

Data Interpretation of Hb and protein Electrophoresis

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Data Interpretation of Hb and protein Electrophoresis

- Hemoglobin electrophoresis
 - Normal Hb
 - Pathological Hb
 - Sickle cell Anemia
 - α -thalassemia
 - β -thalassemia
- Protein electrophoresis
 - Normal
 - Pathological
 - multiple myeloma
 - other pathological profiles

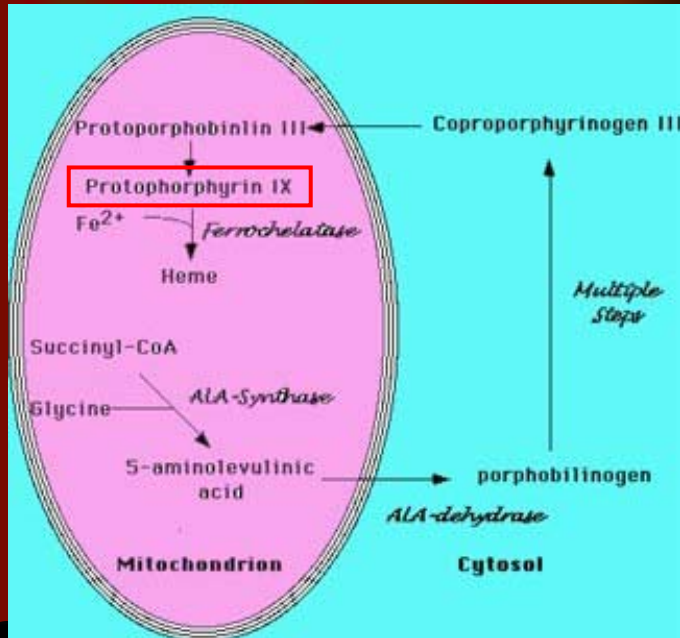
Anemia

- Depletion anemia
- Production defect anemia
 - Aplastic anemia/ marrow replacement
 - factor deficiency
 - Vitamin B12
 - Folic acid
 - iron deficiency
 - hemoglobinopathies

Table 3-4. DIFFERENTIAL DIAGNOSIS OF MICROCYTIC HYPOCHROMIC ANEMIA

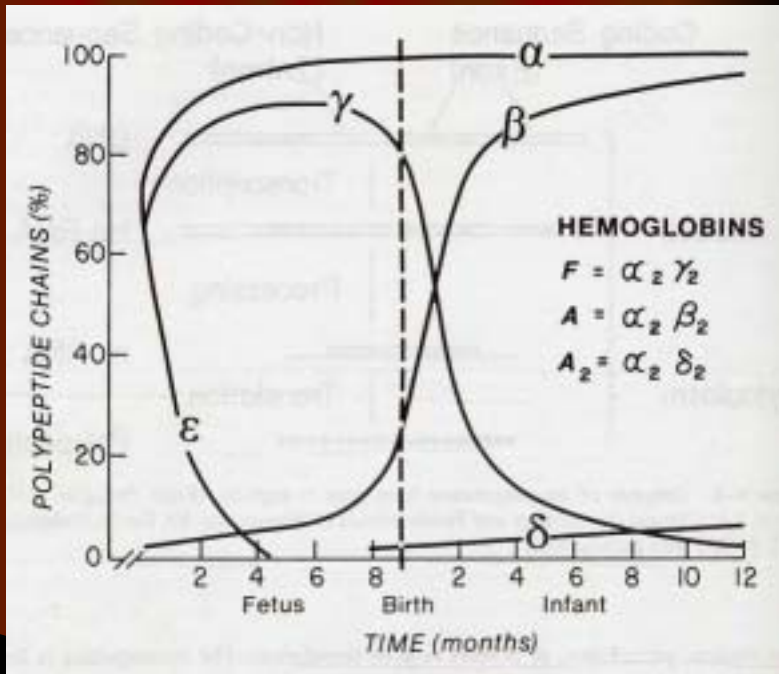
	Serum Iron	TIBC	Serum Ferritin	FEP	HbA ₂	HbF	RDW
Iron deficiency	Low	High	Low	High	nl	nl-low	High
Alpha-thalassemia	High	nl	High	nl	nl	low	
Beta-thalassemia	High	nl	High	nl	High	High (varies)	High
Anemia of chronic disease	Low	Low	High	High	nl	nl	nl
Sideroblastic anemia	High	nl	High	Low	nl	nl	High

TIBC = total iron-binding capacity; FEP = free erythrocyte protoporphyrin; nl = normal; HbA₂ = Hemoglobin A₂; HbF = Hemoglobin F; RDW = red cell distribution width.



Normal Hb in fetal stage

- Embryonic
 - Gower 1 = $\zeta_2\varepsilon_2$
 - Portland 1 = $\zeta_2\gamma_2$
 - Gower 2 = $\alpha_2\varepsilon_2$
- At birth
 - Hb A = $\alpha_2\beta_2$ (25%)
 - Hb F = $\alpha_2\gamma_2$ (75%)



Normal Hb in adult

- Hb A = $\alpha_2\beta_2$ (97%)
- Hb F = $\alpha_2\gamma_2$ (<1 %)
- Hb A₂ = $\alpha_2\delta_2$ (2.5%)

Pathological conditions

- Hemoglobinopathies
 - (a) Structural Hb variants
 - Substitution, addition, or deletion of one or more amino acids of the globin
 - (b) Thalassemias
 - Quantitative defect in globin chain production
 - (c) Combination of (a) and (b)
 - (d) Hereditary persistence of fetal Hb

Nomenclature of Hb variants

- HbA, HbF, and HbS were first discovered
- Additional variants start from HbC
- Too many variants were found(>500)
 - Hb with similar electrophoretic motility
 - distinguished by adding the place of discover
 - Some new Hb were named by pts' family
- New system
 - HbS B6 Glu→ Val (E B6 V)
 - HbC B6 Glu→ Lys (E B6 K)

Classification of Hb variants

- Amino acid substitution
 - e.g. Sickle cell anemia
- Deletion and insertion
 - e.g. thalassemia (α chain deletion)
- Unequal cross over (fusion genes)
- Chain elongation
- Frame shift variants

