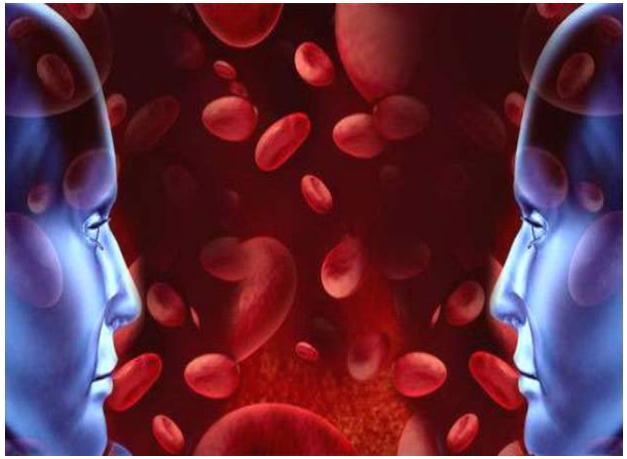
THALASSEMIA



Definition

Thalassemias are group of inherited disorders characterized by reduced or absent of globin chain biosynthesis.

- Thalassemia was defined as a clinical entity in 1925 when Dr.Thomas B.Cooley and his associate Pearl Lee, pediatricians at the Detroit Children's Hospital,

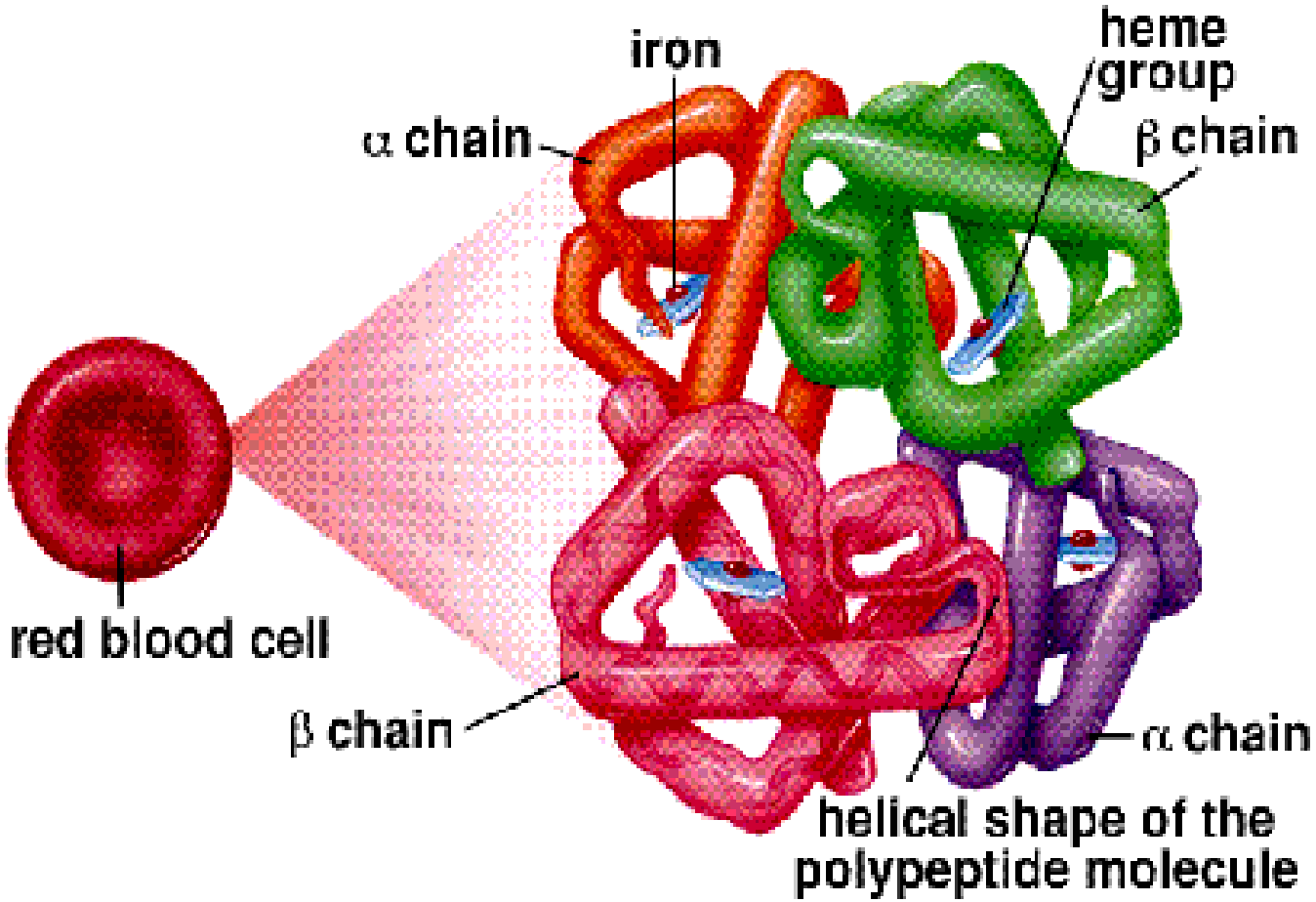
- Earlier it was called as the **anaemia splenica infantum.**

- Whipple and Bradford proposed the name ***Thalassemia.***

Prevalence of Thalassemia

Hemoglobin Structure

- Four subunits
  - two α
  - two β
- Iron
- Heme
- Binds 4 O_2



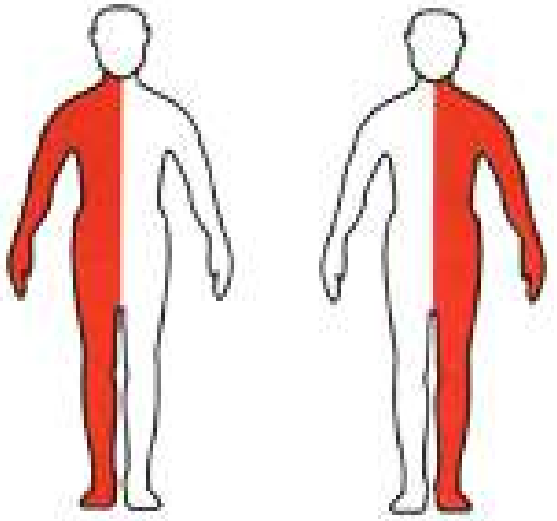
INHERITANCE

- Thalassemias are autosomal recessive disorders.
- Globin of haemoglobin A is made up of 2 alpha and 2 beta chains
- Synthesis of alpha chains is controlled by 2 gene clusters on chromosome 16 and of beta chains on chromosome 11.
- Each globin gene has 3 exons and 2 introns.

