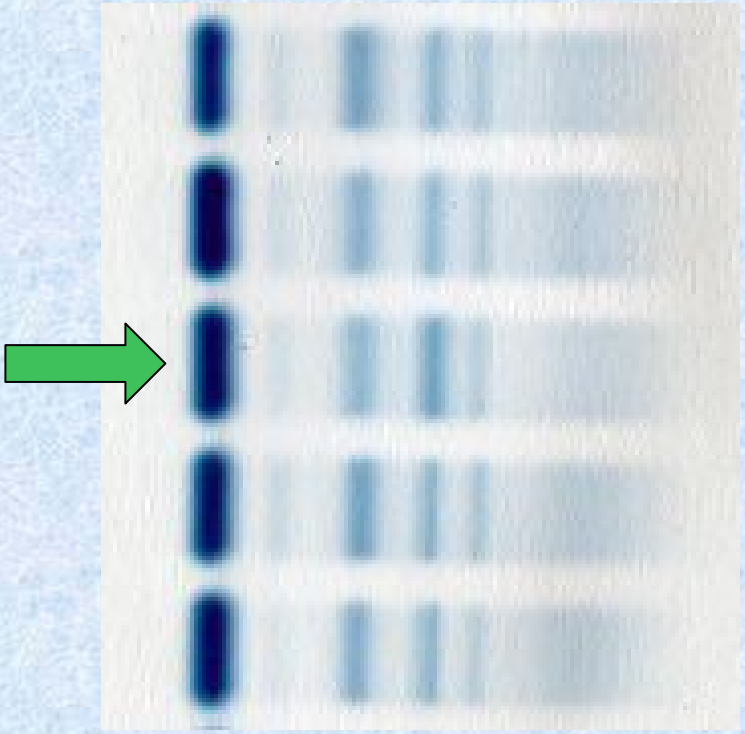
oligoclonal banding, underlying a polyclonal background

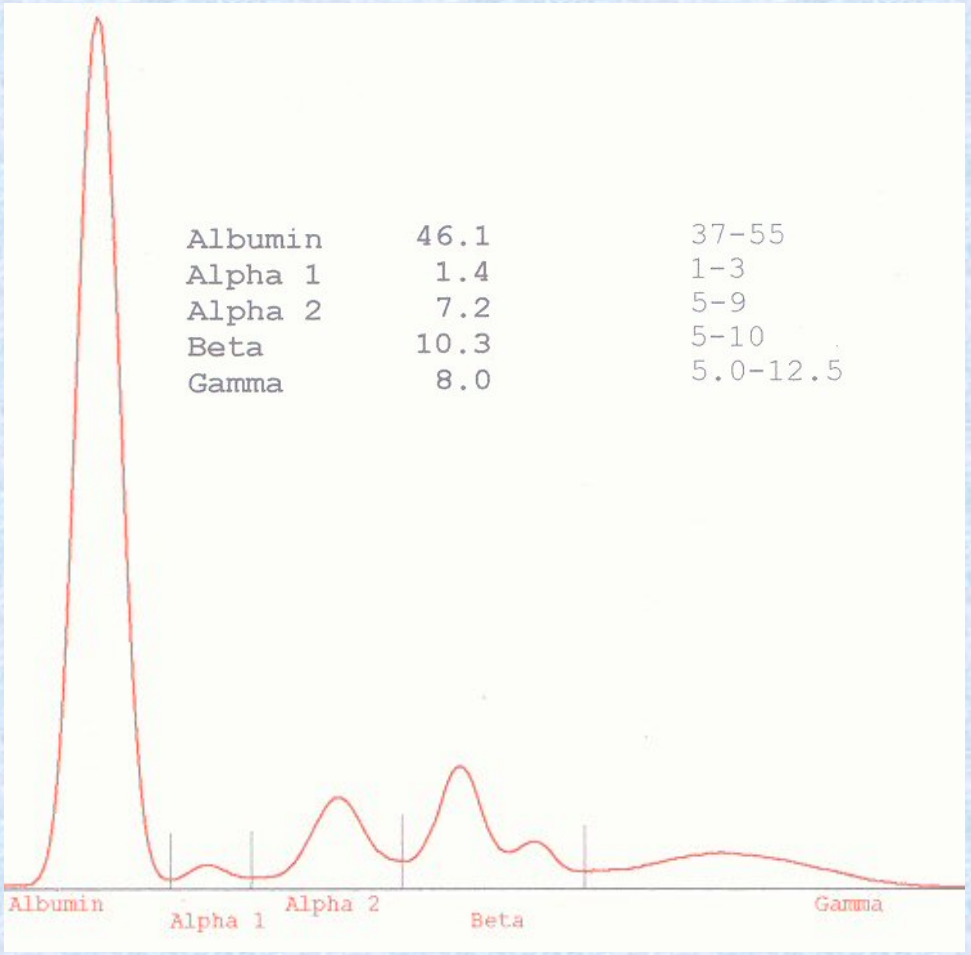
ill-defined restriction, just discernable