Table 36-2 Clinical manifestations associated with some abnormal hemoglobins

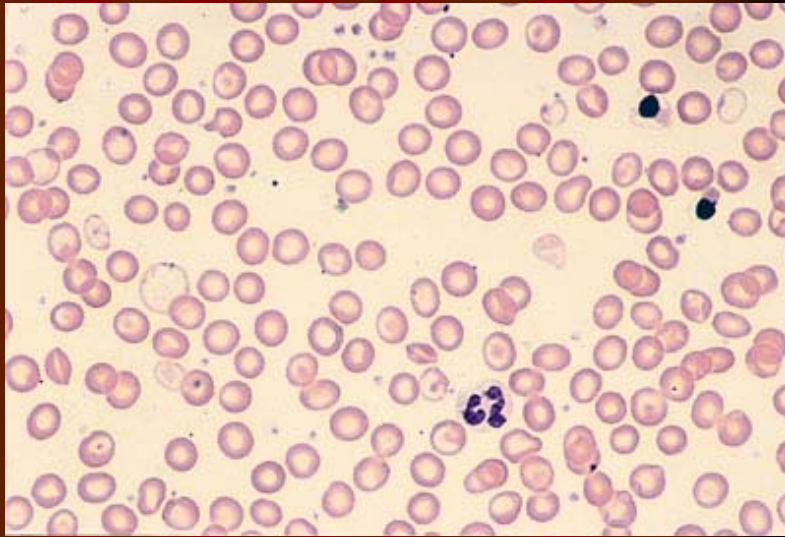
Disorder	Abnormal Hb	Structural change	Comments
hemolytic anemia	H	$\alpha\beta_2 \rightarrow \beta_4$	Unstable hemoglobin occurring in some forms of alpha-thalassemia; precipitation of hemoglobin and hemolysis are accelerated by certain drugs
	S	beta 6 glu \rightarrow val	Forms molecular aggregates when deoxygenated, producing sickle cell anemia in homozygotes
	C	beta 6 glu \rightarrow lys	Low solubility lessens plasticity of red cells, causing hemolytic anemia in homozygotes
Cyanosis caused by methemoglobinemia	M _{Boston}	alpha 58 his \rightarrow tyr	Methemoglobin causes cyanosis in heterozygotes; some also have evidence of hemolytic anemia
	M _{Waco}	alpha 87 his \rightarrow tyr	
	M _{South Park}	beta 92 his \rightarrow tyr	
Cyanosis caused by increased deoxyhemoglobin	Kansas	beta 102 asn \rightarrow thr	Decreased oxygen affinity of hemoglobin causes cyanosis in heterozygotes
	lysochromia		
polycythemia	L _{Chesapeake}	alpha 92 arg \rightarrow gln	Increased oxygen affinity of hemoglobin hinders release of oxygen to tissues, causing compensatory polycythemia in heterozygotes
	Rainier	alpha 92 arg \rightarrow leu	
		beta 145 try \rightarrow cyst	
hydrop fetalis	Bart's	$\alpha\text{-}\gamma_2 \rightarrow \gamma_4$	Unstable hemoglobin with high oxygen affinity occurring in high concentration in stillborn fetuses with homozygous alpha-thalassemia

Clinical consequences of abnormal Hb

- Asymptomatic if they don't interfere with Hb functions → **unstable Hb**
- Produce disease
 - affect the stability, shape, or function of Hb
- Homozygous for abnormal Hb
 - HbS
- Heterozygous are mostly mild
 - HbC, HbD, HbE but not HbA-HbS

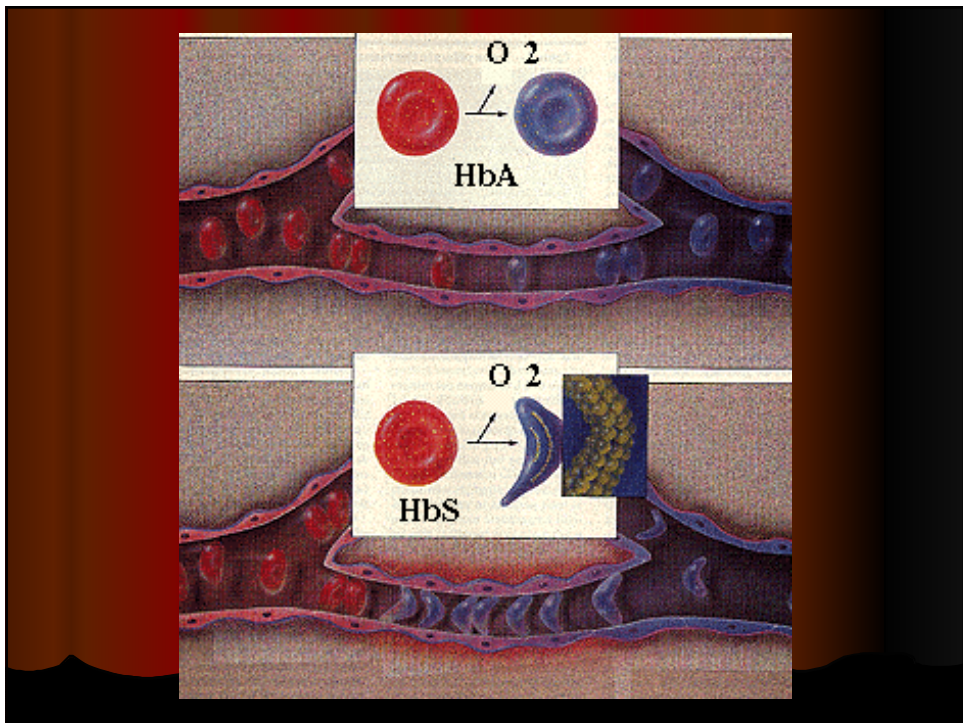
Unstable Hb

- **Hemolytic anemia**
- Hemichrome formation
(Heme iron form various side chain with globin)
- Inclusion body formation (**Heinz bodies**)
- Altered oxygen dissociation
- Altered hemoglobin stability → Increase **methemoglobin** ($\text{Fe}^{++} \rightarrow \text{Fe}^{+++}$) and **sufhemoglobin**
- **Altered solubility** (Target cells)



Sickle cell anemia

- B chain 6th amino acid E → V substitution results in polymerization of deoxy form within the red cells
- The sickled-shape cells block microcirculation
- Stasis → hypoxia and ischemic infarction of liver, kidney, heart, bone, nervous system
- Hemolytic anemia or even DIC