Inheritance

A parent with Beta-Thalassaemia trait

A parent with Beta-Thalassaemia trait

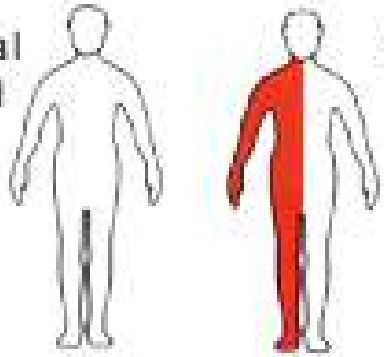


1 25% chance of a child without any trait

2 50% chance of a child with a trait (Minor)

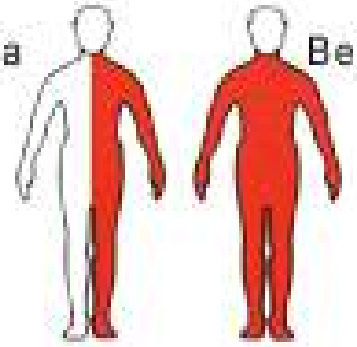
3 25% chance of a child with Beta-Thalassaemia Major

Normal blood

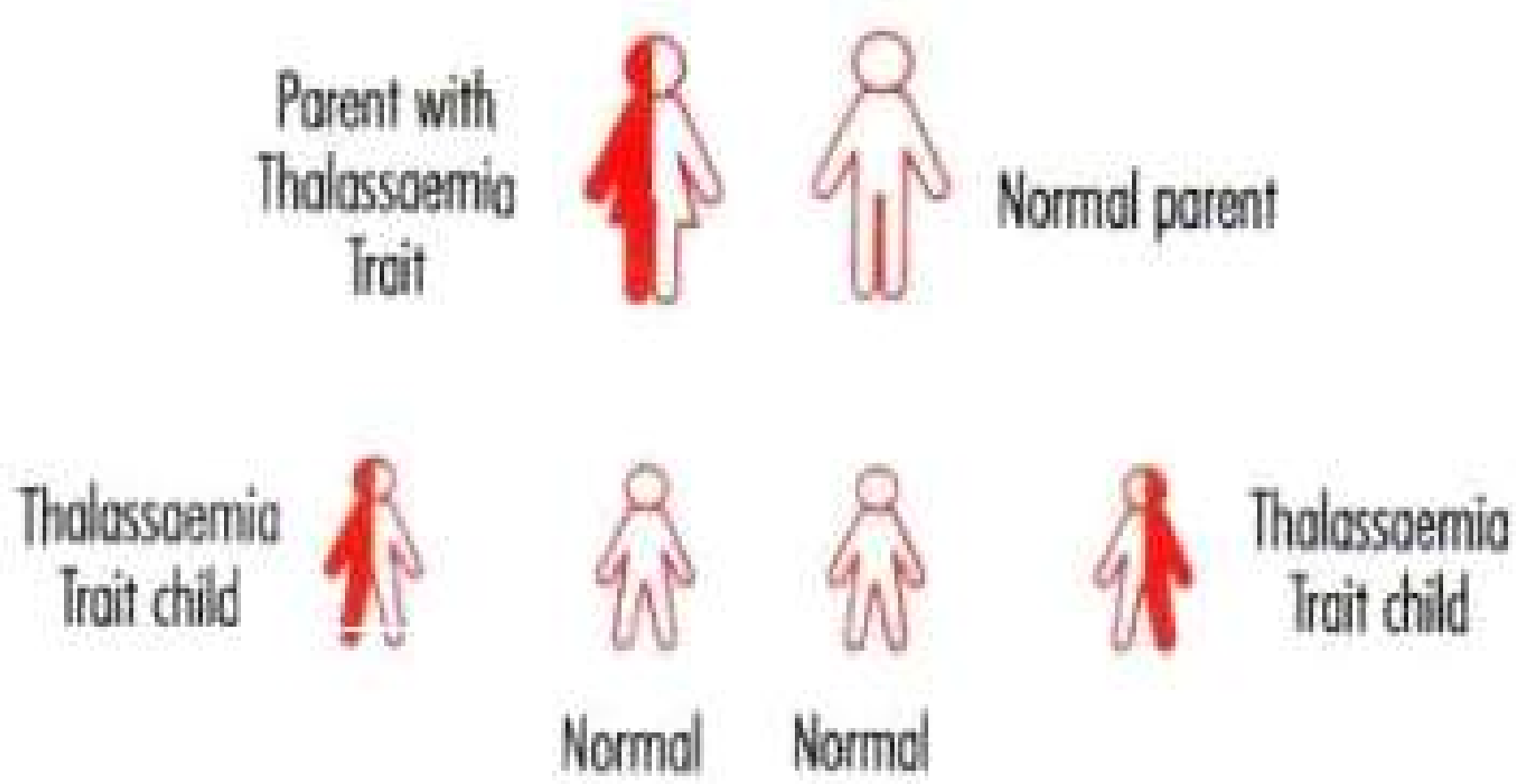


Beta-Thalassaemia trait

Beta-Thalassaemia major



Inheritance



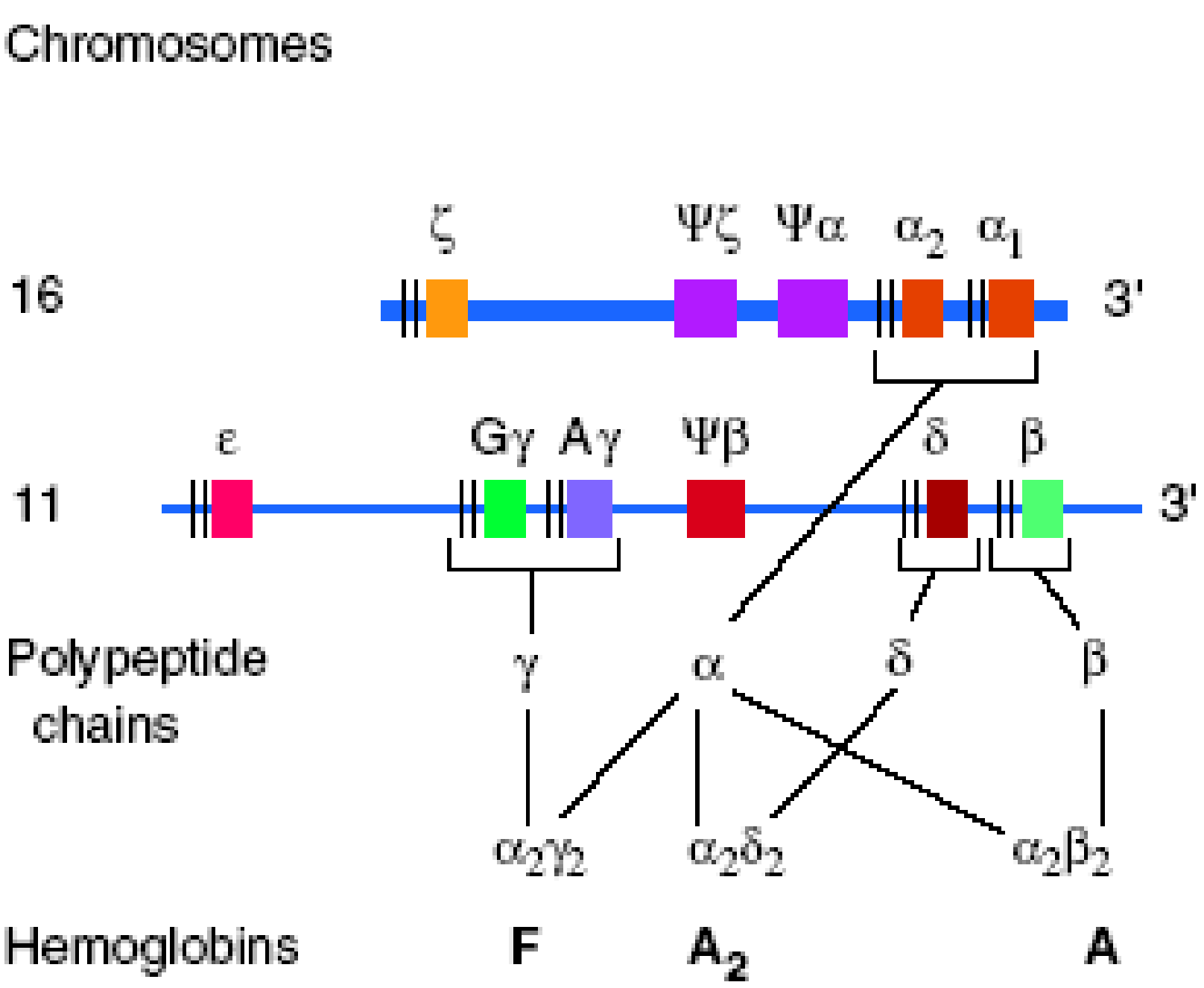

Chromosomes
16
ζ
Ψζ
Ψα
α2
α1
3'
11
ε
Gγ
Aγ
Ψβ
δ
β
3'
Polypeptide chains
γ
α
δ
β
α2γ2
α2δ2
α2β2
Hemoglobins
F
A2
A

Table 2. Types of haemoglobins and globin chains present in normal adult blood and in haemoglobinopathies

Haemoglobin (Hb)	Globin chains	Percentage found in normal adult blood	Clinical state
HbA	$\alpha^2\beta^2$	~97%	Normal
HbA2	$\alpha_2\delta_2$	2–3%	Normal
HbF	$\alpha_2\gamma_2$	<1%	Normal
HbH	β^4	0%	α-thalassaemia
HbBarts	γ^4	0%	α-thalassaemia
	α chain aggregates – insoluble	0%	β-thalassaemia
HbS	$\alpha^2\beta s^2$	0%	Sickle cell disease

Classification of thalassemia

- According to deficient globin chains
  - Alpha thalassemia
  - Beta thalassemia
  - Delta-beta thalassemia
  - Gamma delta beta thalassemia

According to clinical severity

Alpha thalassemia

- Silent carrier
- Thalassemia trait
- HbH disease
- Hb Barts/ Hydrops foetalis syndrome

Beta thalassemia

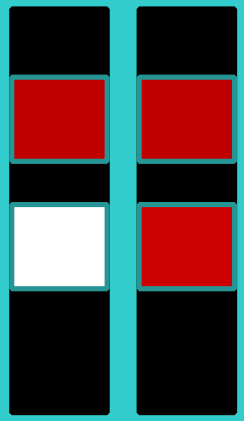
- Thalassemia major
- Thalassemia intermedia
- Thalassemia minor

α - Thalassemia

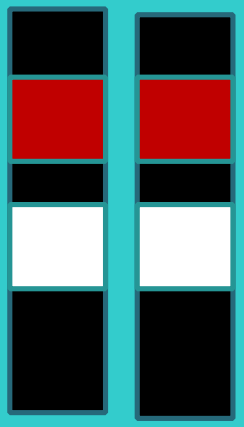
- α chains of globin are not/partly synthesized.
- It is required for both HbA and HbF .
- Majority of α thalassemia cases result from gene deletions.

α-thalassaemias

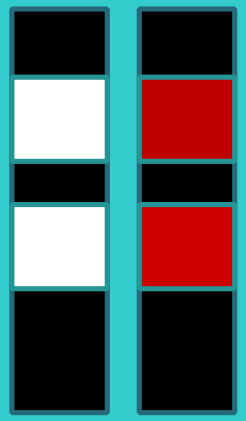
Chromosome 16

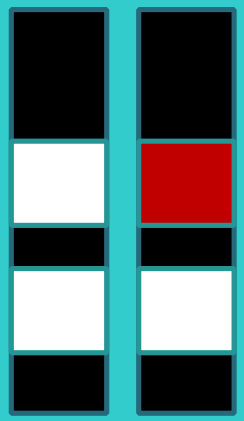


Silent carrier state

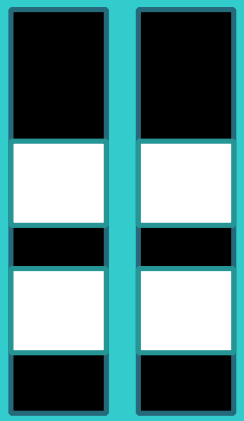


α-thalassaemia trait





Haemoglobin H disease



Homozygous α-thalassaemia

Hydrops foetalis or haemoglobin Barts disease