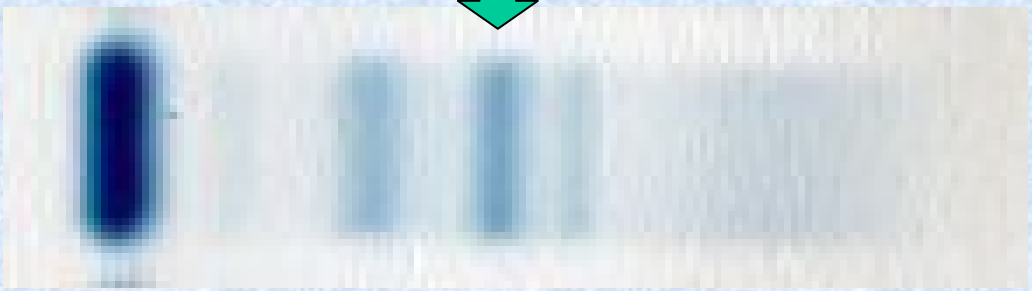

How it feels after reading gels for an hour or two…

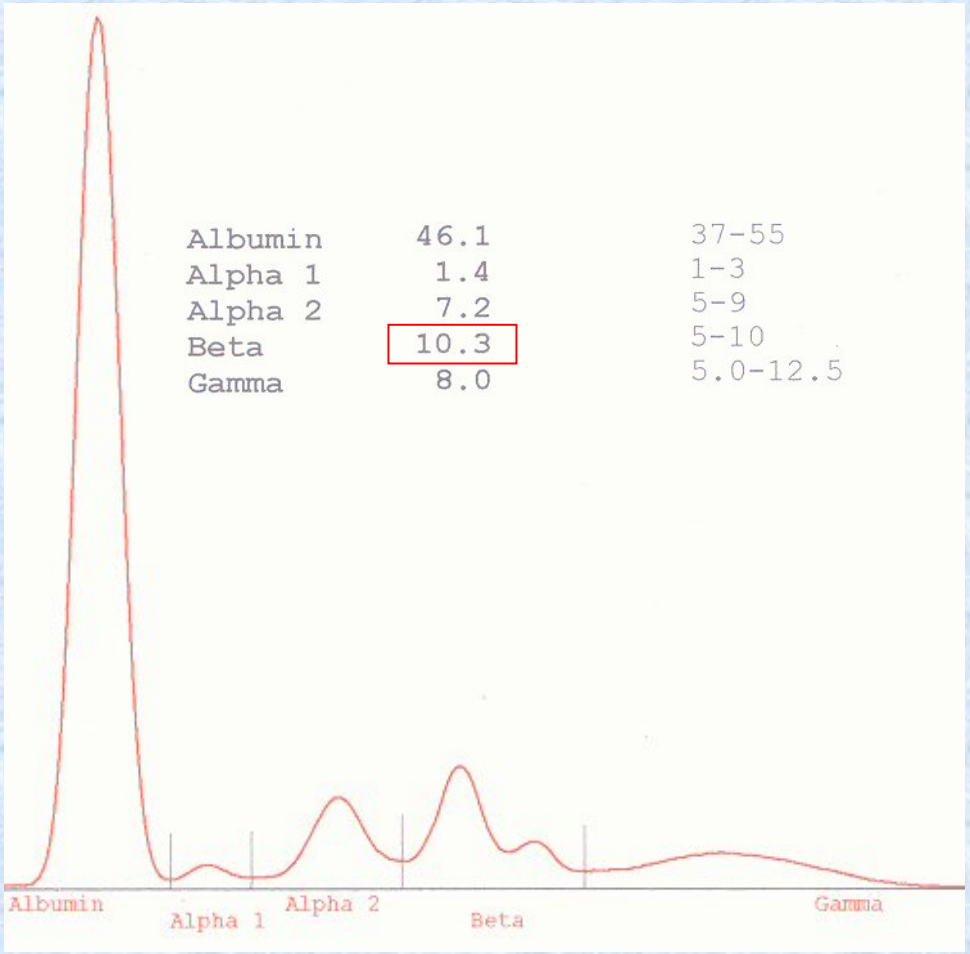
Case 1

Normal profile?