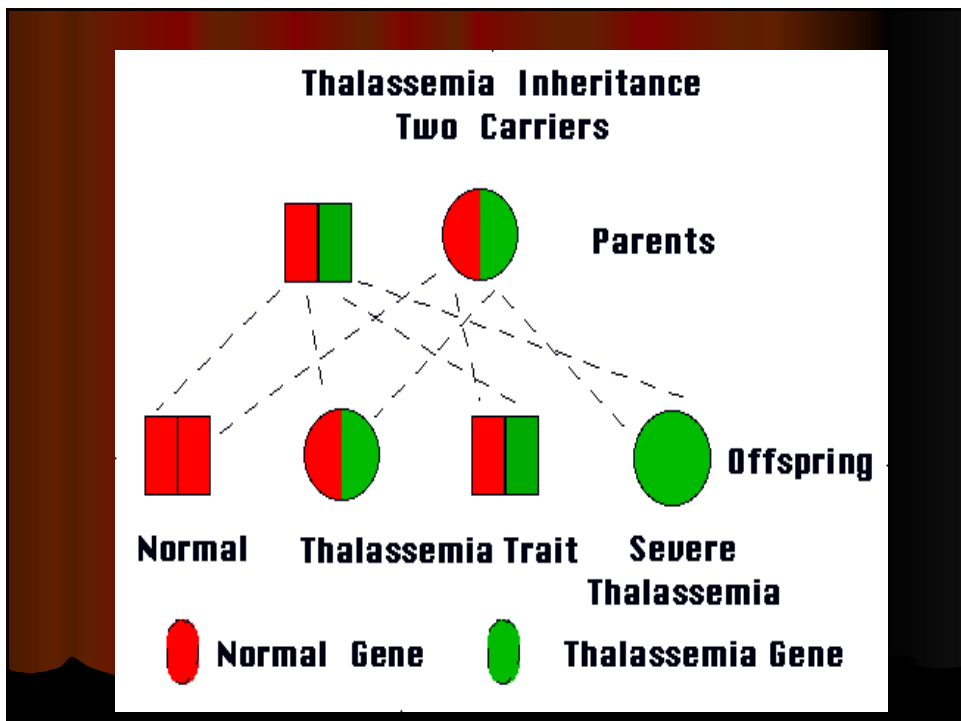


Varying clinical severity of the different sickle syndrome

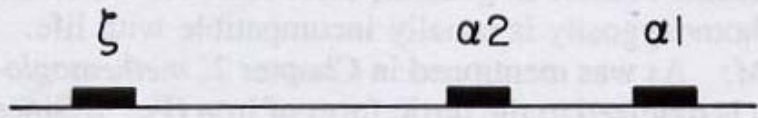
- Sickle cell trait
 - SA (30-40% HbS) (mild severity)
 - SF (70% HbS) (mild severity)
 - SC (50% HbS) (+++ severity)
- Sickle cell anemia
 - hemolytic anemia
 - aplastic crisis
 - vaso-occlusive
- Sickle cell-HbC disease

Thalassemia

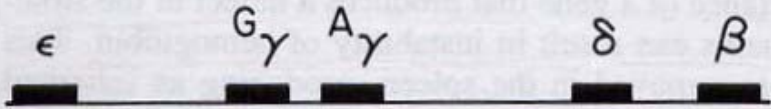
- Decreased rate of globin chain production
 - thalassemia $\gamma, \epsilon, \zeta \rightarrow$ embryonic death
- Classification
 - Thalassemia major
 - thalassemia minor
 - thalassemia minima
- Alpha-thalassemia
- Beta-thalassemia
 - β^+ or β^0 -thalassemia