Mutations causing α thalassemia

- Most cases of α thalassemia result from gene deletion

- Other –

1) Mutation which cause aberrant splicing
2) Mutation of chain terminator codon
3) Mutation which cause instability of α globin chain after translation.

BETA THALASSEMIA

Molecular basis of β-Thalassemias

- Beta (o) thalassemias
  - Complete absence of beta chain synthesis
- Beta (+) thalassemias
  - Reduced synthesis

Mutations causing β-Thalassemia

1) Mutations which affect transcription
2) Mutation that affects splicing of RNA
3) Mutations affecting consensus sequences
4) Polyadenylation mutations
5) Mutations which lead to the formation of the chain termination codon
6) Frame-shift mutations
7) Deletions

Lab Investigations

- Screening tests
- Diagnostic tests
- Prognostic tests

Screening tests

- Red cell indices
- Single tube osmotic fragility test
- Estimation of Hb A2
- Haemoglobin electrophoresis at alkaline pH
- Estimation of Hb F and Hb H inclusion.
- Chorionic villi sampling – 11th week
- Amniocentesis – 16th week

Diagnostic tests

- Electrophoresis
- HPLC

Electrophoresis

- **PRINCIPLE** –

  The term electrophoresis describes the migration of a charged particle under the influence of an electric field. Different haemoglobins have different net charge because of variation in their structure.

- Under the influence of an electric field these charged particles will migrate either to the cathode or to the anode, depending on the nature of their net charge.
- Separation of haemoglobins by electrophoresis → pH 8.4 (alkaline) and pH 6.2 (acid).
- Bands are seen by staining.
- Scanning allows quantification of the hemoglobin present,

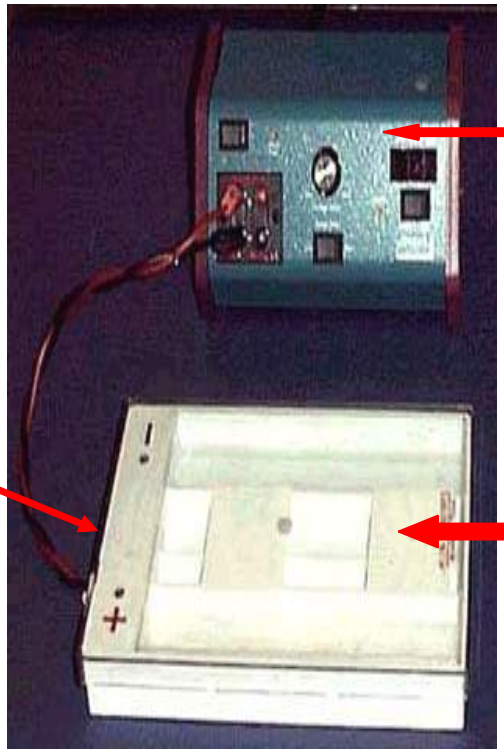
Hemoglobin electrophoresis at *alkali pH*

- Hb: Amphoteric molecule
- Molecular net charge depends on pH of the medium.
- pH > pI (Iso-electric point) : Molecular net charge is negative.
- pH < pI : Molecular net charge is positive.
- pI (Iso-electric point) is the pH where molecular net charge of hemoglobin is zero.