Case 1 (cont.)

Total beta is slightly increased, but is not at a level that would usually merit any comment

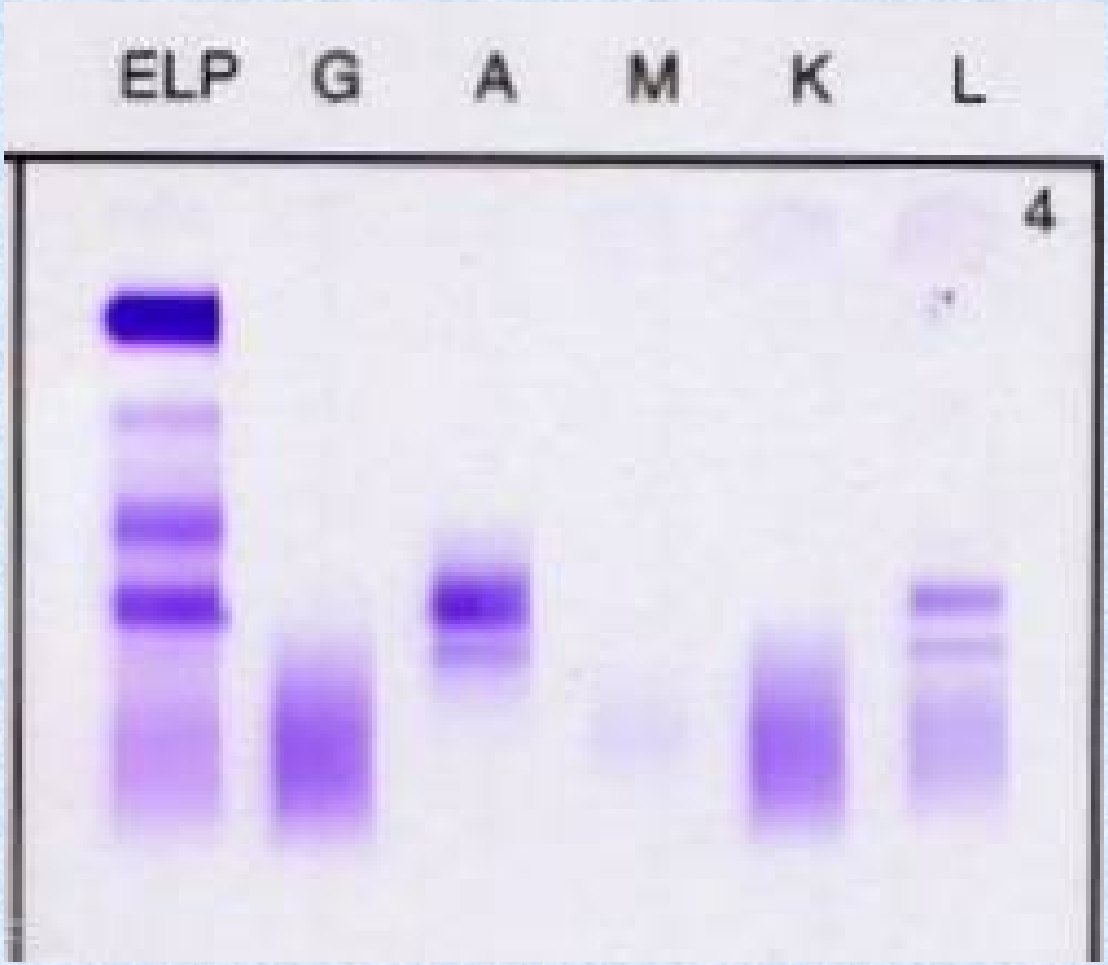
$\beta_1 > \beta_2$ (normal)

$\beta_1 >> \beta_2$ (? abnormal)