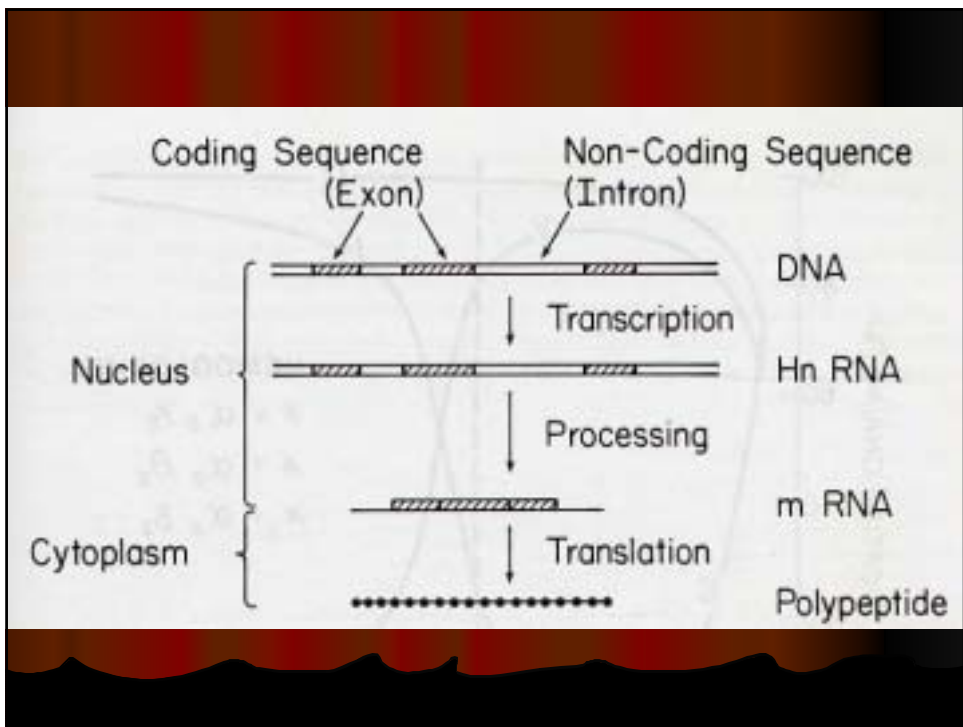
Chromosome 16



Chromosome 11

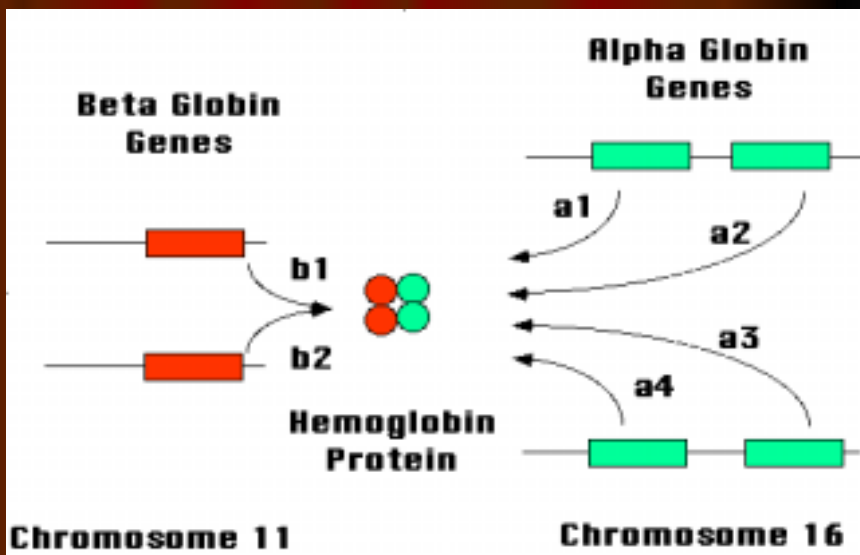


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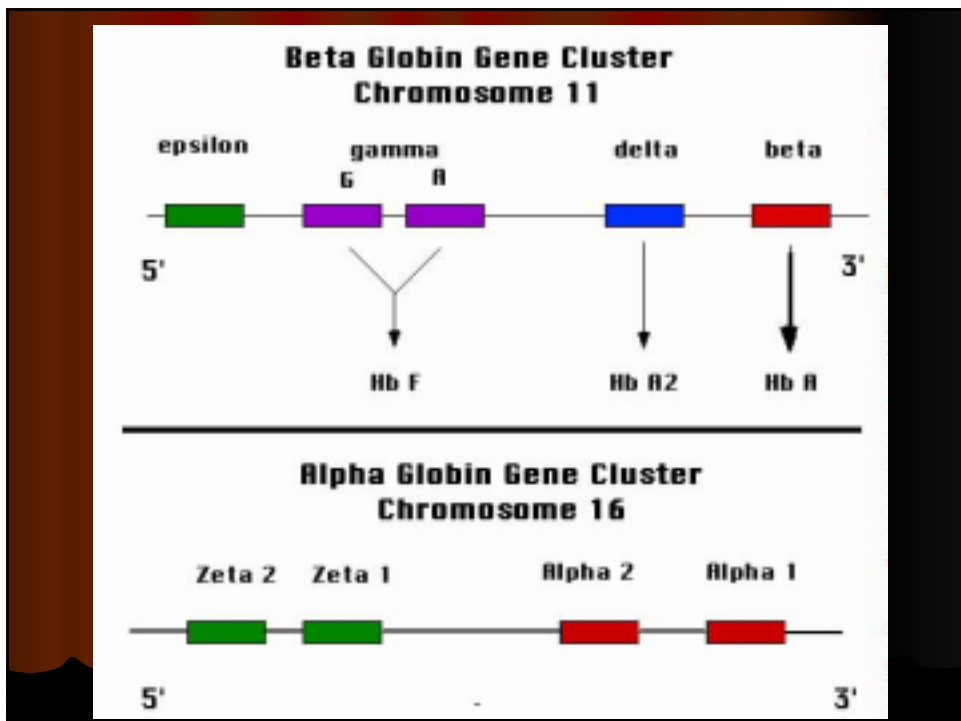
Hemoglobin protein has two alpha subunits and two beta subunits.

- The two chromosomes #11 have one beta globin gene each (for a total of two genes).
- The two chromosomes #16 have two alpha globin genes each (for a total of four genes).
- Each alpha globin gene produces only about half the quantity of protein of a single beta globin gene.



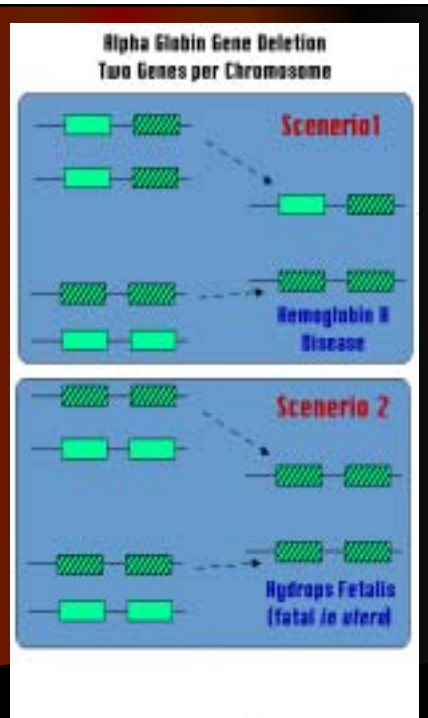
Alpha-thalassemia

- Defective α -chain synthesis
 - No elevation of HbA₂ and HbF
(↑in β -thalassemia)
- Consequence of diminished α -chain synthesis
 - decrease production of HbA, HbF, HbA₂
 - Excess β -chain and γ -chain
 - Hb Bart's (γ_4)
 - HbH (β_4)



The offspring that inherits the double deletion from one parent and the single from the other will have Hemoglobin H disease (Scenario 1).

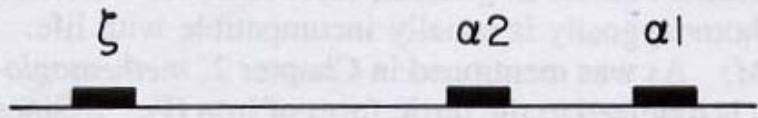
The offspring who inherits **no alpha genes** from the parents dies *in utero* (Scenario 2; hydrops fetalis).