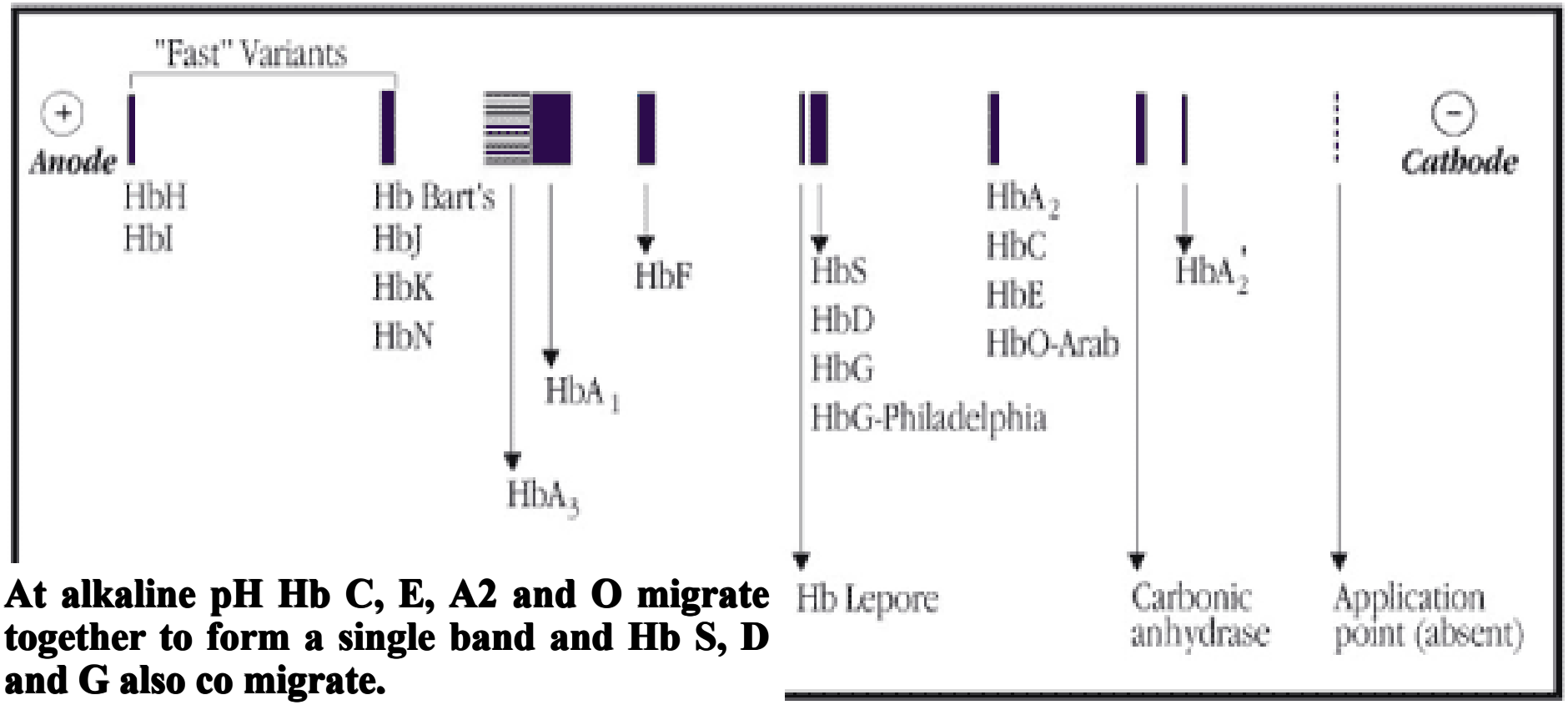
Equipment

Reagent : Tris-EDTA-Borate (TBE) pH 8.4-8.6

Cellulose acetate plate



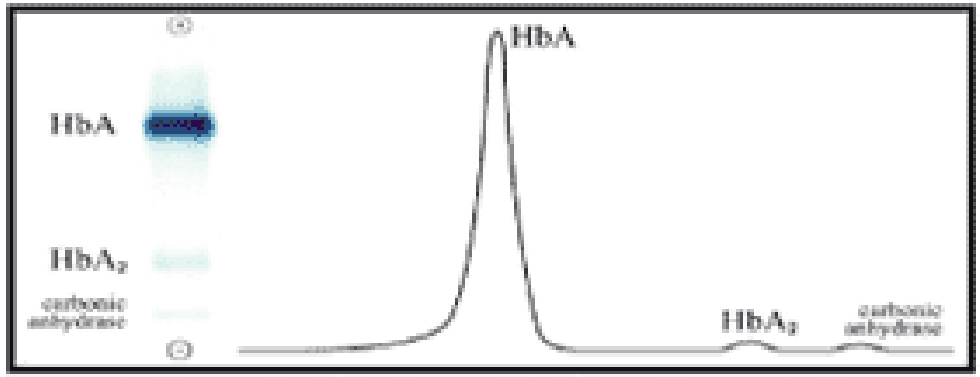
Position of hemoglobin bands on Alkaline Hemoglobin gel



At alkaline pH Hb C, E, A2 and O migrate together to form a single band and Hb S, D and G also co migrate.