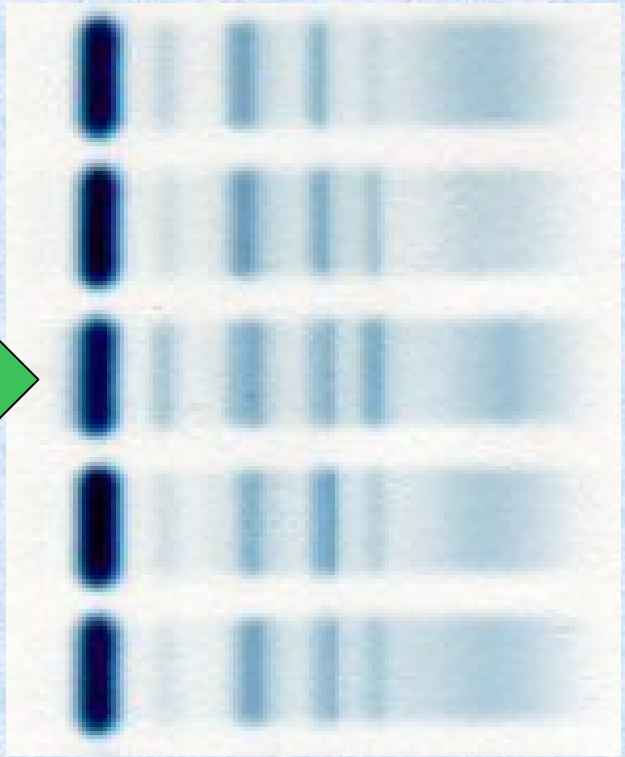
"Immunofixation ordered, to exclude possible paraprotein co-migrating with β_1"

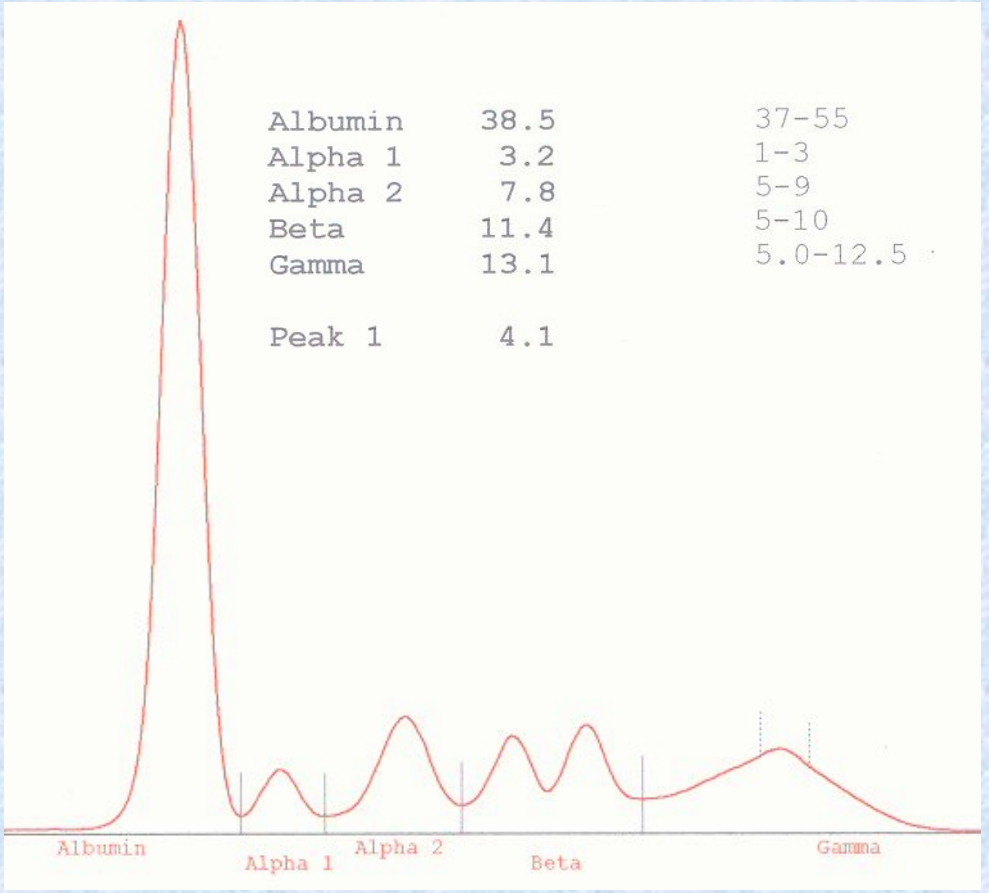
Case 1: serum immunofixation

Shows two monoclonal IgA lambda bands, co-migrating with normal β_1 and β_2 protein bands