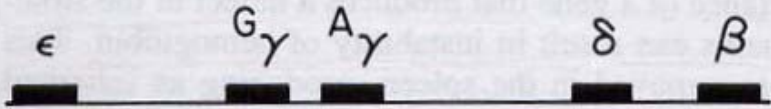
Beta-thalassemia

- Defective β -chain synthesis
 - Diminished (β^+ or β^{++}) or absent (β^0) of β -globin
 - elevation of **HbA₂** & **HbF** in β -thalassemia
- Consequence of diminished β -chain synthesis
 - decrease production of HbA,
 - Excess γ -chain and δ -chain
 - HbF
 - HbA₂
 - No Hb Bart's (γ_4)
 - No HbH (β_4)

Chromosome 16



Chromosome 11



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Hb electrophoresis (alkaline)

- Separation on cellulose acetate (pH8.4)
 (-) A₂ → S → F → A₁ (+)
- Most frequently used
- Resolve most of the major Hbs (A₁, A₂, S, F)
- A₂ can not be separated from C
- Can not resolve HbD, G; and HbC, E

DISEASE	ORIGIN →	A ₂ /C	S	FA
Normal				■
Sickle Cell Trait			■	■
Sickle Cell Disease			■	
Sickle C		■	■	
Sickle-thalassemia			■	
Thalassemia Major				■
Thalassemia Minor				■

Cellulose Acetate pH 8.4

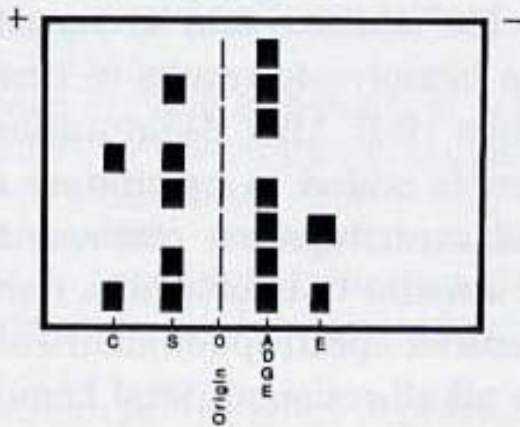


Normal
 Sickle Trait
 Hemoglobin D Trait
 SC Disease
 SE Disease
 Normal Cord Blood
 C Harlem Trait
 Control

Hb electrophoresis (acidic)

- Separation on agarose gel (pH6.0)
 (-) F A □ S C (+)
- Mostly used in confirmation
- E can be separated from C
- HbC, HbS migrate toward the Anode
- HbA, E migrate toward the cathode

Citrate Agar pH 6.0-6.2



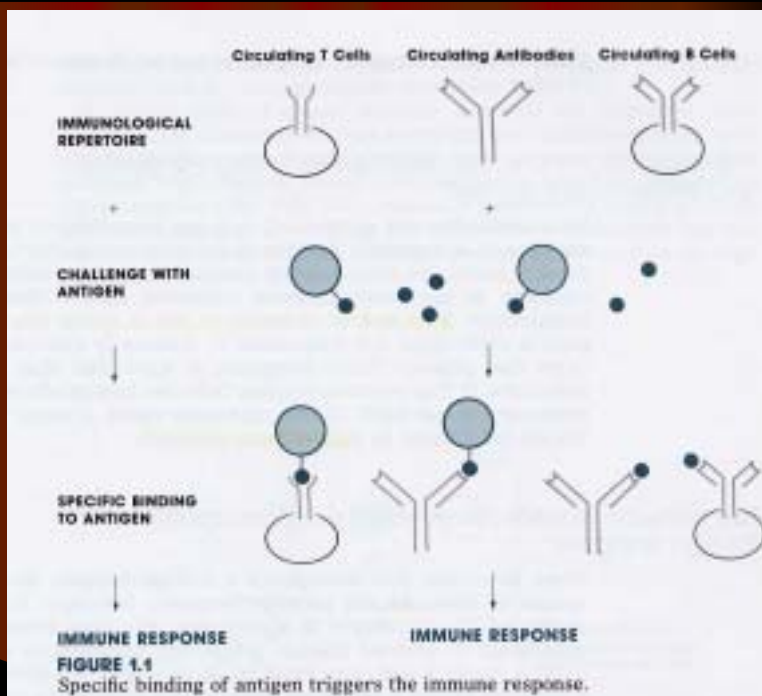
Normal
Sickle Trait
Hemoglobin D Trait
SC Disease
SE Disease
Normal Cord Blood
C Harlem Trait
Control

Protein electrophoresis

- Multiple myeloma and Immunoglobulins
- Data of protein electrophoresis
 - Acute reaction pattern
 - Nephrotic syndrome
 - Chronic inflammation
 - Cirrhosis of liver
 - α 1-antitrypsin deficiency (chronic diseases)
 - polyclonal gammopathies
 - hypogammaglobulinemia
 - Multiple myeloma (M spike)

Issues to be discussed

- Structure and function of Immunogloblins
- Clonal deletion and clonal expansion
- Development of lymphocytic lineage
- Multiple myeloma
- Consequences of multiple myeloma
- Normal patterns of protein electrophoresis
- Pathological patterns