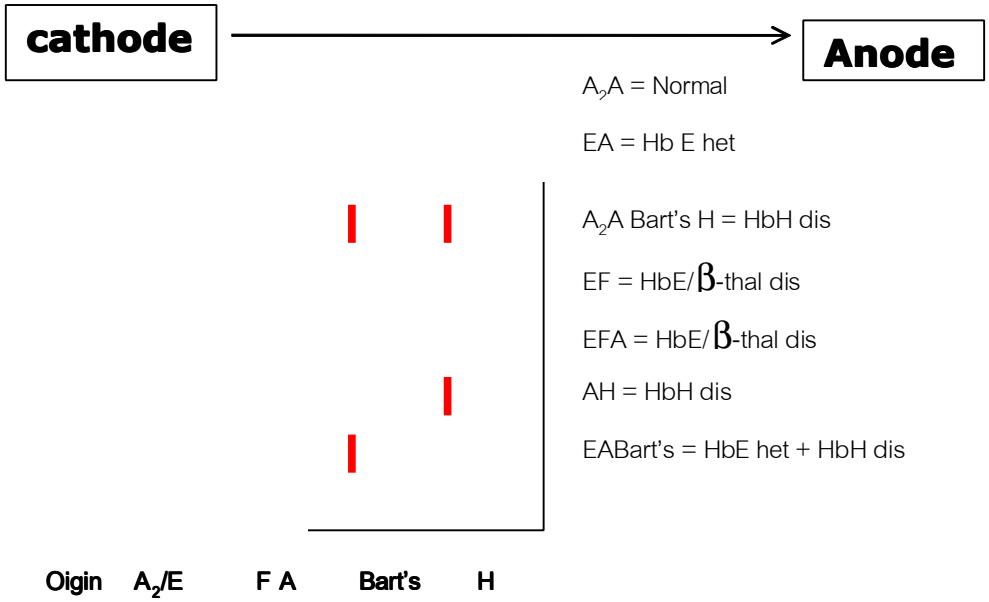
Adult normal values range

Hb type	Tot. Hb.%
HbA	96-99
HbA_2	1-3,5
HbF	≤2

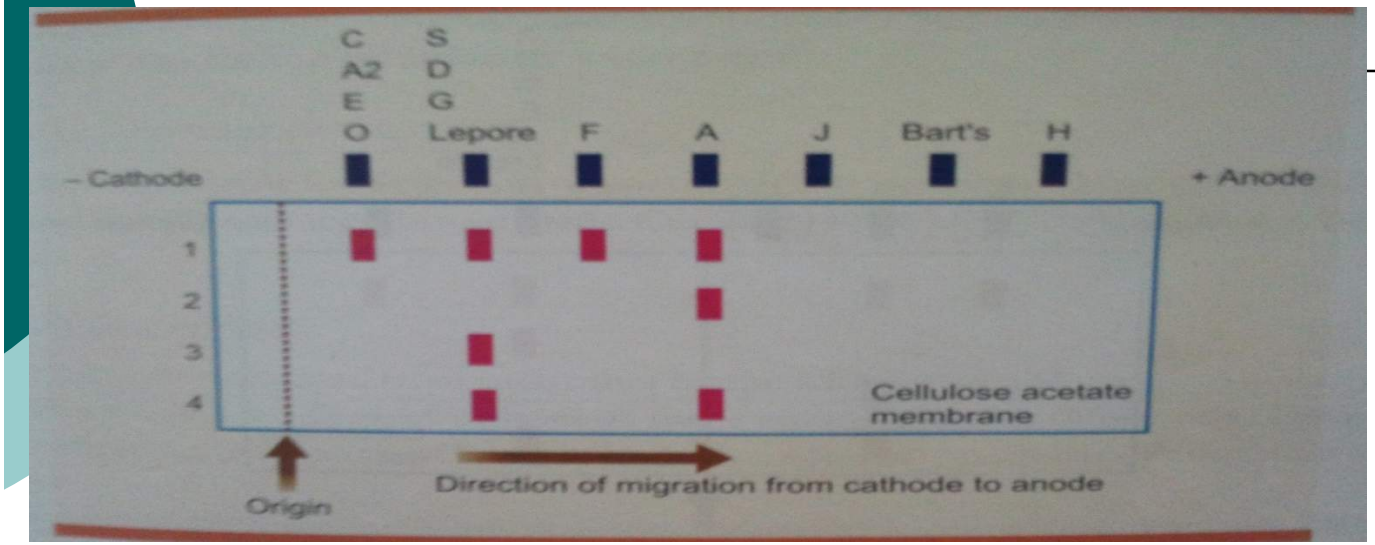


Normal Hemoglobin Pattern

Hb pattern on CAE with TBE pH 8.6

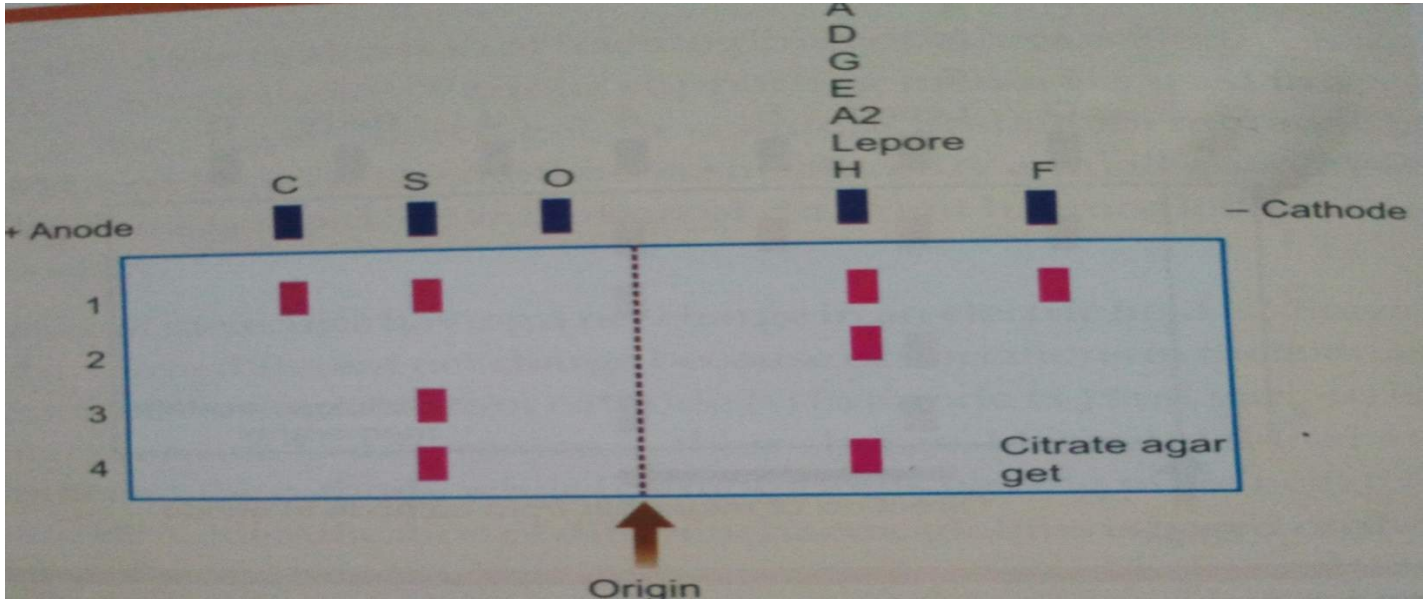


Electrophoretograms



Alkaline pH

Acidic pH



Electrophoresis - Advantages

- Cost effective,
- Widely available,
- Rapid method.

- Gives an estimate of HbA2 level.

- Identifies some variant haemoglobins which are well characterized.