Case 2




Albumin 38.5 37-55
Alpha 1 3.2 1-3
Alpha 2 7.8 5-9
Beta 11.4 5-10
Gamma 13.1 5.0-12.5
Peak 1 4.1
Albumin
Alpha 1
Alpha 2
Beta
Gamma

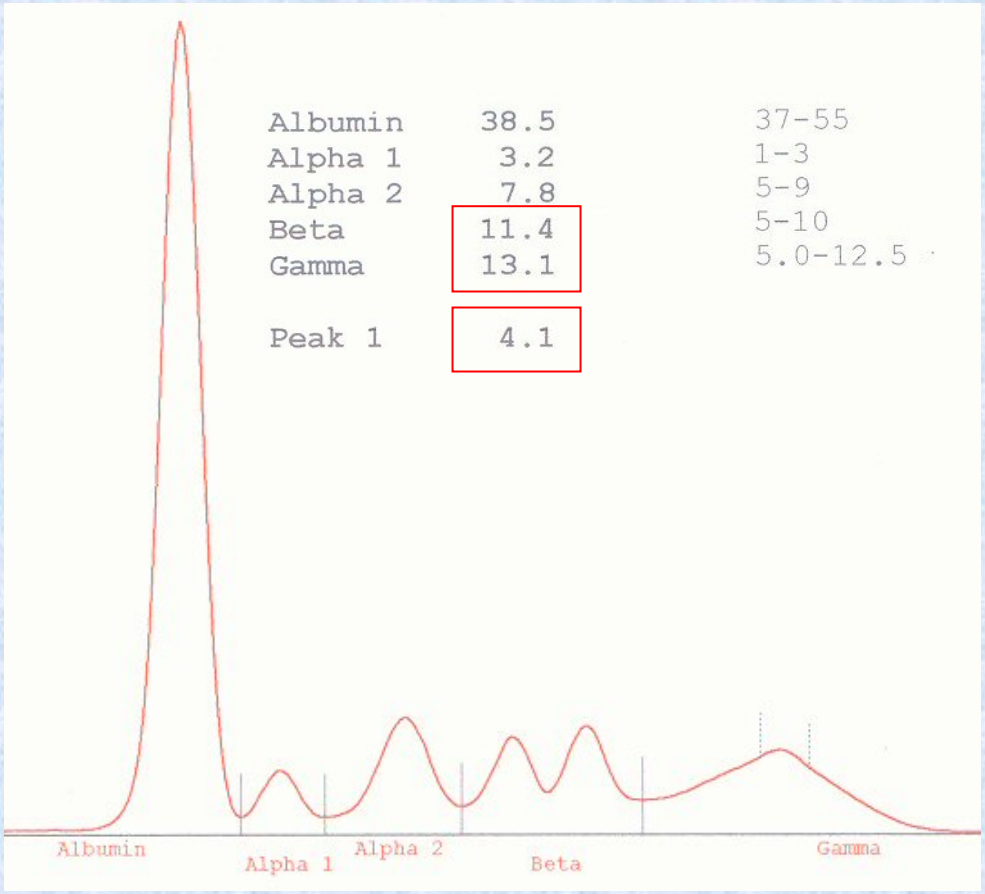
Case 2

$\beta_2 > \beta_1$ (atypical)

"total beta increased, with atypical beta-2 band"

"γ slightly increased"

"An ill-defined band is also noted, underlying the mid-γ region"