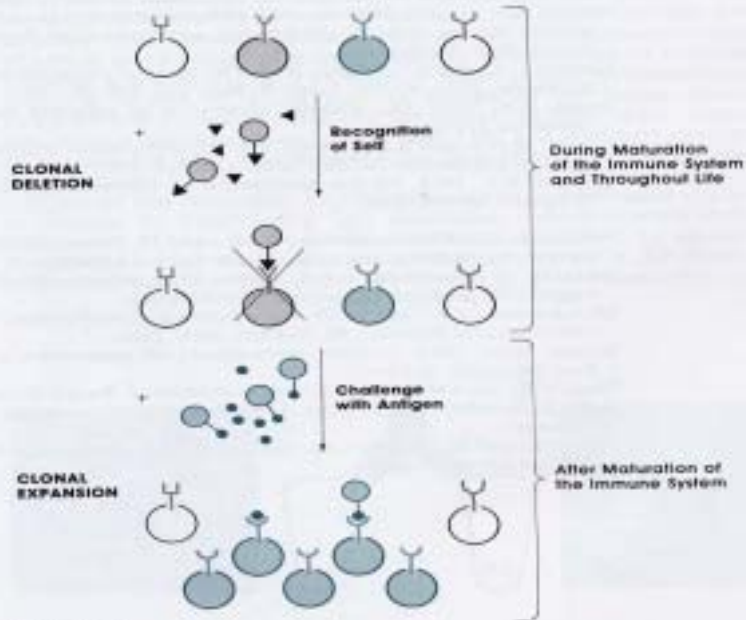





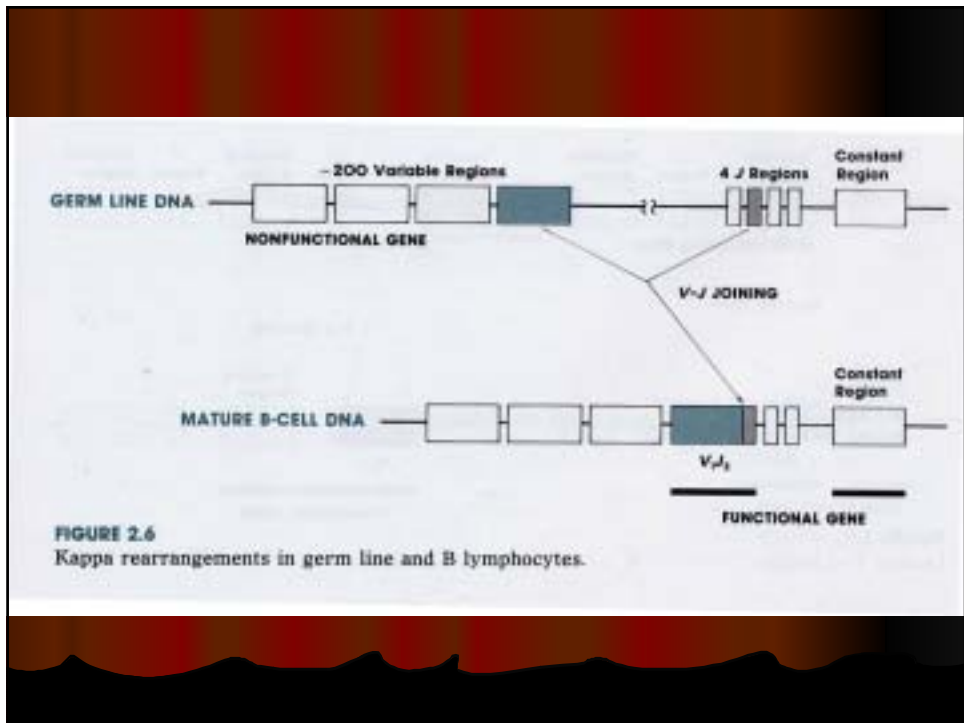


FIGURE 1.3
Clonal selection.

TABLE 2.1
Classes of Antibodies

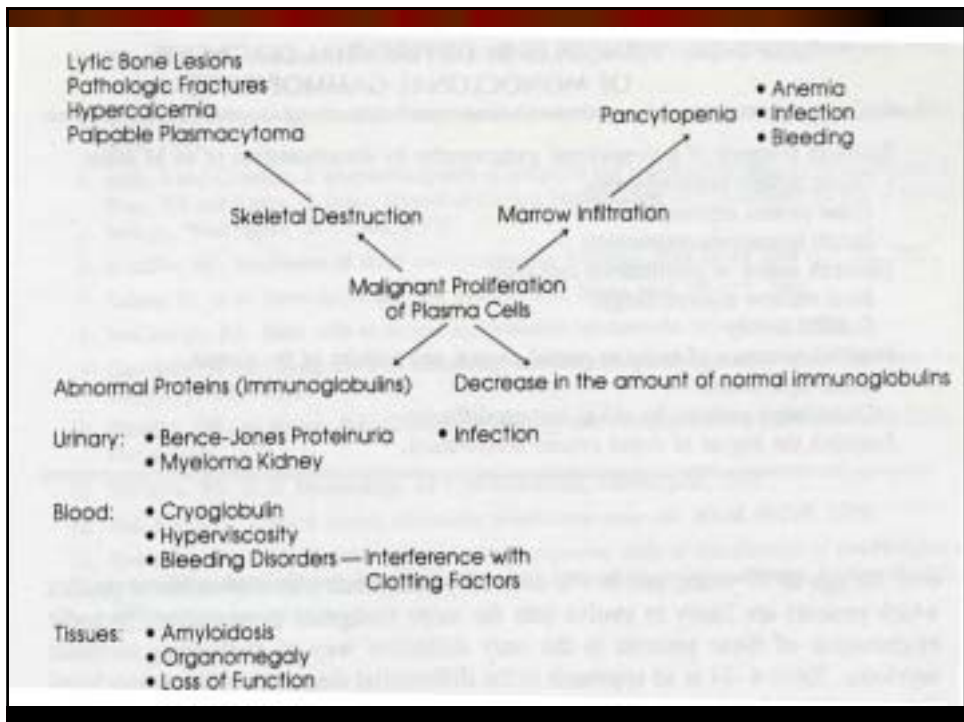
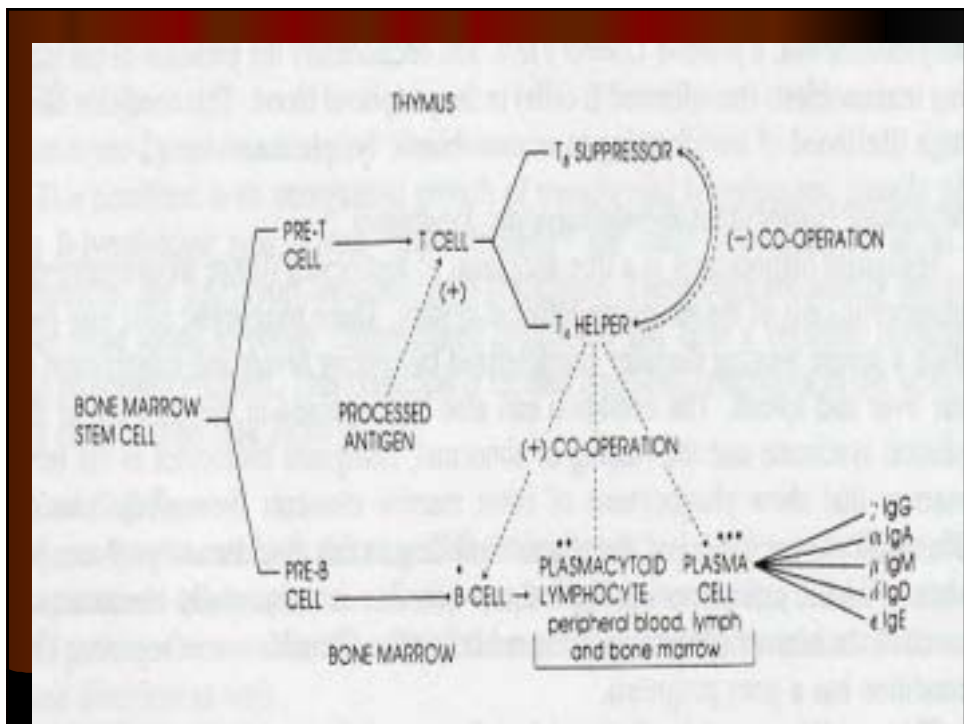
Characteristics	IgG	IgM	IgA	IgE	IgD
Heavy Chain	γ	μ	α	ϵ	δ
Light Chain	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Molecular Formula	$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$	$(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$	$(\alpha_2\kappa_2)_n^*$ or $(\alpha_2\lambda_2)_n$	$\epsilon_2\kappa_2$ or $\epsilon_2\lambda_2$	$\delta_2\kappa_2$ or $\delta_2\lambda_2$
Y Structure					
Valency	2	10	2, 4, or 6	2	2
Concentration in Serum	8–16 mg/ml	0.5–2 mg/ml	1–4 mg/ml	10–400 ng/ml	0–0.4 mg/ml
Function	Secondary response	Primary response	Protects mucous membranes	Protects against parasites (?)	?