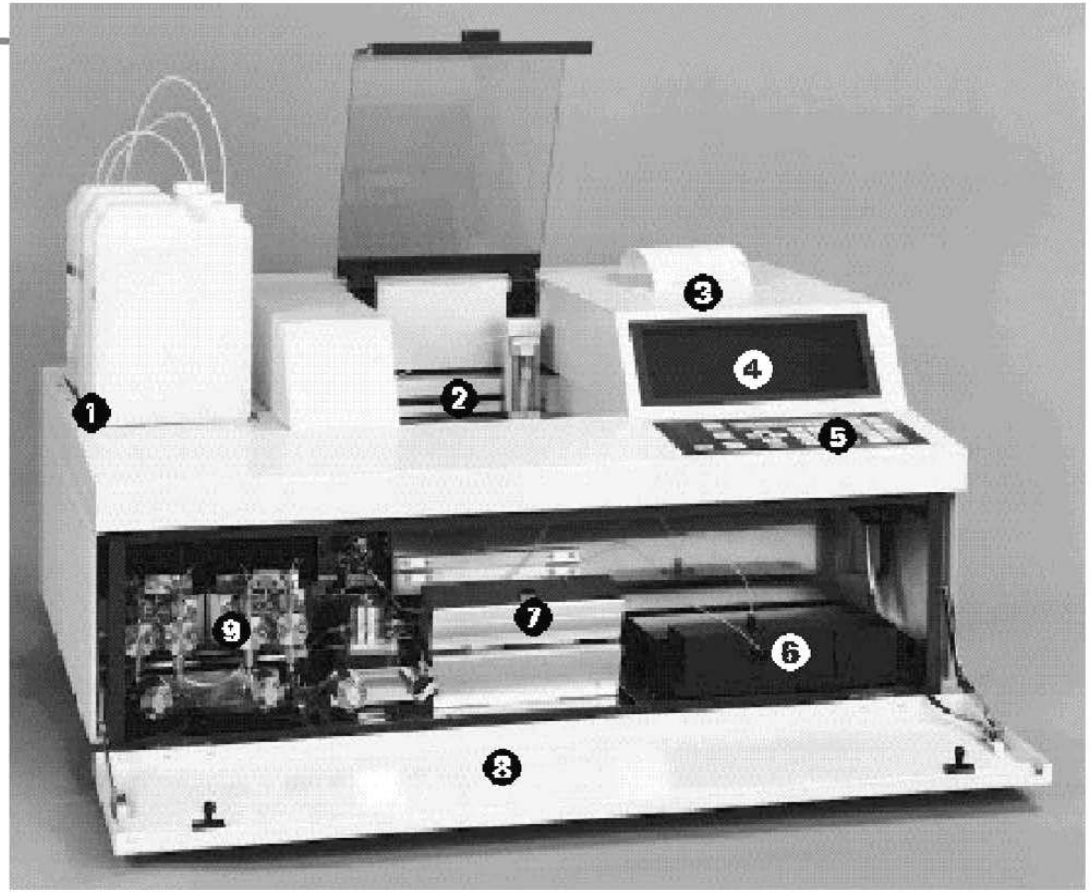
HPLC

PRINCIPLE:

- Positively charge molecules (salt and hemoglobin) bind to the carboxyl groups.
- Haemoglobin molecules are bound and displaced by increasing salt concentration.
- Haemoglobin variants separate out due to variation in charge.

Biorad Variant HPLC

1. Reagent Bottles
2. Autosampler
3. Printer
4. LCD
5. Control Panel
6. Detector
7. Cartridge Thermomodule
8. Front Panel
9. Pump Module

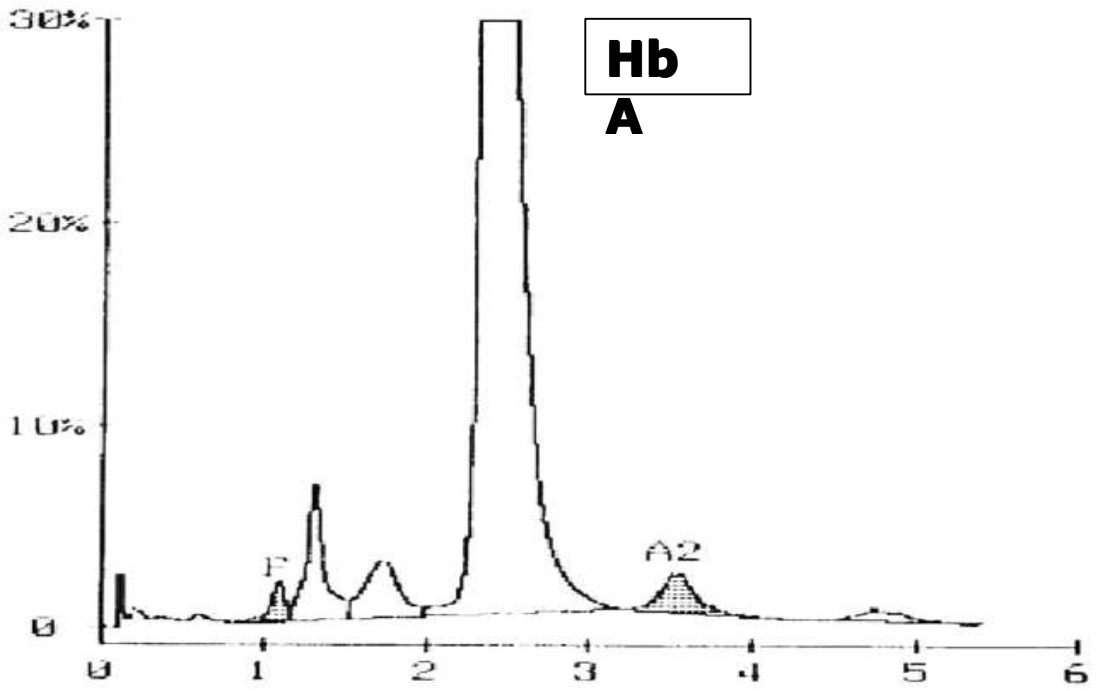


BIO-RAD

Hb A

ANALYTE ID	%	TIME	AREA
F	1.2	1.09	22966
P2	5.0	1.30	98779
P3	4.1	1.73	81235
Ao	85.8	2.37	1708029
A2	2.9	3.53	57396
S-WINDOW	1.2	4.73	22962
	TOTAL AREA		1991367

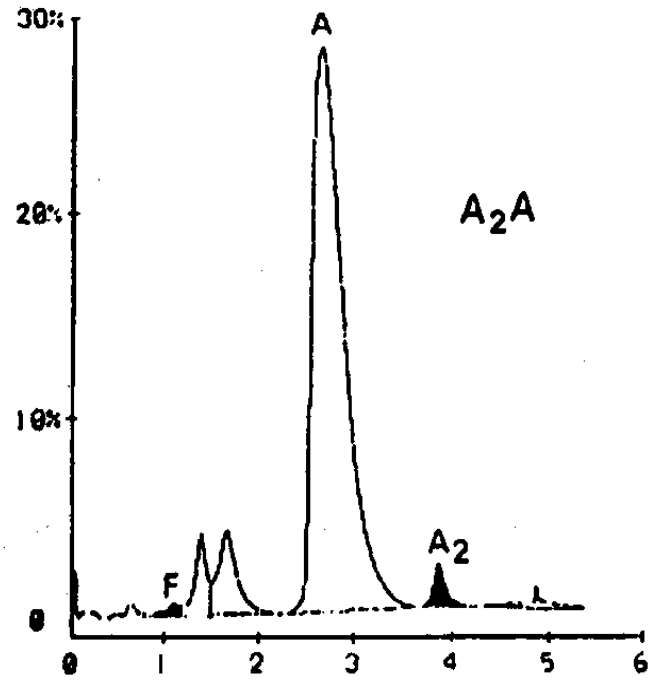
F 1.2% A2 2.9%



VIAL# 18 SAMPLE ID# 0

PEAK ID	%	TIME	AREA
F	0.8	1.08	23520
P2	4.5	1.38	117549
P3	7.8	1.65	202383
Ao	82.3	2.64	2133299
A2	2.8	3.84	69213
Unknown 1	1.8	4.86	45812
	TOTAL AREA		2591776