Case 2: serum immunofixation

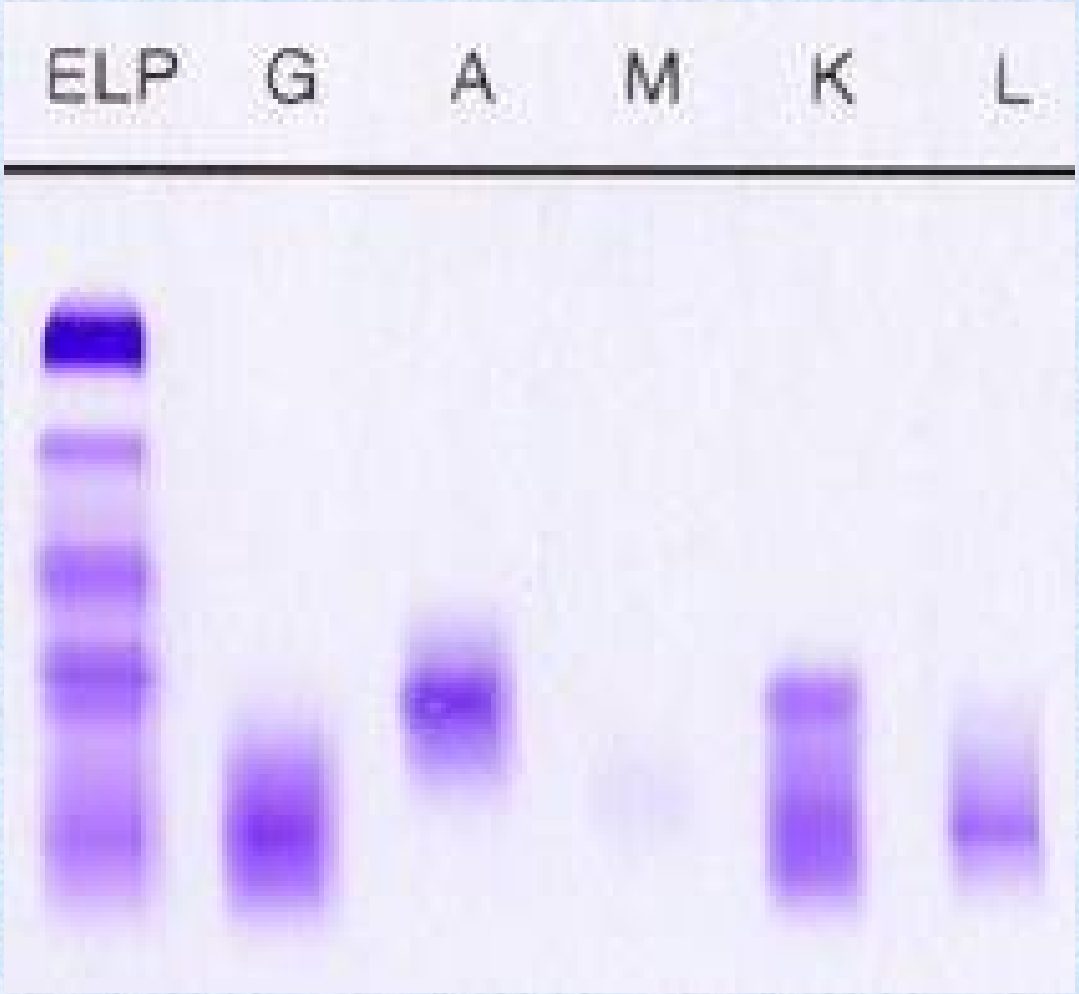
Shows two monoclonal bands:

IgA kappa

(in β_2 position) and

IgG lambda

(in mid-γ region)



Monitoring a co-migrating band

When a paraprotein co-migrates with a normal protein band, it is usually difficult or impossible to differentiate between them

So how do we monitor paraprotein band quantitation to detect progression?

First, do immunotyping to determine the immunoglobulin heavy chain (*eg* IgA), and then monitor total IgA level (every 3-6 mo.)

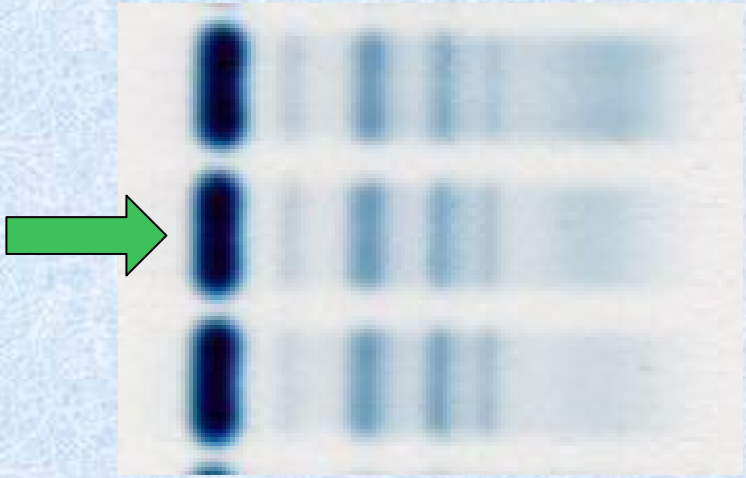
Total beta quantitation can also indicate significant change in an underlying band