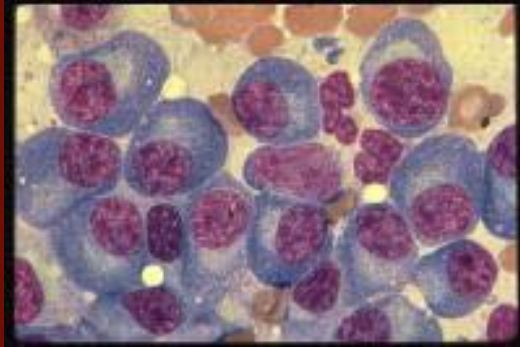
*n = 1, 2, or 3.



Multiple myeloma

- Maturation of plasma cells
- Proliferation of plasmacytoid lymphocytes
- Proliferation of plasma cells
- Principle of data interpretation
 - Blood
 - Hyperviscosity
 - Cryoglobulinemia
 - M spike
 - Bence Jones proteinuria





Bone marrow aspirate demonstrating plasma cells of multiple myeloma. Note the blue cytoplasm, eccentric nucleus, and perinuclear pale zone (or halo).

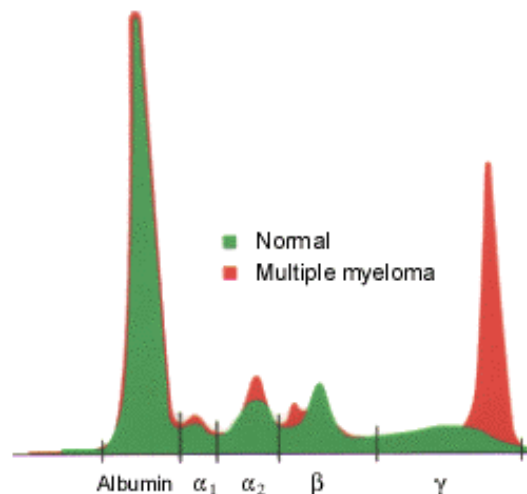


multiple punched-out lesions in a patient with multiple myeloma

Table 4-24. PRINCIPLES IN DIFFERENTIAL DIAGNOSIS OF MONOCLONAL GAMMOPATHIES

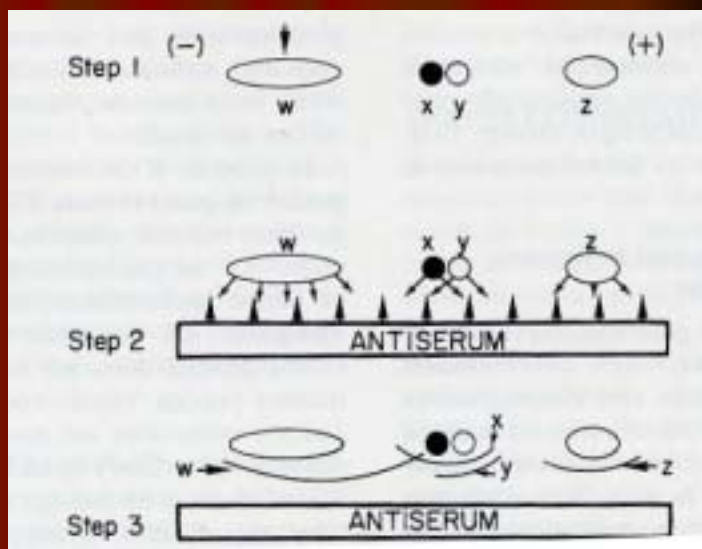
-
- Establish presence of a monoclonal gammopathy by documentation of an M spike.
 - Serum protein electrophoresis
 - Urine protein electrophoresis
 - Serum immunoelectrophoresis
 - Establish nature of proliferating cell type.
 - Bone marrow aspirate/biopsy
 - Skeletal survey
 - Establish presence of entire or partial protein and activity of the protein.
 - Urine light chains
 - Quantitative analysis by radial immunodiffusion
 - Establish the degree of organ system involvement.
-

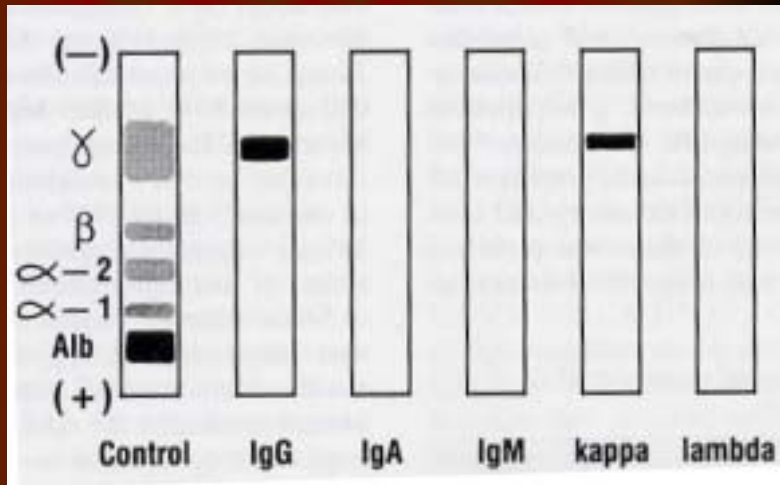
Serum Protein Electrophoresis



Bence Jones proteins

- The multiple myeloma cell clone produces an excess of monoclonal (M proteins) and free light chain proteins.
- The M proteins may be recognized as IgA, IgD, IgG, IgE or IgM, depending on their heavy chain class.
- The light chain proteins may be designated as kappa or lambda. They may precipitate and deposit, producing organ damage. The organ most commonly affected is the kidney.
- When these monoclonal light chains appear in the urine, they are called Bence Jones proteins.