F 0.8% A2 2.8%



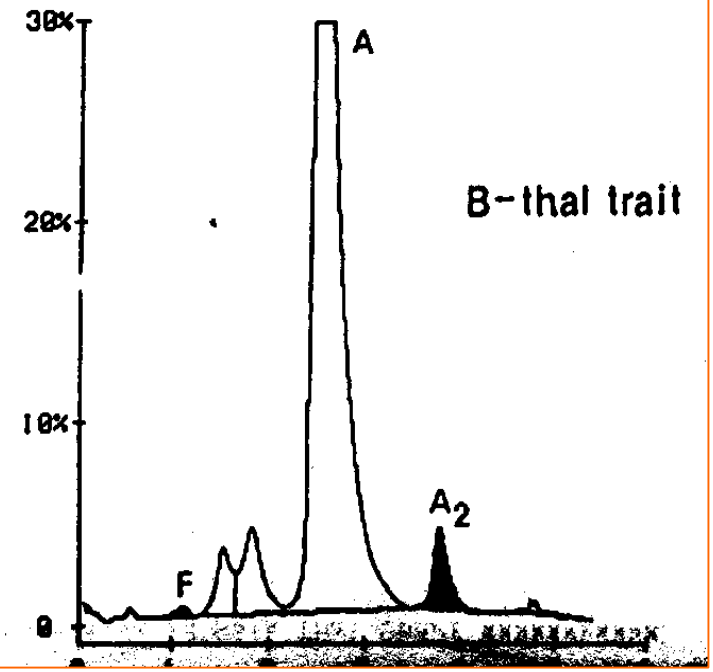
a-thal trait

xxxxxxxxxxxx Beta Thal Short xxxxxxxxxxx
DATE:11/06/94 TIME:23:58:14

VIAL# 81 SAMPLE ID# 0

PEAK ID	%	TIME	AREA
F	0.5	1.10	11466
P2	3.5	1.52	77655
P3	5.9	1.82	129629
Ao	85.3	2.48	1880286
A2	5.0	3.77	106099
	TOTAL AREA		2205135

F 0.5% A2 5.0%



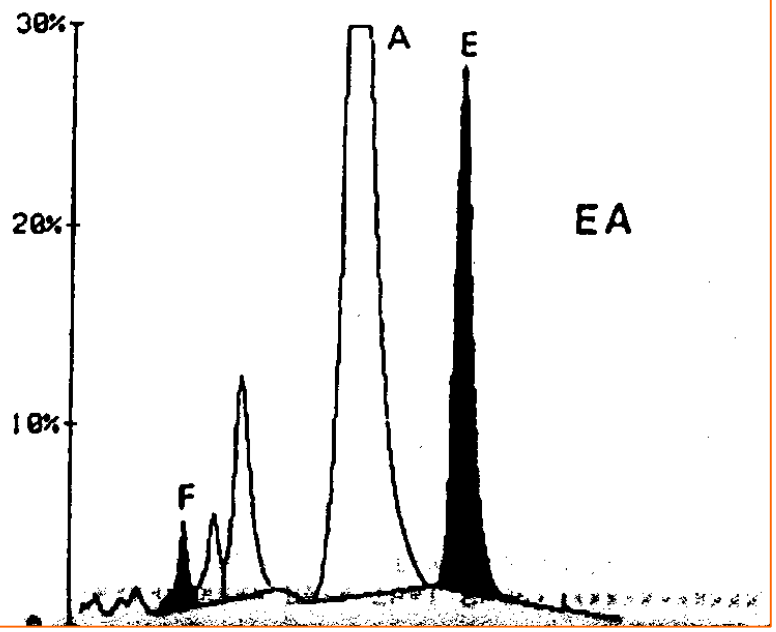
b-thal trait

xxxxxxxxxxxx Beta Thal Short xxxxxxxxxxx
DATE:08/11/93 TIME:12:01:07

VIAL# 15 SAMPLE ID# 0

PEAK ID	%	TIME	AREA
F	2.1	1.11	57366
P2	2.6	1.40	59390
P3	8.7	1.66	200255
Unknown 1	0.1	2.11	2612
Ao	61.9	2.70	1421529
A2	28.2	3.78	553885
TOTAL AREA			2295037

F 2.1% A2 28.2%



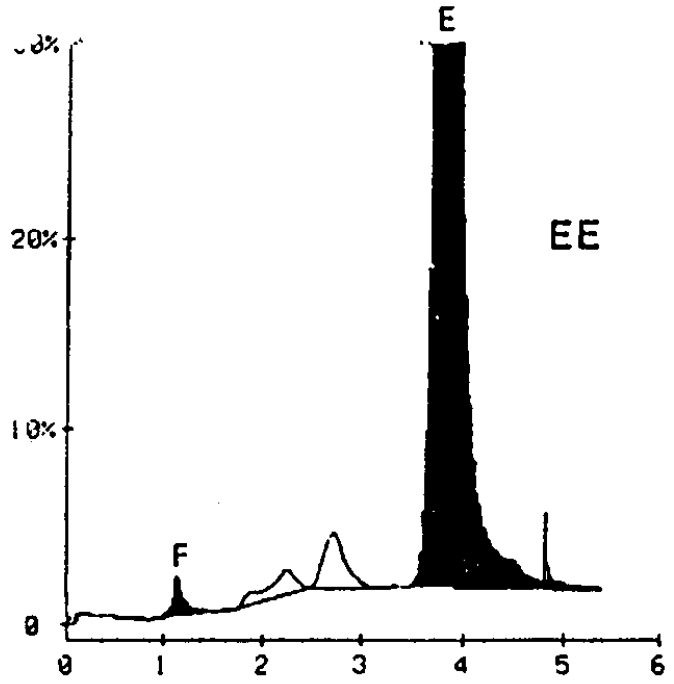
HbE trait

xxxxxxxxxxxx Beta Thal Short xxxxxxxxxxx
DATE:08/09/93 TIME:13:25:13

VIAL# 5 SAMPLE ID# 0

PEAK ID	%	TIME	AREA
F	1.1	1.13	13599
Unknown 1	1.8	2.25	19880
Ao	2.8	2.72	31656
A2	93.2	3.77	1052592
TOTAL AREA			1117727