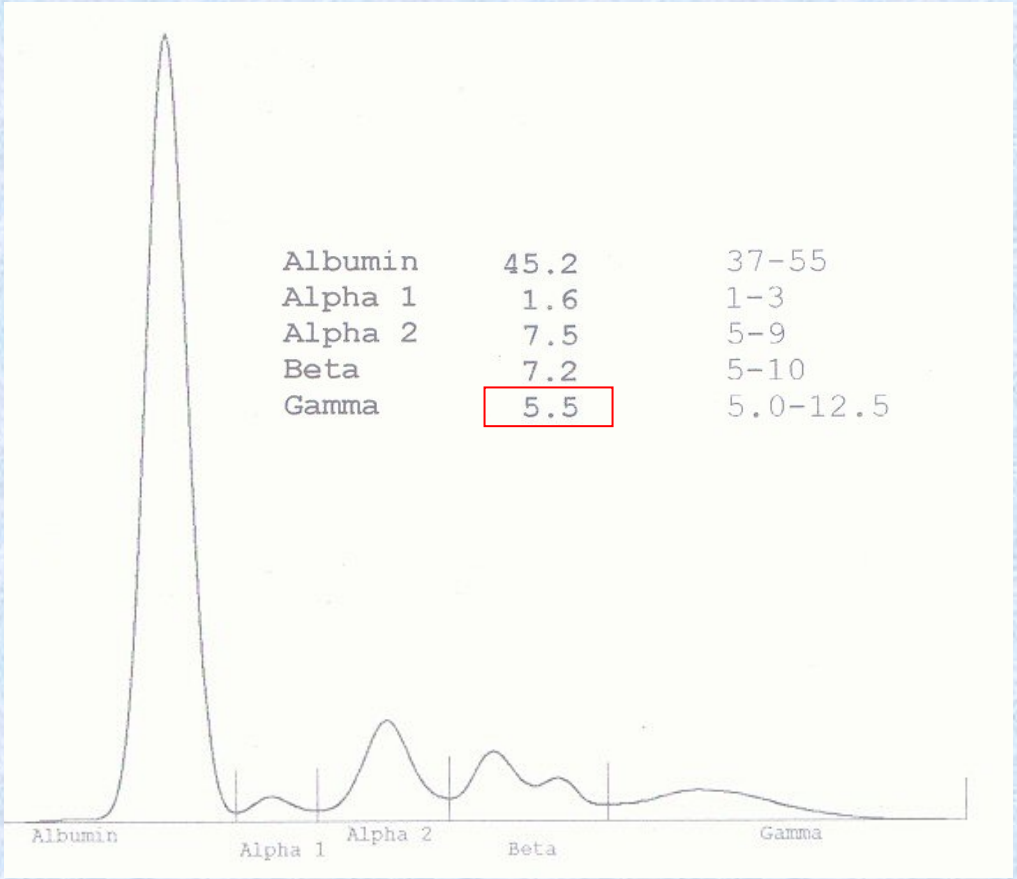
Case 3

Low normal gamma globulins

"No paraprotein band seen.