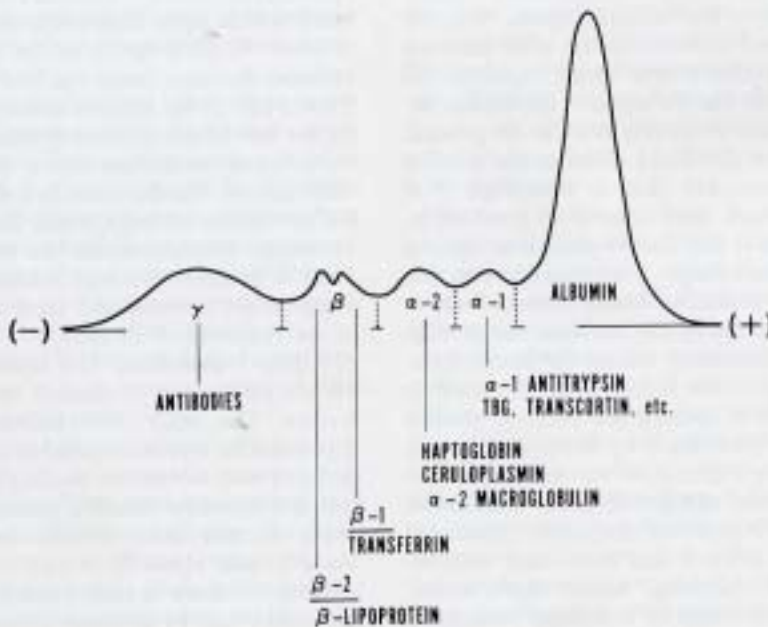


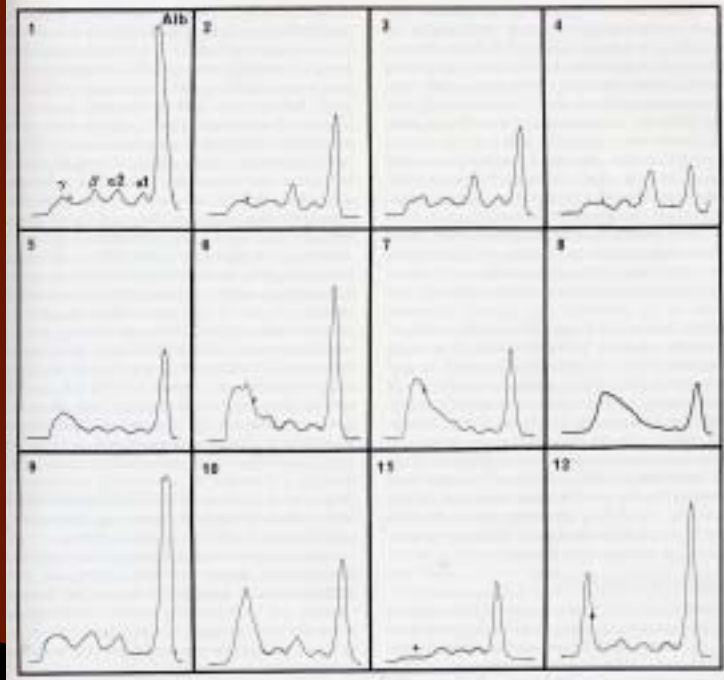
Routine laboratory

- Pancytopenia, abnormal coagulation, hypercalcemia, azotemia, elevated alkaline phosphatase and erythrocyte sedimentation rate, and hypoalbuminemia.
- Proteinuria, hypercalciuria, or both. Urine dipstick tests may not indicate the presence of Bence Jones proteinuria.
- All patients with suspected multiple myeloma require a 24-hour urinalysis by protein electrophoresis to determine the presence of Bence Jones proteinuria and kappa or lambda light chains.

Protein electrophoresis

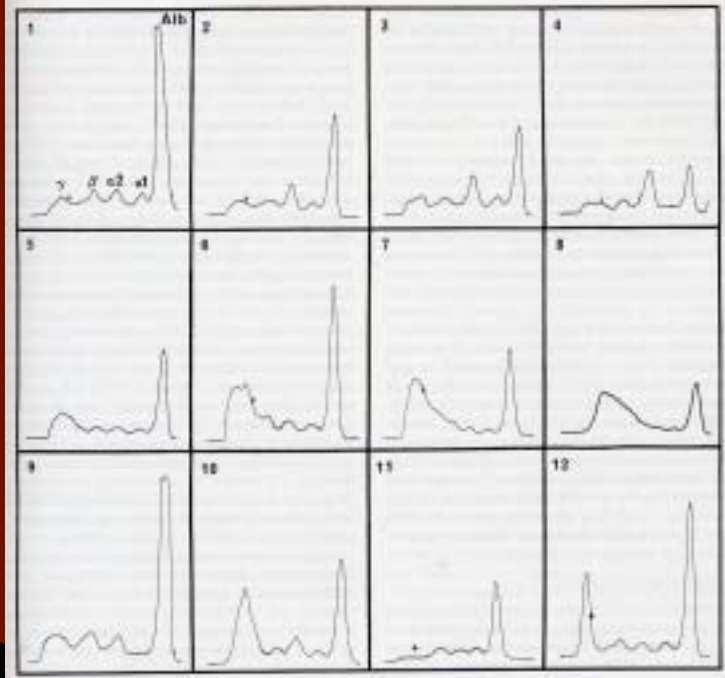
- Supporting matrix
 - Molecular charge
 - Agarose, cellulose acetate
 - Molecular charge and size
 - Starch and polyacrylamide
- pH and ionic strength of buffer
 - pH 8.6 → most proteins are negative charge
 - barbital, Tris-barbital, boric acid, Tris and EDTA
- Visualization
 - Coomassie brilliant blue, Ponceus S (albumin > globulin)
 - Amido black (agarose gel), bromphenol blue,





Data interpretation of protein electrophoresis

- Protein electrophoresis
- Data interpretation of
 - Normal
 - Acute reaction pattern (2, 3)
 - Nephrotic syndrome (4)
 - Chronic inflammation (5)
 - Cirrhosis of liver (6, 7, 8)
 - α 1-antitrypsin deficiency (chronic diseases) (9)
 - polyclonal gammopathies (10)
 - hypogammaglobulinemia (11)
 - Multiple myeloma (M spike) (12)



姓名

學號

1. Normal	2	3	4
5	6	7	8
9	10	11	12