F 1.1% A2 93.2%



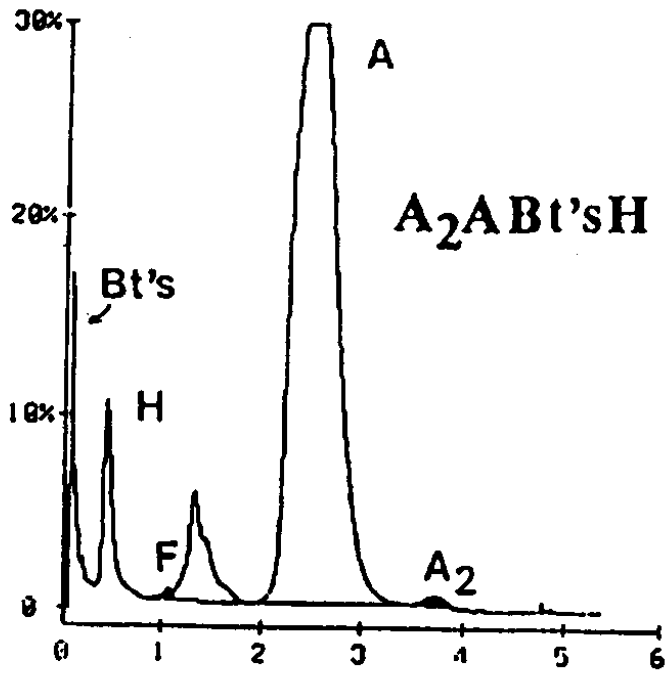
Homo E

xxxxxxxxxxxx Beta Thal Short xxxxxxxxxxx
DATE:07/12/94 TIME:16:59:49

VIAL# 12 SAMPLE ID# 0

PEAK ID	%	TIME	AREA
F	0.4	1.86	8134
P2	7.2	1.32	139456
Ao	91.2	2.48	1771823
A2	1.3	3.72	22368
	TOTAL AREA		1941781

F 0.4% A2 1.3%

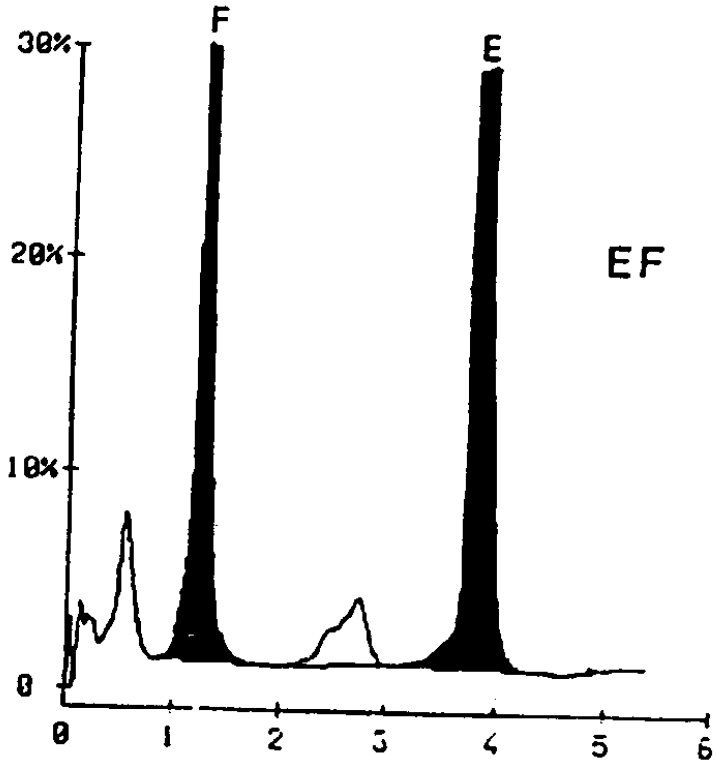


Hb H disease in newborn

VIAL# 7 SAMPLE ID# 0

PEAK ID	%	TIME	AREA
F	40.6	1.25	576015
Ao	8.4	2.73	86658
A2	50.4	3.73	666228
	TOTAL AREA		1349101

F 40.6% A2 50.4%



HbE/βº-thalassemia

HPLC Advantages

- Method of choice for screening for Hb variants;
- for quantification of HbA2 + HbF concentrations
- Neonatal screening.
- Quicker and more sensitive than standard techniques for detecting HbF .
- Indeed alkaline gel electrophoresis cannot detect HbF in healthy adults or those with marginally increased Hb F.

HPLC Disadvantages

- Hbs may co-elute or may elute before instrument peak integration.
- The measurement of Hb A2 is complicated in individuals with Hbs because the Hb A2 is falsely increased by the presence of Hb S adducts.

DNA Analysis

- Indicated when the hemoglobinopathy is not confirmed by other methods.
- When the underlying mutation is important for management.
- For genetic counseling → particular mutation or deletion.

DNA Analysis (cont…)

- WBCs, amniocytes, or chorionic tissue may be utilized for diagnosis of various α and β globin chain abnormalities.

- Southern blot hybridization.
- PCR - amplifies globin gene.
- PCR can be used to detect unknown mutations.

Globin Chain Synthesis

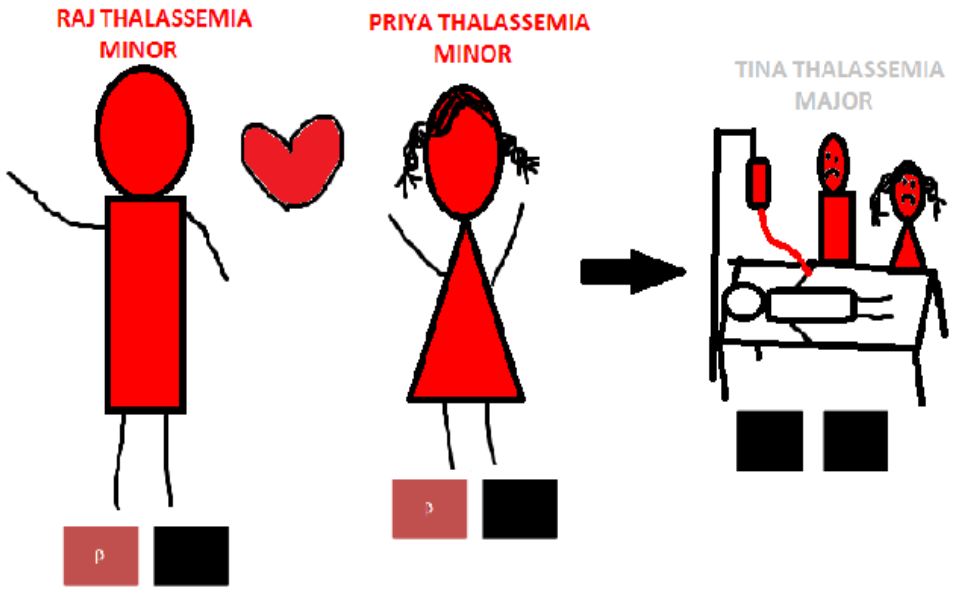
- It is helpful when electrophoretic and other usual haematological studies fail to diagnose.
- α : β ratio. Normal ratio is about 1.0.
- It is reduced in alpha thalassemia and increased in beta thalassemia

PROGNOSTIC TEST

Biochemical parameter
Serum ferritin (µg/l)
Hemoglobin (g/l)
Serum iron (µmol/l)
Serum TIBC (µmol/l)
TISP (%)
Transferrin concentration (mg/l)

TIBC = Total iron binding capacity; TISP = Transferrin iron saturation percentage

THANK YOU


RAJ THALASSEMIA
MINOR
PRIYA THALASSEMIA
MINOR
TINA THALASSEMIA
MAJOR