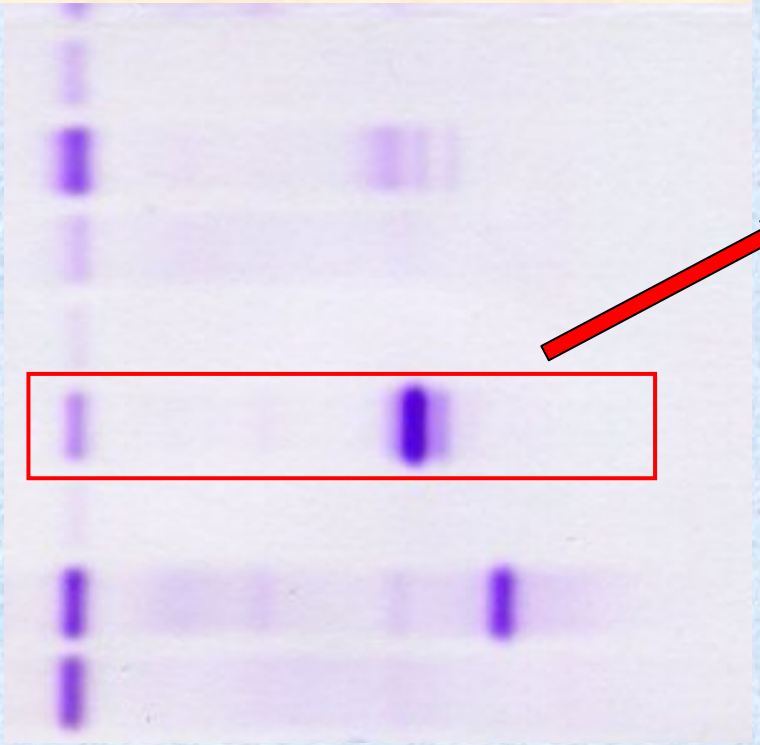
"Suggest AM urine for monoclonal light chains"

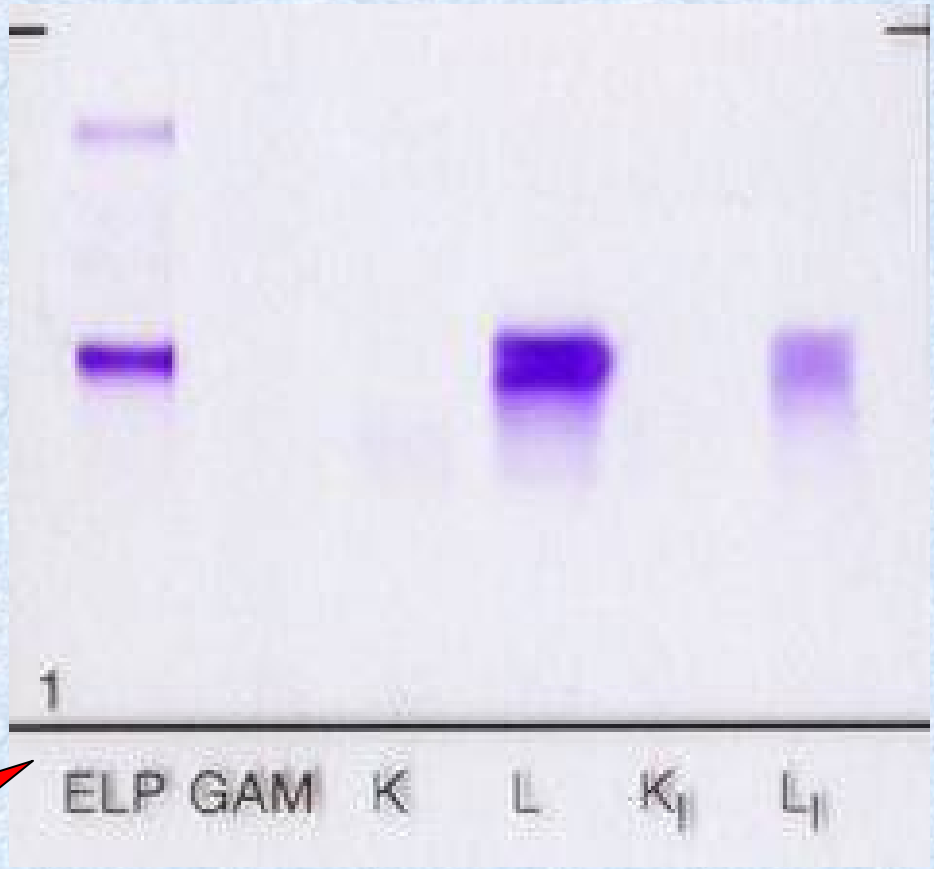




Case 3:

urine protein electrophoresis & immunofixation





Monoclonal lambda light chains

Monoclonal gammopathies: the utility of urine electrophoresis

Protein electrophoresis of AM urine may demonstrate monoclonal light chains that are undetectable by serum electrophoresis

I hope no one's lost..?